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Review

A systematic review of experimental infections with enterotoxigenic *Escherichia coli* (ETEC)☆

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ABSTRACT

Volunteer challenge with enterotoxigenic *Escherichia coli* (ETEC) has been used for four decades to elucidate the pathogenesis and immune responses and assess efficacy of various interventions. We performed a systematic review of these studies and a meta-analysis of individual patient-level data (IPD) from a subset of studies using standard methodology.

We identified 27 studies of 11 ETEC strains administered to 443 naive subjects at doses from 1×10^6 to 1×10^{10} colony forming units (cfu). Diarrhea attack rates varied by strain, dose and enterotoxin. Similar rates were seen at doses of 5×10^8 to 1×10^{10} cfu with the three most commonly used strains B7A, E24377A, H10407. In IPD analysis, the highest diarrhea attack rates were seen with strains B7A, H10407 and E24377A. The H10407 induced significantly higher stool output than the other strains. Additionally, the rate of output was different across strains.

The risk of diarrhea, abdominal cramps, nausea and headaches differed significantly by ETEC strain. An increased risk of nausea, abdominal cramps and headaches was seen for females. Baseline anti-LT IgG titers appeared to be associated with a decrease risk of diarrhea outcomes, a trend not seen with anti-LT IgA or seen consistently with anti-colonization factor antibodies. Neither early antibiotic treatment nor diarrhea duration significantly affected the frequency or magnitude of serologic responses.

These studies have served as an invaluable tool in understanding disease course, pathogenicity, innate immune responses and an early assessment of product efficacy. When designing and planning experimental ETEC infection studies in this age of increased ethical scrutiny and growing appreciation of post-infectious sequelae, better understanding of available data is essential.

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1. Introduction

Over the past 40 years, the enterotoxigenic *E. coli* (ETEC) human challenge model has been used to elucidate the pathogenesis and immune responses associated with ETEC infection as well as to test the efficacy of investigational drugs and vaccines. The initial experimental infection, published in 1971, was a landmark study establishing ETEC as the organism responsible for causing acute, cholera-like illness in a U.S. soldier in Vietnam [1]. In this classic paper, researchers demonstrated that while porcine and human isolates of disease-causing *E. coli* were both capable of inducing fluid excretion in rabbit ileal loops, only human isolates were capable of causing disease in human subjects. It was later discovered that the difference in the two strains was the species-specific tropism of the intestinal colonization factor fimbriae.

Since this Landmark publication, ETEC has been established as the most common cause of diarrhea in travelers as well as in young children in resource-limited regions of the world. As such, it has also become the focus of vaccine development efforts [2,3]. Since an immune correlate of protection has yet to be established and physiologically relevant animal models are lacking, researchers have frequently relied on the use of vaccine-challenge studies in the early clinical development of investigational products. This mechanism has been supportive for vaccine development efforts for cholera, an enteric pathogen with similar disease mechanisms [4] as well as other non-enteric pathogens [5]. The basic concept of the ETEC challenge study is to select a well-characterized, antibiotic susceptible organism that has been associated with diarrhea and related gastrointestinal symptoms. Under close inpatient supervision, the strain is fed to volunteers at a dose that induces diarrhea. Illness is often curtailed by early antibiotic treatment. Preliminary protective efficacy is then calculated by comparing the diarrhea attack rates in subjects receiving an investigational product with subjects receiving placebo.

Here, we have performed a systematic review to thoroughly examine the published literature and other unpublished data and compiled aggregate information regarding the pathogenicity, virulence, and immune responses observed in experimental ETEC infections. Our purpose was to understand the relationship between clinical manifestations of infection and ETEC virulence factors as well as identify potentially important host-specific factors similarly associated with clinical outcomes. The expected outcome is a better understanding of experimental ETEC infections with regards to factors inherent to the CF-toxin profiles of the ETEC strains tested, and factors external to the organism that may affect pathogenicity such as inoculum preparation and administration procedures, variability in study populations. Our findings can be applied to the design and interpretation of future studies with previously untested ETEC strains.

2. Methods

This study was a systematic review of the published and unpublished literature to evaluate specific outcomes in subjects participating in experimental ETEC infection studies using the accepted principles of good methodological design [6,7]. The methodology included the formulation of an analytic framework with the development of key questions to be answered by systematic reviews of the scientific literature. For each question, the systematic review included eligibility criteria for available evidence, standardized data abstraction, critical appraisal of the quality of the evidence, analysis of the data (including a determination of the appropriateness of applying meta-analysis), and interpretation of the results.

In addition to study-level information, a subset of studies with known similarities in specific outcomes was identified for the compilation and analysis of individual patient data. In addition to evaluating factors inconsistently reported in the published literature, analysis of pooled individual patient-level data (IPD) affords the opportunity for more detailed analyses while avoiding some of the potential biases inherent in analyzing summary statistics of study participants [8].

2.1. Search strategy

A comprehensive retrieval of information was conducted by initially performing searches of electronic bibliographies including MEDLINE, EMBASE, CINAHL, and the Cochrane Library. All searches were limited to human studies and started with the term *ETEC* which was then followed by the addition of the following terms: *infection*, *efficacy*, *experimental*, *inpatient* and *challenge*. In addition, MEDLINE searches were conducted using major medical subject headings (MeSH) determined from articles known to be eligible. Additionally, a manual search of the bibliographies of retrieved articles was performed. Conference proceedings, book chapters and technical reports were also reviewed to identify potential studies. Because this study was not limited to published articles, we consulted with experts in the field of ETEC research to identify any previously unidentified eligible studies. Studies had to be completed and/or otherwise available prior to January 2009 to be included in this analysis. All articles, publications and abstracts were reviewed to determine if they met the eligibility criteria, assessed by two independent reviewers (CP and PS).

2.2. Inclusion/exclusion criteria

This study was limited to experimental infection studies in which subjects received live, unattenuated strains of ETEC bacteria either as part of the development of an experimental challenge model, for characterization of strain pathogenicity and/or

immunogenicity or as controls for the evaluation of a vaccine, prophylaxis or treatment product. Subjects receiving an investigational product prior to, or after ingesting the ETEC inoculum were not included in the analysis. The search was limited to studies reported in the English language.

2.3. Data abstraction

Two reviewers (CP and SI) extracted the data using a pre-tested data extraction form. Bibliographic information, study design description, study years, geographic location, population characteristics, primary outcome measures, inoculum and strain information and other study characteristics necessary to assess the key parameters and to evaluate heterogeneity were included. For studies involving a vaccine or treatment arm, only data from the placebo control arm were extracted. Abstraction was not blinded to any study characteristic such as author, journal or year of publication.

Data were entered separately by each of the reviewers into a Microsoft Access database. Discrepancies in data points were evaluated by a third party and resolved by consensus. Results were tabulated from individual studies.

2.4. Data analysis

Heterogeneity was assessed using a χ^2 heterogeneity statistic, and potential sources of heterogeneity were assessed graphically by Forest plots and non-parametric methods (e.g., Kruskal–Wallis, Mann–Whitney *U*-test) to compare differences in incidence between two or more groups of a given study characteristic. In the case of parameters where only a few studies were found, a median and range of estimates were reported. For summary purposes, point estimates and standard 95% confidence intervals were combined using a random-effects model with methodology developed by DerSimonian and Laird [9]. As the principle purpose of this systematic review was to summarize studies reporting diarrhea incidence following experimental infection, publication bias was not assessed; as such, the concern for non-published findings due to negative studies or disappointing results was considered minimal.

The independent study characteristics that were evaluated included strain and quantity of ETEC administered, inoculum administration procedures and volunteer characteristics. These were assessed in relation to their effect on multiple outcomes such as diarrheal attack rates, disease severity, incubation periods, non-diarrheal symptoms and qualitative immune responses to both the colonization factor and the toxin (when appropriate).

2.5. Individual patient level data analysis

For the IPD analysis, only studies for which IPD was obtained were included. Also, these analyses were limited to studies utilizing the same inoculum administration procedures and outcome definitions (diarrhea, immune response, etc.). This analysis included 7 separate studies evaluating 12 strain/dose combinations and a total of 134 subjects. All but one of the studies (evaluating two doses of two separate ETEC strains) were performed by the same principal investigator (PI) at the same clinical facility. For that study, we evaluated the impact of the different clinical site and PI on the clinical outcomes. The lack of significant differences in study populations, or clinical or immunologic outcomes for the lone strain/dose combination in question, led us to combine the data from the multiple studies as if they were performed as a single clinical trial instead of utilizing multilevel and/or hierarchical models to allow for adjustment of between-trial variance (deemed of minimal impact for this unique dataset) [10,11]. Outcomes evaluated were the same as those for the over-arching meta-analysis. Post hoc analyses of non-parametric continuous variables were performed using a bonferroni-adjusted alpha for pair-wise comparisons.

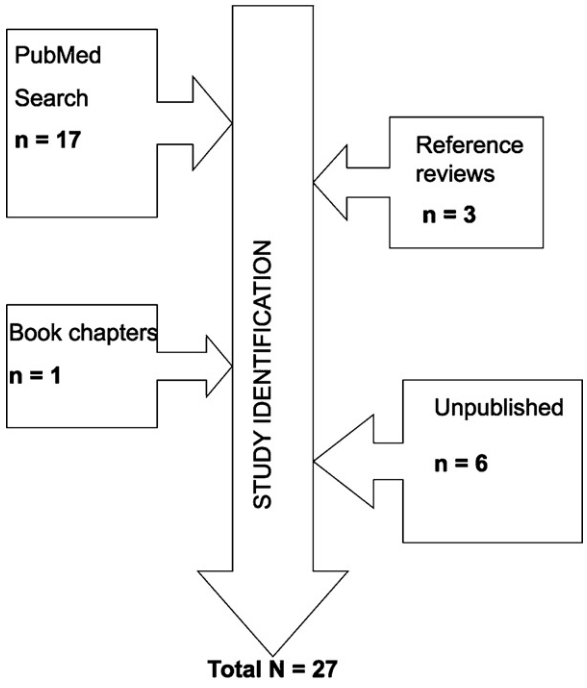

Fig. 1. Flow diagram for studies included in systematic review.

3. Results

3.1. Meta-analysis

A total of 27 studies were identified for inclusion (Fig. 1). A thorough review of the published literature identified a total of 22 reports of experimental ETEC infection. However, 4 publications reported different aspects of only 2 different clinical trials [12–15], yielding 19 individual studies. One additional study was identified in two separate book chapters with topics related to ETEC vaccine pathogenicity and vaccine development [16,17]. We also included 6 unpublished studies for which three principal investigators have been extensively involved. These included administration of 7 different ETEC strains (E24377A, H10407, LSN03-016011/A, WS0115A, DS26-1) in experimental infection or preliminary protective efficacy studies at the inpatient facilities at Johns Hopkins University. We excluded one study that did not report any data on clinical outcomes following ETEC ingestion [18]. A complete listing of all included studies is shown in Table 1 .

The majority (70%) of the published studies were printed in a 12-year span between 1977 and 1988. However, the past decade has seen an increase in the number of experimental ETEC infection studies, many of which are currently unpublished. Eleven of the 27 studies (41%) performed to date have been to fully define and understand the experimental human ETEC infection model with various ETEC strain/dose combinations. The additional studies were performed to utilize these models to evaluate vaccine candidates ($n = 8$), antibiotics ($n = 1$) or other prophylactic and/or treatment intervention ($n = 7$). Variability in the outcomes reported and types of summary effect estimates utilized was also variable across studies.

Over the past four decades of experimental ETEC infection studies, the diarrhea definition has been quite varied. The first definition utilized by Dupont et al. was “3 watery stools/24-h period” [1]. Subsequently, the diarrhea definition was modified to allow for

Table 1
List of experimental ETEC infection studies that met inclusion criteria for systematic review and meta-analysis.

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
[1] (1971)	Dupont	A	TSA	No	Milk	A	B2C	1E8	5	2 (40)	100% colonization; no abx treatment
								1E10	5	3 (60)	100% colonization; no abx treatment
							B7A	1E8	5	1 (20)	80% colonization; no abx treatment
								1E10	5	4 (80)	100% colonization; no abx treatment
[61] (1977)	Levine	A	TSA	No	Milk	B	214-4	1E6	4	0 (0)	50% somatic response; 0% LT response
								1E8	5	2 (40)	All illnesses lasted 1 day; median incubation period = 45 h; 75% colonized; 80% somatic response; 0% LT response
								1E10	5	4 (80)	Median illness duration: 3 days; median incubation period = 20.8 h; 1 subject vomited; 100% colonized; 80% somatic response; 0% LT response
[12,13] (1978)	Evans/Satterwhite	A	CFA	Yes	PBS	NR	H10407	1E6	7	0 (0)	1 subject with 1 LLS, anorexia, nausea and severe abdominal pain; 50% serocon to CFA/I (GMT = 6.5); 33% serocon to LT (GMT = 29)
								1E8	7	6 (86)	Mean # LLS: 9.2; 3 subjects with abdominal pain, 2 with vomiting; 67% serocon to CFA/I (GMT = 17.9); 43% serocon to LT (GMT = 39.0)
[62] (1979)	Levine	A	TSA	Yes	PBS	B	B7A	1E6	6	3 (50)	100% colonization; 1 (17%) with fever; 33% serocon to LT and somatic antigens; all 3 subjects with diarrhea protected from homologous re-chall at 1×10^8
								1E8	11	7 (64)	100% colonization; 2 (18%) with fever; 89% serocon to LT and somatic antigens; 6 of 7 (86%) with diarrhea protected from diarrhea upon homologous re-chall at 1×10^8
								1E8	12	7 (58)	Mean incubation: 45 h; mean volume: 0.9 L; mean # LLS: 4.7; 2 (17%) with nausea/vomiting; 7 (58%) with malaise; 92% serocon to LT and 83% to somatic antigens; 1 of 4 (25%) with diarrhea protected from diarrhea upon heterologous re-chall at 1×10^9 with E2528-C1
							E2528-C1	1E9	6	2 (33)	Mean incubation: 16 h; mean volume: 0.5 L; mean # LLS: 4.5; 67% serocon to LT and 33% to somatic antigens
[63] (1980)	Levine	A	TSA	Yes	PBS	C	H10407	1E8	4	2 (40)	1 subject with nausea and vomiting but only 1 LLS; 3 (75%) subjects with anorexia and abdominal cramps; 75% serocon to LT and 100% to O antigen
							214-4	1E8	4	4 (100)	Range of # LLS: 3-19; range of total volume: 0.5–3.5 L; 100% with abdominal cramps; 75% with nausea; 100% serocon to O antigen and 0% to LT

Table 1 (*Continued*)

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
[64] (1981)	Clements	D	TSA	Yes	$NaHCO_3$	D	TD225-C4	1E10	5	2 (40)	Mean incubation: 9.6 h; mean #LLS: 3.5; mean total volume: 0.6 L; mean duration: 18.0 h; 20% with fever, 0% vomiting, 80% with abd. cramps, 40% with anorexia, 60% with malaise; 100% colonization
							214-4	1E8	5	4 (80)	Mean incubation: 24.5 h; mean #LLS: 5.0; mean total volume: 0.7 L; mean duration: 30.2 h; 20% with fever, 0% vomiting, 60% with abd. cramps, 80% with anorexia, 80% with malaise; 100% colonization
							H10407	1E8	4	3 (75)	Mean incubation: 57.5 h; mean #LLS: 6.7; mean total volume: 1.3 L; mean duration: 21.3 h; 25% with fever, 25% vomiting, 100% with abd. cramps, 25% with anorexia, 0% with malaise; 100% colonization
							B7A	1E8	3	3 (100)	Mean incubation: 23.4 h; mean #LLS: 4.7; mean total volume: 0.5 L; mean duration: 30.2 h; 100% colonization
								1E10	8	5 (63)	Mean incubation: 14.4 h; mean #LLS: 11.8; mean total volume: 1.5 L; mean duration: 60.8 h; 100% colonization
[36] (1982)	Black	C	CFA	Yes	$NaHCO_3$	E	H10407	5E8–5E9	41	31 (76)	After diarrhea onset, subjects randomized to treatment (11 to placebo); For placebo-treated subjects: mean duration: 82.1 h; mean # LLS: 12.0; mean volume: 2.2 L; 55% vomiting, 91% abd cramps (lasting 3.6 days), 91% anorexia (lasting 3.2 days); 100% colonization
[65] (1982)	Levine	B	CFA	Yes	PBS	D	B7A	1E10	6	4 (67)	Mean volume: 0.6 L; mean # LLS: 4.3; 100% colonization
							H10407	1E7	11	3 (27)	Mean volume: 1.2 L; mean # LLS: 15.3; 100% colonization
								5E8	8	7 (88)	Mean volume: 3.0 L; mean # LLS: 12.3; 100% colonization
								5E8	7	7 (100)	Mean volume: 4.0 L; mean # LLS: 18.0; 100% malaise and 86% vomited; 2 (29%) required IV fluids; 100% colonization
[14,15] (1983)	Graham	D	CFA	Yes	PBS	C	H10407	2.7E8	16	9 (56)	Initial phase included 32 subjects randomized to prophylaxis with bismuth subsalicylate ($n = 16$) or placebo ($n = 16$). After diarrhea onset, subjects randomized to treatment with bismuth subsalicylate ($n = 6$) or placebo ($n = 5$). For the 5 placebo recipients: mean # LLS over 48 h: 7.0; 80% nausea, 40% vomiting, 100% abd cramps, 40% headache, 60% fever
[66] (1984)	Evans	B	CFA	Yes	PBS	NR	H10407	4E8	5	2 (40)	No data on additional ETEC-associated symptoms provided

Table 1 (*Continued*)

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
							H1765	4E8	6	5 (83)	No data on additional ETEC-associated symptoms provided
[67] (1984)	Levine	A	TSA	Yes	PBS	NR	E24377A	5E8	14	9 (64)	50% seroconversion to CS1 and 50% to CS3; No data on additional ETEC-associated symptoms provided
[16,17] (1986)	Levine	B	NR	NR	NR	NR	E24377A	5E8	6	6 (100)	Study published in 2 book chapters; no additional data provided
[30] (1988)	Evans	B	CFA	Yes	PBS	F	H10407	5E9	5	5 (100)	Mean stool weight at peak of illness: 765 g;
[31] (1988)	Evans	B	CFA	Yes	PBS	F	H10407	5E9	9	8 (89)	Mean total weight for 24 h during peak illness: 900 g; mean time to 1st LLS: 30.9 h; 88% colonization
[40] (1988)	Tacket	D	NR	Yes	$NaHCO_3$	G	H10407	1.2E9	10	9 (90)	Subjects received $NaHCO_3$ buffer over 2 days prior to and for 5 days after ETEC admin as part of immunoprophylaxis. Mean diarrhea volume: 1.5 L; mean diarrhea onset time: 48 h; 80% abdominal cramps, 60% vomiting, 90% malaise, 20% fever, 90% anorexia; 60% received early abx.; 100% colonization; 40% seroconversion to CFA/I, 50% to LT and 100% to O antigen
[68] (1994)	Tacket	B	NR	Yes	$NaHCO_3$	NR	E24377A	3E9	10	10 (100)	Mean volume: 1.5 L; mean # LLS: 8.6; 90%, 40% and 90% IGA ASC responses to CFA/II, CS1 and CS3, respectively
[41] (1998)	Freedman	D	NR	Yes	$NaHCO_3$	G	H10407	1E9	10	7 (70)	Subjects received $NaHCO_3$ buffer over 2 days prior to and for 5 days after ETEC admin as part of immunoprophylaxis. Mean volume: 1.3 L; mean # LLS: 7.4; 100% abd cramps, 60% anorexia, 50% headache, 30% malaise; 100% serologic response to CFA/I, LPS and LT
[69] (1999)	Tacket	D	NR	No	Apple-sauce	G	E24377A	1E8	10	3 (30)	Mean volume: 0.8 L; mean # LLS: 5.3; 100% colonization; 100% seroconversion to LT and 20% to CS3
[70] (2007)[c]	Coster	A	TSA	Yes	$NaHCO_3$	C	B7A	1.5E9	8	5 (63)	Mean weight: 1.0 kg; mean onset time: 10 h; 25% mod-sev abd cramps, 25% mod-sev headache, 13% mod-sev nausea, 13% mod-sev loss of appetite, 0% mod-sev fever; 100% colonization
								1.4E10	8	8 (100)	Mean weight: 0.9 kg; mean onset time: 12 h; 38% mod-sev abd cramps, 38% mod-sev headache, 38% mod-sev nausea, 0% mod-sev loss of appetite, 25% mod-sev fever; 100% colonization
							H10407	1.2E8	7	6 (86)	Mean weight: 1.9 kg; mean onset time: 43 h; 71% mod-sev abd cramps, 57% mod-sev headache, 43% mod-sev nausea, 71% mod-sev loss of appetite, 43% mod-sev fever; 100% colonization

Table 1 (*Continued*)

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
								1.4E9	8	7 (88)	Mean weight: 1.9kg; mean onset time: 34 h; 100% mod-sev abd cramps, 63% mod-sev headache, 63% mod-sev nausea, 75% mod-sev loss of appetite, 38% mod-sev fever; 100% colonization
[21] (2007)	McKenzie	B	CFA	Yes	$NaHCO_3$	G	E24377A	6E8	20	20 (100)	Mean weight: 1.1 kg; mean # LLS: 9.7; 40% received IV fluids; mean time to diarrhea onset: 29 h; mean time to abx: 51 h
2008 [71]	McKenzie	B	CFA	Yes	$NaHCO_3$	G	E24377A	3E9	16	13 (81)	Median volume: 0.9 L; median # LLS: 6; median max. 24 h, volume: 0.9 L; median time to diarrhea onset: 24.1 h; median duration: 32.2 h; 0% fever, 67% malaise; 73% abdominal cramps; 47% nausea; 33% headache; 7% vomiting; 31% IV fluids; 50% early abx
Unpub[c]	McKenzie	A	CFA	Yes	$NaHCO_3$	G	H10407	1.1E9	5	5 (100)	Median volume: 1.5 L; median # LLS: 13; median max. 24 h, volume: 1.0 L; median time to diarrhea onset: 25.7 h; median duration: 46.6 h; 0% fever, 80% malaise; 80% abdominal cramps; 60% nausea; 80% headache; 40% vomiting; 20% IV fluids; 100% early abx
Unpub[c]	McKenzie	D	CFA	Yes	$NaHCO_3$	G	H10407	1E9	11	9 (82)	Subjects received $NaHCO_3$ buffer over 2 days prior to and for 3 days after ETEC admin as part of immunoprophylaxis. Median volume: 1.9 L; median # LLS: 10; median max. 24 h, volume: 1.2 L; median time to diarrhea onset: 22.8 h; median duration: 53.8 h; 27% fever, 55% malaise; 73% abdominal cramps; 36% nausea; 73% headache; 18% vomiting; 45% IV fluids; 55% early abx
[72] [c]	McKenzie	A	CFA	Yes	$NaHCO_3$	G	LSN03-016011/A	7.0E8	5	3 (60)	Median volume: 0.8 L; median # LLS: 6; median max. 24 h, volume: 0.6 L; median time to diarrhea onset: 11.7 h; median duration: 27.3 h; 0% fever, 60% malaise; 80% abdominal cramps; 20% nausea; 20% headache; 40% vomiting; 20% IV fluids; 60% early abx
								6.2E9	8	7 (88)	Median volume: 1.0 L; median # LLS: 6; median max. 24 h, volume: 0.5 L; median time to diarrhea onset: 11.7 h; median duration h: 10.4; 0% fever, 63% malaise; 75% abdominal cramps; 25% nausea; 25% headache; 25% vomiting; 13% IV fluids; 38% early abx

Table 1 (*Continued*)

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
[72] [c]	McKenzie	D	CFA	Yes	$NaHCO_3$	G	LSN03-016011/A	5E9	12	6 (50)	Subjects received $NaHCO_3$ buffer over 2 days prior to and for 5 days after ETEC admin as part of immunoprophylaxis. Median volume: 0.8 L; median # LLS: 5; median max. 24 h, volume: 0.5 L; median time to diarrhea onset: 21.9 h; median duration: 34.5 h; 0% fever, 33% malaise; 50% abdominal cramps; 42% nausea; 33% headache; 8% vomiting; 0% IV fluids; 25% early abx
[72] [c]	McKenzie	A	CFA	Yes	$NaHCO_3$	G	WS0115A	4E8	5	1 (20)	Median volume: 1.2 L; median # LLS: 5; median max. 24 h, volume: 1.2 L; median time to diarrhea onset: 16.7 h; median duration: 10.0; 0% fever, 20% malaise; 40% abdominal cramps; 20% nausea; 20% headache; 0% vomiting; 20% IV fluids; 20% early abx
								3E9	6	2 (33)	Median volume: 0.7 L; median # LLS: 6; median max. 24 h, volume: 0.4 L; median time to diarrhea onset: 6.8 h; median duration: 46.9 h; 0% fever, 50% malaise; 50% abdominal cramps; 33% nausea; 67% headache; 0% vomiting; 20% IV fluids; 0% early abx
								9.2E9	9	4 (44)	Median volume: 0.7 L; median # LLS: 5; median max. 24 h, volume: 0.4 L; median time to diarrhea onset: 25.1 h; median duration: 36.0 h; 0% fever, 22% malaise; 22% abdominal cramps; 11% nausea; 33% headache; 0% vomiting; 20% IV fluids; 0% early abx
							DS26-1	4E8	5	0 (0)	Only ETEC-associated symptom was 40% headache; 0% IV fluids; 0% early abx
Unpub[c]	McKenzie	A	CFA	Yes	$NaHCO_3$	G	E24377A	7.3E8	10	8 (80)	Median volume: 0.8 L; median # LLS: 7; median max. 24 h, volume: 0.6 L; median time to diarrhea onset: 50.0 h; median duration: 47.3 h; 0% fever, 50% malaise; 63% abdominal cramps; 25% nausea; 13% headache; 0% vomiting; 10% IV fluids; 20% early abx
								3.1E9	5	4 (80)	Median volume: 1.3 L; median # LLS: 9; median max. 24 h, volume: 0.9 L; median time to diarrhea onset: 22.3 h; median duration: 53.0 h; 20% fever, 80% malaise; 100% abdominal cramps; 60% nausea; 40% headache; 0% vomiting; 40% IV fluids; 40% early abx

Table 1 (*Continued*)

Ref (pub. yr.)	Primary Author or Investigator	Study type[a]	Agar	$NaHCO_3$ buffer	Inoc. solution	Dia. Def.[b]	Strain	Dose (cfu)	*N*	*N* (%) diarrhea	Comments
								4.9E9	5	4 (80)	Median volume: 0.7 L; median # LLS: 7; median max. 24 h, volume: 0.7 L; median time to diarrhea onset: 22.5 h; median duration: 29.8 h; 0% fever, 40% malaise; 60% abdominal cramps; 60% nausea; 40% headache; 20% vomiting; 20% IV fluids; 40% early abx

abx: antibiotic; AR: attack rate; LLS: loose or liquid stools (frequently referred to as grade 3, 4 or 5 stools [19]); mod-sev: moderate to severe; NR: not reported; serocon: seroconversion; #: number.

[a] Study types: (A) pathogenesis; (B) vaccine efficacy; (C) antibiotic treatment; (D) other treatment and/or prophylaxis.

[b] Diarrhea definitions: (A) ≥3 LLS in 24 h.; (B) ≥3 LLS in 24 h or 1 LLS ≥ 200 mL; (C) ≥2 LLS in 24 h; (D) ≥3 LLS or ≥2 LLS at ≥200 mL in 48 h or 1 LLS ≥300 mL; (E) ≥2 LLS; (F) ≥2 LLS + 1 somatic complaint; (G) ≥2 LLS in 48 h at ≥200 mL or 1 LLS ≥300 mL.

[c] These studies used ETEC strains for which the seed lots were manufactured under Good Manufacturing Practices.

a volume quantification of either 1 or 2 loose stools over a 24 or 48 h time period. In 1978, Levine et al. established a grading system for scoring stools which has been subsequently used to classify loose stools as those coded as grade 3 ("thick liquid"), 4 ("opaque-watery") or 5 ("rice-water") [19]. This grading system has been consistently utilized since, although the number and/or quantity of stools required to meet the diarrhea definition remained inconsistent. The most common diarrhea outcome definition, used in 41% of the studies is ≥200 mL of Grade 3, 4 or 5 stools within a 48 h period or 1 Grade 3, 4 or 5 stool totaling ≥300 mL.

Eleven different strains have been utilized in these studies and summary information about each is provided in Table 2. The three most commonly administered strains are H10407, E24377A and B7A all three of which express both the LT and ST enterotoxins. As shown in Fig. 2, the percent of subjects reaching the primary outcome of diarrhea has varied within and across a range of dose-strain combinations. The majority of studies (88.5%) involved volunteer pretreatment with sodium bicarbonate prior to administration of the challenge inoculum. Most commonly the vehicle to administer the challenge strain was also sodium bicarbonate (52%), although saline (33%), milk (7%) and apple sauce (4%) have also been used.

When limiting our analyses to the 3 most utilized strains, B7A, H10407 and E24377A, we found that the diarrhea attack rate was dose-dependent with increasing doses associated with higher attack rates ($p < 0.01$). Interestingly, when evaluating these strains at doses of 5×10^8 and higher cfu, there was no difference in diarrhea attack rates (heterogeneity chi-square $p = 0.07$) across any of the strains at doses up to 1×10^{10}, with an overall attack rate of 87% (95% CI 82, 92) and strain-specific attack rates of 78% (95% CI: 64, 92), 89% (95% CI: 79, 98) and 87% (95% CI: 81, 93) for B7A, E24377A

Table 2
Detailed information on strains of ETEC that have used for experimental human infection.

Strain name	Initial strain description	Serotype	CF(s)	Toxin(s)	Country/region of origin	Clinical information on index case	Range of doses administered
214-4	[73]	Not typeable	Unknown	ST	Mexico	Isolated from 29 year-old Caucasian male physician with travelers' diarrhea characterized by watery diarrhea, abdominal cramps, malaise, nausea and fever	1E6–1E10
B2C	[1]	O6:H16	CS2, CS3	LT/ST	Vietnam	Diarrhea case in US military adult	1E8
B7A	[1]	O148:H28	CS6	LT/ST	Vietnam	Diarrhea case in US military adult serving in Vietnam	1E6–1E10
DS26-1	[72]	O8:H9	CS19	LT	Saudi Arabia	Isolated in 1990 from a U.S. soldier with diarrhea while on deployment during Operation Desert Shield	5E8
E24377A	[74]	O139:H28	CS1, CS3	LT/ST	Egypt	Traveler returning from Egypt with ETEC disease	1E8–3E9
E2528-C1	[75]	O25:NM	Unknown	LT	Caribbean	Cruise ship diarrhea outbreak	1E9
H10407	[76]	O78:K80:H11	CFA/I	LT/ST	Bangladesh	Severe case of watery diarrhea	1E6–5E9
H1765	[66]	O6:K15:H16	CFA/II	LT/ST	Bangladesh	Unknown	4E8
LSN03-016011/A	[72]	O8:H-	CS17	LT	Turkey	Isolated from 29 year-old U.S. female military with acute, watery diarrhea shortly after arrival at Incirlik Airbase in Turkey	5E8–5E9
TD225-C4	[64]	O75:H9	Unknown	LT	Mexico	Case of diarrhea	1E10
WS0115A	[77]	O114:H-	CS19	LT/ST	Egypt	Isolated from stool of 12-month old Egyptian female suffering from watery diarrhea in Abees, Egypt	5E8–5E10

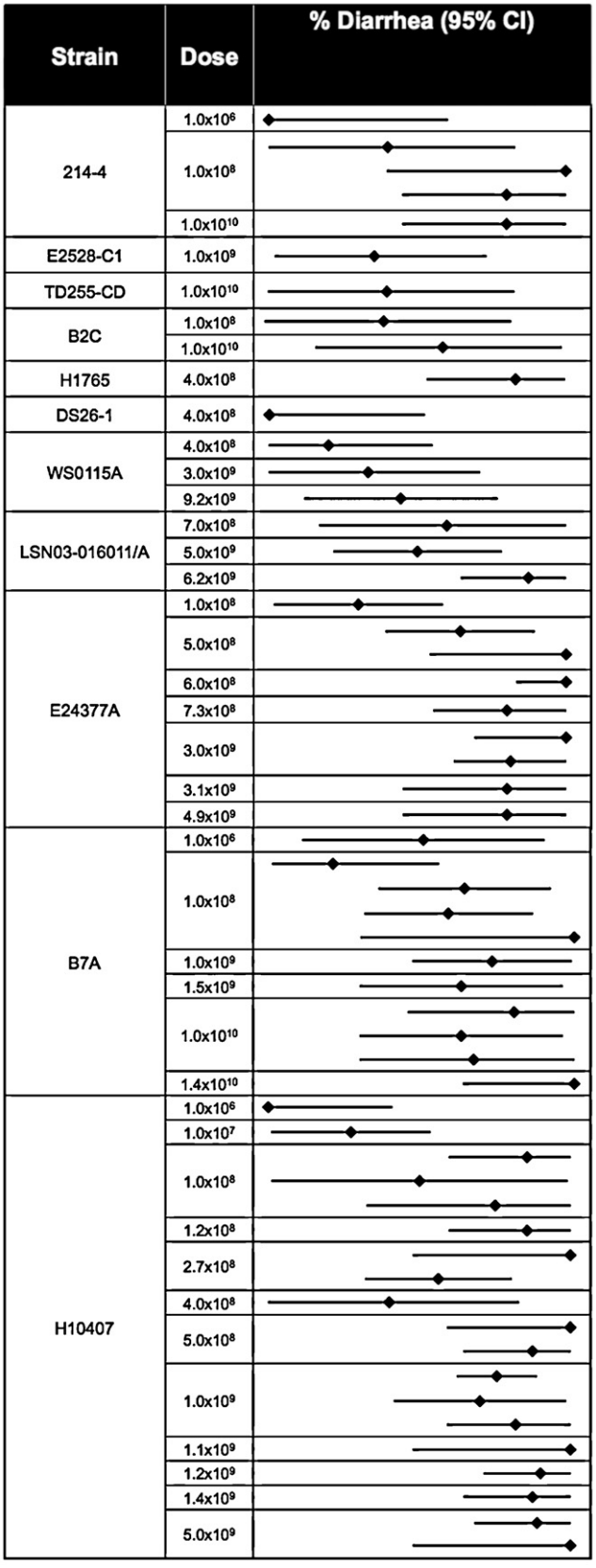

Fig. 2. Point estimate and 95% confidence interval for the proportion of subjects meeting the primary outcome of diarrhea for all included dose and strain combinations. Diamond: point estimate; horizontal line: 95% confidence interval.

and H10407, respectively. The diarrhea attack rates of strains with similar toxin phenotypes at the ≥5E8 dose showed significant heterogeneity, with the highest rates following administration of LT/ST strains (84%; 95% confidence interval {CI}: 78, 89; heterogeneity chi-square $p = 0.02$) followed by ST only strains (80%; 95% CI: 52, 100; no p-value as only 1 study included) and LT only strains (57%; 95% CI: 33, 81; heterogeneity chi-square $p = 0.02$). For the purposes of this analysis, we excluded the DS26-1 strain which did not induce diarrhea at any of the inoculum doses administered. We found no significant effect on diarrhea rates with changing inoculum solution, agar used for strain growth or fasting time, although our power was relatively limited to find these effects.

3.2. Individual patient level data

3.2.1. Clinical data

The individual patient-level data is shown in Table 3 and includes 133 subjects administered 1 of 6 ETEC strains at doses of 1E8 to 1E10. The highest diarrhea attack rates were seen with B7A at ~1E10 cfu (100.0%), H10407 at ~1E9 cfu (87.5%) and E24377A at ~5E9 cfu (80.8%). Strains expressing CS17 (LSN03-016011/A) and CS19 (WS0115A) also caused diarrhea, although at lower attack rates and a strain expressing CS19 (DS26-1) caused no diarrhea. There was a higher proportion of severe diarrhea among all doses of H10407 compared to the other strains. The only dose/strain combination that resulted in a similar proportion of severe diarrhea was the B7A strain at the 1E10 inoculum.

There were also apparent strain differences in the non-diarrheal symptoms following ETEC inoculation. While abdominal cramps/pain, nausea, malaise and headache were relatively common across all doses and strains (excluding DS26-1), vomiting was seen most frequently following inoculation with the strains H10407, B7A and LSN03-016011/A. Similarly, fever was relatively infrequent in all strains except H10407 and B7A. While most strains demonstrated a range of severity in these ETEC-associated symptoms, the H10407 strain exhibited markedly more severe symptoms. The need for intravenous fluids (I.V.) was relatively rare, possibly due to the high frequency of early antibiotic treatment across dose/strain combinations. Although not shown here, all subjects were encouraged to consume oral rehydration solution or other oral fluids at diarrheal onset, potentially decreasing the need for I.V. fluids.

A more detailed description of the diarrhea episode among subjects meeting the diarrhea definition is shown in Table 4. Regardless of dose, H10407 exhibited a significantly higher number of unformed stools (all $p < 0.013$ except: B7A {$p = 0.014$}, E24377A {$p = 0.022$}), total volume (all $p < 0.013$) and maximum 24 h volume (all $p < 0.013$ except: E24377A {$p = 0.022$}) than the other strains. The increase in the total number of unformed stools and total diarrhea may be explained by the longer duration of the diarrheal episode seen with both evaluated doses of the H10407 strain. Interestingly, the time to antibiotic treatment was quite variable across dose/strain combinations, likely reflecting differences in strain pathogenicity and clinical treatment algorithms.

The median time to the first loose stool was quite variable, and ranged from 6.8 to 50.0 h post-inoculation. Fig. 3 shows a detailed representation of stool volume accumulation and number of stools for the four strains resulting in the highest diarrhea attack rates. The volume of loose stools at the highest H10407 dose (1×10^9) increases at a higher rate than the other strains regardless of dose, and continues to increase throughout the observation period. In contrast, the output for the 'low dose' (1×10^8) of H10407 is relatively consistent with the other dose/strain combinations until the 72 h timepoint when it increases remarkably. The stool volume and count for the 'low dose' (5×10^8) of strain E24377A falls between

Table 3
Description of primary outcomes following experimental infection with enterotoxigenic *Escherichia coli* (ETEC).

Strain	H10407		E24377A		B7A		LSN03-016011/A		WS0115A		DS26-1	
Approximate dose	1E8	1E9	5E8	5E9	1E9	1E10	5E8	5E9	5E8	5E9	1E10	5E8
N	7	24	10	26	8	8	5	20	5	6	9	5
% male	57.1	58.3	90.0	76.9	87.5	50.0	80.0	60.0	60.0	66.7	88.9	60.0
% African American	42.9	70.8	80.0	76.9	50.0	37.5	100.0	85.0	100.0	100.0	88.9	100.0
Median (IQR) age	46.2 (37.4, 49.6)	31.6 (25.4, 40.3)	43.0 (21.0, 47.0)	37.5 (22.0, 43.0)	43.2 (25.6, 51.2)	37.2 (28.1, 43.8)	34.9 (32.9, 35.8)	31.6 (25.1, 41.1)	40.3 (38.6, 44.5)	34.3 (24.8, 41.3)	25.5 (24.0, 31.2)	28.7 (19.8, 30.3)
% diarrhea	85.7	87.5	70.0	80.8	62.5	100.0	60.0	65.0	20.0	33.3	44.4	0.0
Mild	0	12.5	20.0	19.2	25.0	0.0	20.0	15.0	0.0	0.0	0.0	0.0
Moderate	0	4.2	30.0	23.1	0.0	25.0	0.0	30.0	20.0	33.3	33.3	0.0
Severe	85.7	70.8	20.0	38.5	37.5	75.0	40.0	20.0	0.0	0.0	11.1	0.0
% abdominal cramps	85.7	83.3	62.5	76.0	62.5	62.5	80.0	60.0	40.0	50.0	22.2	0.0
Mild	14.3	16.7	25.0	28.0	37.5	25.0	0.0	10.0	40.0	0.0	11.1	0.0
Moderate	0	29.2	37.5	24.0	12.5	0.0	20.0	30.0	0.0	50.0	11.1	0.0
Severe	71.4	37.5	0.0	24.0	12.5	37.5	60.0	20.0	0.0	0.0	0.0	0.0
% Nausea	71.4	50.0	25.0	48.0	37.5	62.5	20.0	35.0	20.0	33.3	11.1	0.0
Mild	28.6	4.2	25.0	24.0	25.0	25.0	0.0	65.0	20.0	16.7	16.7	0.0
Moderate	0	8.3	0.0	12.0	12.5	0.0	0.0	20.0	0.0	0.0	0.0	0.0
Severe	42.9	37.5	0.0	12.0	0.0	37.5	20.0	15.0	0.0	16.7	0.0	0.0
% Malaise	57.1	62.5	50.0	64.0	0.0	37.5	60.0	45.0	20.0	50.0	22.2	0.0
Mild	0	8.3	12.5	20.0	0.0	12.5	0.0	20.0	20.0	0.0	22.2	0.0
Moderate	14.3	25	25.0	24.0	0.0	12.5	20.0	10.0	0.0	16.7	0.0	0.0
Severe	42.9	29.2	12.5	20.0	0.0	12.5	40.0	15.0	0.0	33.3	0.0	0.0
% headache	57.1	70.8	12.5	36.0	37.5	87.5	20.0	30.0	20.0	66.7	22.2	40.0
Mild	0	4.2	0.0	16.0	12.5	50.0	0.0	25.0	20.0	16.7	22.2	20.0
Moderate	14.3	4.2	12.5	12.0	12.5	25.0	20.0	5.0	0.0	0.0	0.0	0.0
Severe	42.9	25	0.0	8.0	12.5	12.5	0.0	0.0	0.0	50.0	0.0	20.0
% vomiting	42.9	33.3	0.0	8.0	0.0	25.0	40.0	15.0	0.0	0.0	0.0	0.0
Mild	0	4.2	0.0	4.0	0.0	12.5	40.0	5.0	0.0	0.0	0.0	0.0
Moderate	14.3	4.2	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0
Severe	28.6	25	0.0	4.0	0.0	12.5	0.0	5.0	0.0	0.0	0.0	0.0
% lightheaded	57.1	47.8	25.0	28.0	25.0	25.0	20.0	10.0	0.0	30.0	11.1	0.0
Mild	14.3	13.0	12.5	16.0	25.0	25.0	0.0	10.0	0.0	33.3	11.1	0.0
Moderate	14.3	26.1	0.0	4.0	0.0	0.0	20.0	0.0	0.0	16.7	0.0	0.0
Severe	28.6	8.7	12.5	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% fever	42.9	25.0	0.0	4.0	12.5	25.0	0.0	0.0	0.0	0.0	0.0	0.0
Mild	28.6	16.7	0.0	4.0	12.5	25.0	0.0	0.0	0.0	0.0	0.0	0.0
Moderate	14.3	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Severe	0.0	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% intravenous fluid	0.0	25.0	10.0	30.7	0	12.5	20.0	5.0	0.0	0.0	0.0	0.0
% early abx. treatment	85.7	70.8	20.0	46.2	12.5	75.0	60.0	30.0	20.0	0.0	0.0	0.0
% seroconversion												
2-fold												
LT IgG	57.1	95.8	100.0	92.3	75.0	62.5	80.0	80.0	80.0	83.3	66.7	40.0
LT IgA	71.4	91.7	100.0	69.2	87.5	62.5	80.0	65.0	60.0	50.0	55.6	40.0
CF IgG	100.0	70.8	40.0[a]	7.7[a]	37.5	12.5	100.0	100.0	60.0	100.0	88.9	20.0
CF IgA	100.0	50.0	70.0[a]	11.5[a]	50.0	12.5	100.0	95.0	100.0	83.3	100.0	40.0
4-fold												
LT IgG	42.9	83.3	100.0	88.5	37.5	25.5	80.0	75.0	60.0	50.0	44.4	20.0
LT IgA	42.9	83.3	90.0	65.4	62.5	50.0	80.0	65.0	60.0	50.0	55.6	40.0
CF IgG	100.0	70.8	40.0	7.7	37.5	12.5	60.0	85.0	40.0	33.3	44.4	0.0
CF IgA	100.0	50.0	40.0	3.9	50.0	12.5	100.0	85.0	60.0	83.3	100.0	40.0
% ASC response												
LT IgA	85.7	79.2	100.0	76.9	100.0	75.0	ND	ND	ND	ND	ND	ND
CF IgA	100.0	37.5	100.0[b]	96.2[b]	87.5	12.5	ND	ND	ND	ND	ND	ND

[a] Shown are response rates to CS3 (CS1 response rates 1×10^8: IgG = 50.0, IgA = 70.0; 1×10^9: IgG = 69.2, IgA = 65.4).
[b] Shown are response rates to CS3 (CS1 response rates 1×10^8 = 100.0; 1×10^9 = 92.3).

Table 4
Description of diarrheal episode {presented as median (interquartile range)} among subjects meeting diarrhea definition by strain and approximate dose.

Strain	Approx. dose	# Loose stools	Total volume	Time to first loose stool	Maximum 24 h volume	Duration	Time to antibiotic treatment
H10407	1E8	8.5 (5.0, 17.0)	1923.0 (1125.0, 2673.0)	47.8 (29.2, 62.2)	948.5 (573.0, 1243.0)	81.3 (33.0, 118.2)	81.6 (57.6, 103.2)
	1E9	10.0 (8.0, 16.0)	1643.8 (1325.0, 2379.3)	27.6 (24.6, 42.2)	984.0 (715.5, 1534.0)	58.4 (47.5, 84.0)	48.0 (38.4, 108.2)
E24377A	5E8	6.0 (4.0, 9.0)	786.5 (383.9, 1139.0)	11.7 (6.6, 13.5)	556.1 (326.0, 845.0)	27.3 (19.8, 29.7)	29.7 (17.0, 33.2)
	5E9	6.0 (4.0, 8.0)	979.0 (479.6, 1183.8)	14.2 (11.6, 22.0)	508.8 (386.0, 771.0)	29.2 (10.4, 41.3)	119.8 (16.5, 119.9)
B7A	1E9	5.0 (4.0, 10.0)	822.0 (587.0, 915.0)	9.4 (7.3, 11.0)	435.0 (382.0, 651.0)	42.1 (41.9, 126.0)	156.0 (144.0, 156.0)
	1E10	5.5 (3.0, 7.5)	825.0 (684.5, 998.5)	13.9 (11.0, 15.1)	543.5 (491.0, 809.5)	30.0 (13.6, 50.8)	60.0 (36.0, 132.0)
LSN03-016011/A	5E8	7.0 (4.0, 10.0)	753.3 (556.2, 1612.5)	50.0 (24.9, 87.4)	556.2 (264.0, 1033.5)	47.3 (25.2, 71.3)	120.3 (96.3, 120.3)
	5E9	8.0 (5.0, 10.0)	910 (624.7, 1209.6)	22.6 (17.7, 26.6)	771.2 (519.0, 999.0)	35.6 (15.5, 54.3)	48.3 (24.3, 120.3)
WS0115A	5E8	5.0 (–)	1186.0 (–)	16.7 (–)	1186.0 (–)	10.0 (–)	27.0 (–)
	5E9	6.0 (5.0, 7.0)	676.5 (402.0, 951.0)	6.8 (5.1, 8.4)	448.5 (402.0, 495.0)	46.9 (22.2, 71.5)	120.0 (–)
	1E10	4.5 (3.5, 5.0)	737.8 (420.5, 927.0)	25.1 (19.6, 26.3)	439.8 (391.1, 624.4)	36.0 (26.0, 53.8)	120.0 (–)
DS26-1	5E8	–	–	–	–	–	–

the low and high doses of H10407 until the 96 h mark where they are exceeded by the low dose of H10407. The other dose/strain combinations show increases in stool volume and count through the 72–84 h post-inoculation timepoint at which point they tend to level off. Importantly, there was variability in the median time to antibiotic treatment (Table 4), although this alone does not account for the temporal variability in diarrhea output.

Using multivariate models, we found several important predictors of clinical outcomes. The risk of diarrhea, malaise and headache was variable by ETEC strain. Specifically, when using H10407 as the reference, subjects receiving the WS0115A strain had a significantly decreased risk of diarrhea (Relative Risk {RR} = 0.63, $p = 0.03$) and headache (RR = 0.47, $p = 0.04$). Similarly, the risk of headache was decreased in subjects receiving either the LSN03-016011/A (RR = 0.35, $p < 0.01$) or E24377A (RR = 0.44, $p < 0.01$) strains and subjects receiving the B7A strain had a borderline significant decreased risk of malaise (RR = 0.36, $p = 0.06$). Regardless of strain administered, the risk of headache increased with increasing inoculum dose (RR = 1.23, $p = 0.05$) and was higher in females than in males (RR = 1.39, $p = 0.07$). Being of female gender also increased the risk of reporting nausea (RR = 1.92, $p < 0.01$) and abdominal pain or cramps (RR = 1.39, $p = 0.07$). The risk of nausea was also higher in subjects of Caucasian race (RR = 1.49, $p = 0.04$), compared to those of African-American race. We found no effect of dose on the risk of any other outcomes (other than headache) likely due to the narrow range of relatively high inocula evaluated. In contrast, increasing baseline LT IgG levels were associated with a decreased risk of diarrhea (RR = 0.85, $p = 0.02$). However, this association was not consistent when stratified by diarrhea severity (data not shown). There was no association with baseline LT IgA or any titers to the homologous colonization factors.

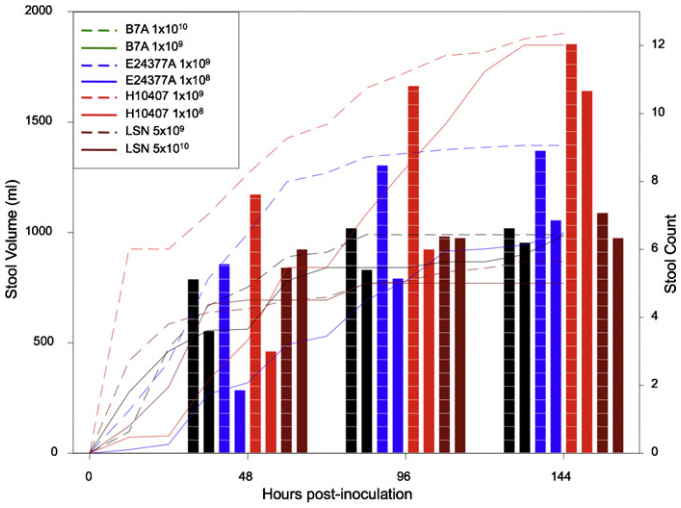

Fig. 3. Volume and number of loose stools over observation period by strain and dose. Bars: stool count; Lines: stool volume.

3.2.2. *Immunology data*

Serologic and ASC responses to LT and homologous colonization factor were relatively common for all dose/strain combinations. Overall, baseline serologic titers to LT (IgA GMT: 85, IgG GMT: 10) were low across all studied dose/strain combinations as were serologic titers to homologous fimbriae (CFA/I: IgA GMT:4, IgG GMT:16; CS1: IgA GMT:296, IgG GMT:764; CS3: IgA GMT:376, IgG GMT:378; CS6: IgA GMT:4, IgG GMT:17; CS17: IgA GMT:3, IgG GMT:13; CS19: IgA GMT: 17, IgG GMT: 31). Neither anti-LT IgA baseline titers nor anti-CF IgG or IgA appeared to correlate with diarrhea risk or severity. Similarly, baseline levels of antibody secreting cells (ASCs) specific to either LT or the homologous CF were low with only 5 subjects presenting with at least 1 ASC per 10^6 peripheral blood mononuclear cells (PBMC). All 5 subjects had diarrhea post-inoculation (1 severe, 2 moderate and 2 mild) likely reflecting no association between low level ASCs at baseline and diarrhea risk following inoculation.

Early antibiotic treatment did not appear to reduce the frequency or magnitude of serologic or ASC responses to LT or homologous CF with the majority of subjects exhibiting a response to both antigens (data not shown). Additionally, there was no significant association between the time to antibiotic treatment and maximum LT or CF titers (data not shown). Similarly, the duration of the subject's diarrheal episode had no significant impact on resultant maximum LT or CF titers (data not shown).

4. Discussion

A systematic review of 27 studies on 11 ETEC strains highlights variability in inoculum preparation and administration as well as

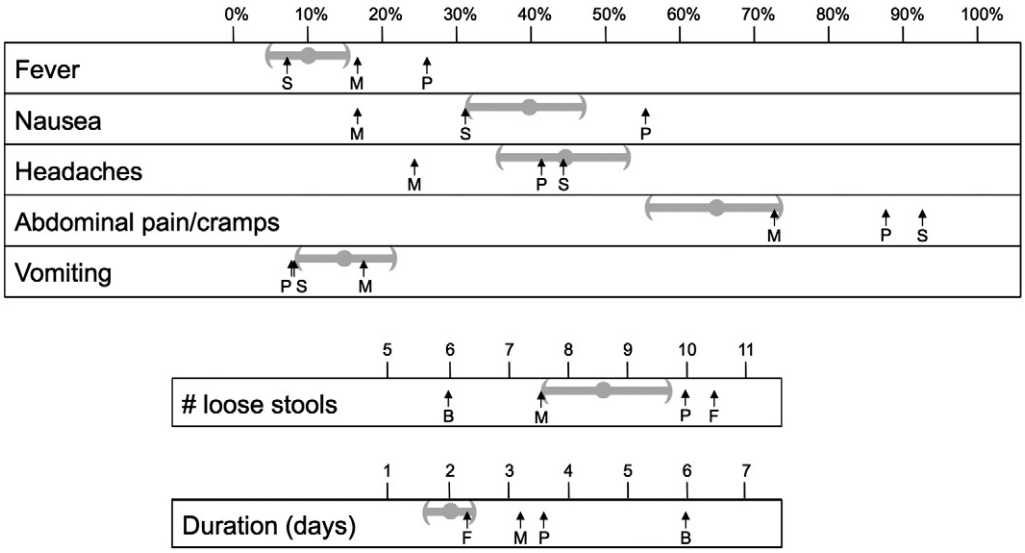

Fig. 4. Similarities and discrepancies between ETEC-associated clinical symptoms from adults with travelers' diarrhea and participants in experimental ETEC infection studies. Each of the letters reference a specific epidemiologic study of adult travelers' diarrhea. References are as follows: B = [23], F = [22], M = [24], P = [28], S = [25]. The ⟨⟩ represents the average point estimate with 95% confidence intervals calculated from the individual patient-level data.

differences in clinical and immunological outcomes across studies. We were able to calculate general estimates of diarrhea attack rates for the 3 most utilized strains, B7A, E24377A and H10407; however, report variability complicated calculations of aggregate outcome measures for given strains and/or doses. Furthermore, utilization of individual patient-level data enabled summative estimates of disease severity parameters and identification of strain and host-specific factors associated with specific clinical outcomes.

4.1. Report variability

A total of 27 different studies of ETEC experimental infection have been performed to date, the majority of which are available in the peer-reviewed literature. Of the published studies, variability existed in data reported and methods of analysis. Specifically, while all authors provided information on the number of subjects that met the primary diarrhea outcome, very few provided detailed information on the disease course of those subjects. Additional information on other ETEC-associated symptoms, including their severity, requirements for intravenous therapy, time from antibiotic treatment to microbial cure, and duration of ETEC-associated symptoms after antibiotic treatment would guide future protocol and informed consent development. Additionally, specific summary measures describing the diarrheal episode such as total stool volume, number of stools passed and time to event information would be of equal importance to increase comparability of data across different strains and doses. In an effort to standardize reporting and identify minimum data elements from experimental infection studies, we recommend a consortium be formed to define critical data elements similar to what has been done with travelers' diarrhea treatment trials [20].

4.2. Similarity to natural infections

To date, the results of experimental infection models have shown a great diversity in disease severity across a range of doses for a given strain, and among ETEC strains at similar doses. Some have argued that the most rigorously studied strains induce ETEC disease inconsistent with that observed in natural settings such as among naive travelers to an endemic region [21]. While there are numerous epidemiological studies on traveler's diarrhea, few have reported ETEC-specific outcomes and clinical presentation in a naive, adult population. As shown in Fig. 4, the estimates from those studies can be directly compared to estimates obtained from human challenge studies described here.

Most recently, Frech et al. described the results of a placebo-controlled clinical trial evaluating an LT skin patch vaccine in travelers to Guatemala or Mexico [22]. Placebo recipients with ETEC-attributable disease experienced a median of 2.2 days of diarrhea and a median of 10.5 (range: 5–30) loose stools. A slightly longer duration (median of 6 days) and slightly lower total number of loose stools (median of 6 loose stools) was reported by Bolin et al. in a similar population traveling to these 2 countries [23]. Among Finnish travelers to Morocco, Matilla et al. reported a median diarrhea duration of 3.1 days and a total output of 7.5 loose stools with onset occurring approximately 6 days after arrival into the country [24]. In addition to diarrhea, subjects with ETEC also reported abdominal pain (71%), nausea/vomiting (18%), fever (18%), headaches (24%) and myalgias (19%). The rates of these concurrent ETEC-related symptoms are similar to those reported by Sanders et al. in U.S. military personnel involved in a training exercise in Thailand [25].

Although in these studies ETEC has tended to be classified as a more mild disease, it can clearly present as a more severe dehydrating illness requiring intravenous rehydration [26]. The drivers of disease severity are largely unknown; though likely represent an interplay between host and pathogen factors. One factor that has inconsistently shown a significant association with increased disease severity is ST production either alone or in combination with LT [27,28]. This is consistent with what has been observed in experimental infection studies, although the data for LT-only producing strains are limited. To date, only 4 LT-only strains have been administered: LSN03-016011/A, DS26-1, E2528-C1, TD255-C4 (Table 2). While doses have ranged from 5E8 to 1E10, diarrhea attack rates have been relatively low across the dose/strain combinations with relatively infrequent and less severe associated symptoms. However, this clearly does not explain all the variation observed within the experimental infection studies as strains producing ST only and LT and ST in combination have resulted in a continuum (i.e., from mild to severe) of clinical symptoms at various inoculum levels.

Table 5
Products assessed using the ETEC human challenge model.

Reference	Year	Author	Product (route)	Strain	Dose (cfu)	Result
[64]	1981	Clements	Lactobacillus acidophilus	TD225-C4	1×10^{10}	No protection against diarrhea
			Lactobacillus bulgaricus (oral)	214-4	1×10^{8}	No protection against diarrhea
				H10407	1×10^{8}	No protection against diarrhea
				B7A	1×10^{8}	No protection against diarrhea
				B7A	1×10^{10}	No protection against diarrhea
[65]	1982	Levine	Type 1 somatic pili (intramuscular)	H10407	5×10^{8}	Significant protection from diarrhea
				H10407	5×10^{8}	Nonsignificant decrease (37.5%) in diarrhea attack rates compared to controls
				H10407	1×10^{7}	No protection against diarrhea
				B7A	1×10^{10}	No protection against diarrhea
[14]	1983	Graham	Bismuth subsalicylate (oral)	H10407	1×10^{8}	Significant protection from diarrhea
[66]	1984	Evans	CFA/I and CFA/II (oral)	H10407	4×10^{8}	No protection against diarrhea
				H1765	4×10^{8}	No protection against diarrhea
			CFA/I (subcutaneous prime followed by two oral doses 1 week apart)	H10407	4×10^{8}	25% lower diarrhea attack rate compared to subjects receiving oral vaccination only
[16,17]	1986	Levine	E1392-75-2A: live attenuated strain expressing CS1 and CS3 (oral)	E24377A	5×10^{8}	Significant protection from diarrhea
[30]	1988	Evans	Killed whole cell strain H10407 (oral)	H10407	5×10^{9}	Significant protection from diarrhea
				B2C	3×10^{8}	Diarrhea attack rate of 25% (no comparable placebo comparator
				CFA/I; O63:H-	Not reported	Diarrhea attack rate of 25% (no comparable placebo comparator)
[31]	1988	Evans	Killed whole cell strain H10407 (oral)	H10407	5×10^{9}	Significant protection from diarrhea
[40]	1988	Tacket	Bovine milk IgG against common ETEC O serogroups (oral)	H10407	1.2×10^{9}	Significant protection
[68]	1994	Tacket	CS1 and CS3 encapsulated in biodegradable microspheres (intestinal tube)	E24377A	3×10^{9}	Nonsignificant decrease (30.0%) in diarrhea attack rates compared to controls
[41]	1998	Freedman	Bovine milk IgG against CFA/I (oral)	H10407	1×10^{9}	Significant protection from diarrhea
[69]	1999	Tacket	Enteric-coated capsules containing bovine milk IgG against CFA/I, CS3 and CS6	E24377A	1×10^{8}	Significant protection from diarrhea
[21]	2007	McKenzie	LT (skin patch)	E24377A	6×10^{8}	No protection against moderate to severe diarrhea; amelioration of disease
[71]	2008	McKenzie	PTL-003: live attenuated strain expressing CS1 and CS3 (oral)	E24377A	3×10^{9}	No protection against moderate to severe diarrhea

4.3. Model utility

It can be argued that the ETEC volunteer challenge model is an overly rigorous and artificial method for assessing preliminary efficacy of ETEC vaccine candidates. As reported by McKenzie et al., an LT skin patch vaccine failed to protect against diarrhea in an experimental infection study with the strain E34277A, although there were trends toward a less severe illness in the vaccinated subjects [21]. However, when that vaccine (with minor modifications in the administration) was evaluated in a relatively small logistics field trial, a statistically significant reduction was observed in the rate of moderate to severe diarrhea of any cause and in the number of loose stools and duration of illness among those who developed ETEC-associated diarrhea [22]. Those data were not corroborated in an expanded field trial and subsequent development of the ETEC patch vaccine was halted [29]. While the results of the expanded efficacy study have not been released in detail, it would be of interest to assess the correlation between efficacy effect estimates between that study and the original vaccination/challenge study.

To date, the only ETEC vaccine that has shown statistically significant protection in vaccination-challenge studies is the oral, colicin E2 treated, whole-cell vaccine evaluated by Evans et al. [30,31]. However, lack of additional clinical development of that vaccine precludes any assessment of the

utility of the human challenge model to predict field efficacy.

Bismuth subsalicylate has been shown to afford significant protection (protective efficacy = 76.3%) against ETEC-associated diarrhea in the experimental infection model [14,15]. These results correlated well with several field studies evaluating the efficacy of bismuth subsalicylate against all-cause diarrhea [32]. Specifically, in field studies of diarrhea in travelers to endemic regions, prophylaxis with bismuth subsalicylate yielded an approximate 65% efficacy against all-cause diarrhea [33–35]. While bismuth subsalicylate prophylaxis is not the same as active vaccination, these data highlight the potential utility of these models in predicting outcomes in large scale clinical trials in travelers to ETEC endemic settings.

Although markedly different than disease prevention, Black et al. assessed trimethoprim and trimethoprim-sulfamathoxazole (Bactrim) in the treatment of subjects with experimentally induced ETEC diarrhea [36]. They saw a significant decrease in the number of loose stools and in diarrhea duration among those receiving the study drugs compared to those receiving placebo. This is consistent with studies of travelers' diarrhea treatment with these antibiotics [37–39].

Tacket et al. evaluated the ability of bovine milk antibodies raised against ETEC expressing CFA/I to confer passive protection against diarrhea in volunteers challenged with H10407 [40]. This was followed by a study by Freedman et al. demonstrating significant protection utilizing bovine milk antibodies against only the CFA/I fimbriae [41]. Unfortunately, despite these positive findings, no field studies utilizing either of these formulations have been conducted precluding a direct comparison between the two settings.

A listing of the prophylactic products assessed using the ETEC human challenge model is included in Table 5, and a review of ETEC vaccine candidates, including those assessed using these models has been previously reported [42]. It should be noted that the studies referenced above only compare and contrast field epidemiological studies with experimental infection studies in adults. This fails to represent the population with the highest disease burden, children living in ETEC endemic regions. This population is not one in which experimental ETEC infection would be ethically justifiable. This represents a potential challenge even if the experimental infection model is deemed an adequate predictor of field efficacy in an adult traveler population.

4.4. Unique severity with H10407

It has long been recognized that ETEC strain H10407 causes significant disease in experimental infection studies. Although other ETEC strains with similar diarrhea attack rates have been evaluated, H10407 appears to induce a more severe diarrhea. For example, in the study by McKenzie et al. evaluating a bovine milk IgG product, four of the eleven placebo recipients (36%) had over 10 diarrheal stools totaling 2.0–6.5 L. This is similar to the amount of fluid loss seen following an infection with *Vibrio cholera* O1 [43]. In addition to the significant diarrheal output, H10407 is associated with more severe concurrent signs and symptoms with fever and vomiting reported in a relatively high proportion of subjects compared to volunteers challenged with other ETEC strains. Efforts are ongoing to evaluate lower inoculum doses of H10407 [44]. These studies may identify lower inoculum doses that retain a high diarrhea attack rate yet result in a lower frequency of high volume output and less severe concurrent symptoms.

A number of proposed virulence factors have been identified for H10407 including EAST [45], Tia [46,47], Tib [47], leoA [48], EatA [49] and EtpA [50]. However, studies to date are unclear as to the effect each may play in the pathogenesis observed in experimental human infections and may not fully explain the differential effect seen with this particular strain compared to other ETEC challenge strains administered to date. The human challenge model could be used to compare the disease severity produced by H10407 compared to isogenic mutants lacking one or more of these genes. Additionally, this model could be utilized to evaluate host genomics and differences in innate and adaptive immune responses and effects on the host's microbiome.

4.5. Ethics

All clinical trials must weigh the ethical dilemmas involved with putting human subjects at risk against the potential benefit of a future drug, vaccine or other product designed to treat or prevent disease. This is no different in experimental ETEC infections, and in fact may be more difficult to appropriately balance the risk-benefit ratio. The ethical framework developed by Miller and Grady offers a structure by which to evaluate experimental infection studies and can serve as a guide for future ETEC infection research [51]. They highlight seven ethical issues that should be used to evaluate proposed studies, the most pertinent of which is "Risk". While ETEC-associated diarrhea in adults has generally been thought of as a relatively mild, self-limited disease, recent literature has highlighted a potential association with post-infectious sequelae. Two separate meta-analyses have shown a 7-fold increase in the risk of developing post-infectious irritable bowel syndrome (PI-IBS), following an episode of acute infectious gastroenteritis (IGE), of which ETEC is a common cause [52,53] and two studies have found this association in areas of high ETEC prevalence [54,55]. Although the pathogen-specific attributable risk for this post-infectious sequelae is currently unknown, these data suggest a new paradigm in the understanding of infectious diarrhea, including ETEC, and its impact on long-term health. In addition, a growing body of literature has shown a similar association between IGE and inflammatory bowel disease (IBD) [56–58]. Although unlikely to be identified in post-infection follow-ups due to its generally rare occurrence, this association highlights the need for further study of a pathogen that was once thought to cause only a self-limiting diarrhea.

Based on the relatively high inoculum required to elicit diarrhea in a sufficient number of subjects and the inverse association between diarrhea risk and baseline LT IgG titers, one may hypothesize that inclusion of serologic screening, as has been utilized for *Campylobacter jejuni* challenge model development [59,60], may decrease the required inoculum. None of the studies reviewed here reported serologic screening of potential study participants. However, several did report an exclusion for subjects with prior travel to ETEC-endemic regions and/or exclusion for known ETEC exposure. It is unclear what the added impact of baseline serologic screening may have on future utilization of the model.

The experimental human ETEC challenge has proved invaluable in defining and understanding strain pathogenicity, highlighting strain variability in disease manifestation and increasing our knowledge of the human immune response. It has also served as an important tool in the evaluation of vaccines and other experimental therapies. The information gleaned from these studies should guide future experimental ETEC challenge studies of existing and novel ETEC strains. With our expanding understanding of genomics, proteomics and microbiomics, data obtained from human challenge models may provide the opportunity to gain a better understanding of pathogenesis, host factors impacting susceptibility as well as develop more, well-characterized and targeted vaccines and adjuvants thereby increasing efficacy.

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