Prophylactic Efficacy of Hyperimmune Bovine Colostral Antiadhesin Antibodies Against Enterotoxigenic Escherichia coli Diarrhea: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Trial

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(See the editorial commentary by Fleckenstein on pages 1–3.)

Background. Tip-localized adhesive proteins of bacterial fimbriae from diverse pathogens confer protection in animal models, but efficacy in humans has not been reported. Enterotoxigenic Escherichia coli (ETEC) commonly elaborate colonization factors comprising a minor tip adhesin and major stalk-forming subunit. We assessed the efficacy of antiadhesin bovine colostral IgG (bIgG) antibodies against ETEC challenge in volunteers.

Methods. Adults were randomly assigned (1:1:1) to take oral hyperimmune bIgG raised against CFA/I minor pilin subunit (CfaE) tip adhesin or colonization factor I (CFA/I) fimbriae (positive control) or placebo. Two days before challenge, volunteers began a thrice-daily, 7-day course of investigational product administered in sodium bicarbonate 15 minutes after each meal. On day 3, subjects drank 1×109 colony-forming units of colonization factor I (CFA/I)-ETEC strain H10407 with buffer. The primary efficacy endpoint was diarrhea within 120 hours of challenge.

Results. After enrollment and randomization, 31 volunteers received product, underwent ETEC challenge, and were included in the per protocol efficacy analysis. Nine of 11 placebos developed diarrhea, 7 experiencing moderate to severe disease. Protective efficacy of 63% (P = .03) and 88% (P = .002) was observed in the antiadhesin bIgG and positive control groups, respectively.

Conclusions. Oral administration of anti-CFA/I minor pilin subunit (CfaE) antibodies conferred significant protection against ETEC, providing the first clinical evidence that fimbrial tip adhesins function as protective antigens.

Keywords. enterotoxigenic Escherichia coli infections; humans; adhesins; bovine antibodies; passive protection.

A theme in the pathogenesis of many bacterial diseases is the deployment of filamentous surface appendages that attach to critical host surfaces [1]. These fimbriae or pili are composed of a polymeric tract of major protein subunits and often feature an accessory protein at the distal tip with specialized adhesive function. That these minor fimbrial tip components appear to play a critical role early in pathogenesis makes them attractive vaccine targets, though a protective effect in humans has yet to be demonstrated for such a moiety.

Fimbriae serve as intestinal colonization factors (CFs) for enterotoxigenic Escherichia coli (ETEC), an important cause of diarrhea in developing countries and in travelers [2–4]. Class 5 fimbriae are among the most prevalent ETEC fimbrial CFs [5]. Colonization factor I (CFA/I) fimbriae, the class archetype, is composed of the major pilin subunit of CFA/I (CfaB), a helically arrayed polymer of which forms the stalk, and a minor tip-localized pilin subunit of CFA/I (CfaE) [6]. Binding properties have been ascribed to both of these subunits, though mounting evidence suggests that CfaE and related class 5 fimbrial tip proteins function as intestinal adhesins [7–12]. We have recently shown that CfaE functions as a protective immunogen in a neonatal mouse model [13], but similar proof in humans is lacking.

Passive oral administration of hyperimmune bovine milk immunoglobulin (bIgG) has been experimentally evaluated as a preventive or therapeutic modality for a variety of enteric infections including ETEC [14–18]. Notably, hyperimmune bIgG generated against a cocktail of inactivated whole-cell ETEC and against purified CFA/I fimbriae conferred 76%–100% protection against challenge with a prototype CFA/I-ETEC strain in volunteers [14, 15, 19], corroborating other lines of evidence that anti-CF immunity is associated with protection against ETEC diarrhea [20, 21].
We hypothesize that the tip adhesins of class 5 fimbriae represent an intrinsic protective antigen of this important class of human intestinal CFs of ETEC. Using CfaE as the prototype adhesin, we previously engineered and purified a stable, soluble variant of CfaE using donor strand complementation technology \cite{10, 11}. Donor strand complemented CfaE (dscCfaE) was used to generate a hyperimmune anti-CfaE bIgG preparation. Here we report the results of a randomized, double-blind, placebo-controlled clinical trial showing the protective efficacy of this preparation against challenge with a CFA/I-producing ETEC strain.

METHODS

Production of Bovine Vaccines

Donor strand complemented CfaE was expressed and purified using previously described methods \cite{10, 22}. The dscCfaE lot used for bovine vaccination had a purity of 94% and contained <10 endotoxin units (EU) per milligram as measured by the Limulus amebocyte lysate (LAL) assay. CFA/I was produced from ETEC strain WS1933D (ST; CFA/I; O71:H−) at the Walter Reed Army Institute of Research (WRAIR) Pilot Bioproduction Facility (PBF) by previously described methods \cite{13}. Purity of the final CFA/I fimbrial preparation was >98%, and the endotoxin content (by LAL) was 6 × 10⁴ EU/mg.

Production and Characterization of Antiadhesion (CfaE) and Antifimbrial (CFA/I) bIgG Products and Description of Placebo

The bIgG products were prepared by ImmuCell Corporation (Portland, Maine). During the dry period, pregnant cows were immunized by 3 intramuscular injections at 3-week intervals of dscCfaE (500 µg/dose) or CFA/I (250 µg/dose), each admixed with a copolymer-based adjuvant. After parturition, colostrum was collected, and the immunoglobulin G (IgG)–rich whey fraction was obtained as the byproduct of a standard cheese-making process. The whey was further processed by pasteurization, diatomaceous earth clarification, diafiltration, heat treatment, and freeze-drying. Final products were milled to a semifine off-white powder. Each product was characterized for total protein (Kjeldahl method), IgG content (sodium dodecyl sulfate polyacrylamide gel electrophoresis and densitometry), moisture, and bioburden (microbial limits test) using standardized methods and predetermined release criteria to ensure product safety. Powdered lactose-free infant formula (LactoFREE LIPIL Enfamil, Mead Johnson) was used as the placebo. The single-unit doses of each product were dissolved in a solution of 2 g of sodium bicarbonate (U.S. Pharmacopoeia [USP] and food-grade bicarbonate of soda) in 150 mL of water within 2 hours prior to dosing.

Potency was assessed by measuring antibody levels to the homologous antigen by enzyme-linked immunosorbent assay (ELISA) in a 96-well microtiter format. Cross-reactivity of each product to either dscCfaE or CFA/I was also measured. Functional (antiadhesive) antibody activity was measured in an ELISA (ELISA) in a 96-well microtiter format. Cross-reactivity of homologous antigen by enzyme-linked immunosorbent assay within 2 hours prior to dosing.

Bacterial Challenge Strain Preparation

The ETEC challenge strain was H10407 (CFA/I; LTST; O78:H11) \cite{23}. The challenge inoculum was freshly prepared from production cell bank vials that had been produced under Good Manufacturing Practices (GMP) conditions at the WRAIR PBF (lot number 0519). H10407 was grown on CFA agar overnight at 37°C and harvested in sterile saline. Final concentration of colony-forming units (CFU) was determined by optical density and confirmed by the plate count method. Before study initiation, 5 naive volunteers were subject to H10407 challenge to ascertain potency of inocula prepared from the GMP cell bank, using the same procedures for follow-up and outcome definitions as described below (ClinicalTrials.gov identifier NCT00198796).

Passive Vaccination-Challenge Trial Design

The clinical protocol was approved for implementation at Johns Hopkins University by the Western Institutional Review Board and the Committee on Human Research, and by the US Army Human Subjects Research Review Board and registered on ClinicalTrials.gov (NCT00435526). Healthy adult subjects aged 18–45 years were recruited from the Baltimore, Maryland area and screened for participation. Consented subjects were evaluated to assure good health and eligibility through medical history, physical examination, and screening laboratory tests. Eligible subjects were admitted in 2 cohorts to the inpatient research ward at Bayview Medical Center (n = 27) and the General Clinical Research Center (n = 9) of Johns Hopkins Hospital (Baltimore, Maryland) 3 days before challenge and randomly assigned in a 1:1:1 ratio to receive anti-CfaE bIgG, anti-CFA/I bIgG, or placebo in a double-blinded fashion based on blocked (block size = 3) randomization. Other than the pharmacist preparing investigational products, all investigators, data collectors, and subjects were blinded to treatment allocation.

Two days before challenge, subjects began their assigned treatment thrice daily, 15 minutes after each meal. Each unit
dose, set at 1 g blgG for each of the 2 active products (total unit dose weight anti-CfaE, 2.8 g; anti-CFA/I, 2.4 g), and 2.8 g of infant formula powder (placebo) was dissolved in 150 mL water containing 2 g sodium bicarbonate (USP).

On the day of challenge, subjects followed the prescribed routine through breakfast and then fasted for 90 minutes. One minute before challenge, subjects drank 120 mL bicarbonate buffer, and then received approximately $1 \times 10^6$ CFU of strain H10407 in 30 mL of the same sodium bicarbonate solution. Subjects received a second dose of blgG or placebo 15 minutes after challenge. Subjects then received their assigned treatment on the evening of challenge and then thrice daily for an additional 4 days.

Subjects were actively monitored for adverse events including signs and symptoms of gastrointestinal illness during the hospitalization period. This included daily assessments by a study physician, measurement of vital signs 3 times a day, and inspection and weighing of all stools. Stool consistency was graded based on a previously described 5-point scale [24]. Grade 3–5 stools were considered to be loose. Qualitative and quantitative stool cultures were performed daily. Nonlactose fermenting colonies on MacConkey agar were screened for CFA/I production on CFA agar by the colony immunoblot method to quantitate shedding of the ETEC challenge strain. When a stool sample was not available, a rectal swab was obtained for qualitative assessment of ETEC colonization.

Subjects were given oral rehydration with passage of grade 3–5 stool(s) to maintain proper hydration. Intravenous fluid replacement was begun if a subject met preset criteria for high-output diarrhea or displayed signs of hypovolemia, or at the principal investigator’s discretion. All subjects were treated with a 3-day course of ciprofloxacin 5 days after challenge (or orally challenged with ETEC to ensure continued potency of the H10407 GMP cell banks (Table 1). All subjects developed diarrhea, 4 with moderate to severe illness (Table 2).

### Definitions and Trial Endpoints

Diarrhea was defined as at least 1 loose stool of $\geq 200$ g during any 48-hour period, in the 120-hour window after ETEC challenge. Based on the highest 24-hour output over the 120 hours postchallenge, diarrhea was graded as mild (1–3 loose stools of $\leq 400$ g total), moderate (4–5 loose stools or 401–800 g total), or severe (>6 loose stools or >800 g total weight). The primary endpoint for determination of protective efficacy was diarrhea. Prespecified secondary efficacy endpoints included moderate to severe diarrhea and ETEC colonization. Other planned secondary outcomes included evaluation of differences between the placebo group and each test group with respect to time to diarrhea onset, duration of diarrhea, and the total number and volume of loose stools.

#### Serum Antibody Responses

Serum was collected 2 days before challenge and on days 7, 10, and 21 after challenge and stored at $-20$°C until use. Serum immunoglobulin A (IgA) and IgG antibody titers against CFA/I and LT were determined by ELISA as previously described [25]. Seroconversion was defined as a $\geq 2$-fold increase in the endpoint titer with a postchallenge reciprocal titer $>10$.

### Statistical Analysis

The proportion of subjects experiencing an adverse event was compared between each active group and the placebo group using Fisher exact test. Continuous variables were compared between active and placebo groups using the Kruskal-Wallis test. The protective efficacy of immunoprophylaxis with anti-CfaE and anti-CFA/I blgG was calculated using the formula $1 - \text{relative risk}$. A sample size of 10 per arm was selected to provide 72% power to detect $>60\%$ protective efficacy against diarrhea, assuming an attack rate of at least 80% in the placebo group and using a 1-tailed analysis ($\alpha = .10$).

### RESULTS

#### ETEC Challenge Validation

Before trial initiation, 5 naive volunteers were enrolled and orally challenged with ETEC to ensure continued potency of the H10407 GMP cell banks (Table 1). All subjects developed diarrhea, 4 with moderate to severe illness (Table 2).

#### Subjects

Seventy-four subjects were recruited and screened for participation, of whom 36 met all entry criteria and were admitted to

### Table 1. Baseline Characteristics of Subjects in the Pretrial Challenge Validation Group as Well as Those Included in the Interventional Trial (Per Protocol)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pretrial Validation</th>
<th>Placebo</th>
<th>Anti-CfaE blgG</th>
<th>Anti-CFA/I blgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 11)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>27.7 (76)</td>
<td>30.7 (76)</td>
<td>32.3 (8.9)</td>
<td>34.1 (7.4)</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td>Male 2 (40)</td>
<td>7 (64)</td>
<td>7 (70)</td>
<td>9 (90)</td>
</tr>
<tr>
<td></td>
<td>Female 3 (60)</td>
<td>3 (32)</td>
<td>3 (30)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td>African-American 4 (80)</td>
<td>10 (91)</td>
<td>9 (90)</td>
<td>7 (70)</td>
</tr>
<tr>
<td></td>
<td>White 1 (20)</td>
<td>1 (9)</td>
<td>1 (10)</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

Abbreviations: blgG, bovine colostral immunoglobulin G; CFA/I, colonization factor I; CfaE, CFA/I minor tip-adhesin subunit; SD, standard deviation.
the inpatient research ward. Thirty-four were randomized and begun on anti-CfaE bIgG (n = 11), anti-CFA/I bIgG (n = 12), or placebo (n = 11). Of these, 10 in the anti-CfaE bIgG arm and 11 each in the anti-CFA/I bIgG and placebo arms received the H10407 challenge (Figure 1). Prior to challenge, 2 subjects were discharged from the inpatient facility due to events unrelated to the investigational products. No baseline differences were noted across groups in terms of age, sex, or ethnicity (Table 1).

Compliance and Safety
Compliance with oral immunoprophylaxis was complete for all subjects except 1, who missed 1 scheduled dose 2 days after challenge. All doses were taken within 20 minutes (mean, 12 minutes) after each meal. During the 2-day prophylaxis period before challenge, adverse events were infrequently reported with the exception of flatulence (24% of all subjects), with no significant difference between groups. Transient, mild elevations in serum enzymes (alanine and aspartate aminotransferases) were noted in several subjects, with no significant differences between the anti-CfaE bIgG (3/11), anti-CFA/I bIgG (6/12), or placebo (2/11) groups. Return to normal values was documented by the 10th day postchallenge in all affected subjects.

Protective Efficacy
Following H10407 challenge with an inoculum of $1.3-1.4 \times 10^9$ CFU, 9 of 11 placebo recipients developed diarrhea (Table 2). In comparison, 3 of 10 subjects in the anti-CfaE bIgG group and 2 of 11 subjects in the anti-CFA/I group experienced diarrhea. One subject with diarrhea in the anti-CFA/I bIgG group had a documented fever 2 days before challenge and a first loose stool 2 hours before challenge. Based on adjudication by the medical monitor prior to lifting the blind, this subject was excluded from the per protocol efficacy analyses.

In the per protocol analysis, anti-CfaE bIgG conferred significant protection against diarrhea compared to the placebo group (Table 2). Consistent with results of an earlier trial [15], anti-CFA/I bIgG also afforded significant protection (Table 2). In an intention to treat analysis, inclusion of the 1 subject with nonincident diarrhea (see above) resulted in a slightly lower estimate of protective efficacy for anti-CFA/I bIgG (data not available).

**Table 2.** Diarrhea Incidence Rates and Passive Protective Efficacy Against Any Diarrhea and Moderate-to-Severe Diarrhea

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Any Diarrhea (Primary Endpoint)</th>
<th>Vaccine Efficacy</th>
<th>P Value (2-Sided)</th>
<th>Moderate to Severe Diarrhea</th>
<th>Vaccine Efficacy</th>
<th>P Value (2-Sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretrial validation</td>
<td>5</td>
<td>5 (100%)</td>
<td>NA</td>
<td>.03</td>
<td>4 (80%)</td>
<td>NA</td>
<td>.02</td>
</tr>
<tr>
<td>Anti-CfaE bIgG</td>
<td>10</td>
<td>3 (30%)</td>
<td>63%</td>
<td>.03</td>
<td>1 (10%)</td>
<td>84%</td>
<td>.02</td>
</tr>
<tr>
<td>Anti-CFA/I bIgG</td>
<td>10</td>
<td>1 (10%)</td>
<td>88%</td>
<td>.002</td>
<td>0 (0%)</td>
<td>100%</td>
<td>.004</td>
</tr>
<tr>
<td>Placebo</td>
<td>11</td>
<td>9 (82%)</td>
<td>referent</td>
<td>referent</td>
<td>7 (64%)</td>
<td>referent</td>
<td>referent</td>
</tr>
</tbody>
</table>

Abbreviations: bIgG, bovine colostral immunoglobulin G; CFA/I, colonization factor I; CfaE, CFA/I minor tip-adhesin subunit; NA, not applicable.

**Figure 1.** Trial profile. **A.** Number of subjects screened, enrolled, and challenged with enterotoxigenic *Escherichia coli* strain H10407 to validate pathogenicity of fresh-grown bacteria from existing seed banks. **B.** Number of subjects screened, enrolled, randomized, treated, and challenged in the main trial.
shown). Against moderate to severe diarrhea, the point estimates of protective efficacy for both anti-CfaE (84%) and anti-CFA/I bIgG (100%) were significant.

Subjects with diarrhea in the anti-CfaE bIgG group had mild (n = 2) or moderate (n = 1) diarrhea, whereas 7 of the 9 ill subjects in the placebo group experienced moderate (n = 1) or severe (n = 6) disease. Amelioration in the anti-CfaE bIgG group was also reflected in a shortened duration of illness and lower number and volume of loose stools (Table 3). Abdominal cramps, nausea, headache, and malaise were all more commonly reported by placebo recipients than by recipients of either bIgG preparation (Supplementary Table 2).

All challenged volunteers developed positive stool cultures for H10407, indicating that neither bIgG preparation prevented ETEC colonization. Analysis of daily quantitative stool cultures for the challenge organism indicated that the maximal number of H10407 per g of stool was lower in the anti-CfaE bIgG recipients (median, 2 × 10^7 CFU/g) than the placebo group (3.2 × 10^8 CFU/g), suggesting a lower density of colonization. In the positive control anti-CFA/I bIgG group, the maximal concentration of H10407 (3.2 × 10^7 CFU/g) was also a log lower than that of the placebo group.

Serologic Responses to H10407 Challenge
A majority of subjects in the placebo group manifested IgG seroconversion to CFA/I and IgG and IgA seroconversion to LT (Table 4). In comparison, seroconversion rates to these antigens were generally lower in the passive bIgG treatment groups (Table 4).

**DISCUSSION**

We have shown that orally administered hyperimmune anti-CfaE bIgG confers significant, clinically meaningful protection against the primary outcome of diarrhea following a rigorous challenge with CFA/I-producing ETEC. Moreover, higher efficacy was evident against a secondary outcome of moderate to severe diarrhea, suggestive of a gradient in protection with increasing severity. Administration of antiadhesin antibodies did not impact pathogen excretion rates per se, but quantitative stool culture data indicated a diminished intensity of shedding in those receiving the antiadhesin as well as the antifimbrial antibody preparation. Findings in the anti-CFA/I bIgG positive control group bolster previously reported results showing that homologous antifimbrial antibodies conferred passive protection against CFA/I-ETEC challenge [15]. In our trial, purified CFA/I for bovine vaccination was prepared from a wild-type ST-only ETEC strain displaying a heterologous O-antigen rather than from strain H10407 as done by Freedman et al [15], thus alleviating the possible confounding effect of O-specific and/or LT antibodies in protection (Supplementary Table 1).

Effectively, this is the third in a progression of trials using the same volunteer challenge model that has incrementally shown the protective efficacy of bovine milk immunoglobulins first against a cocktail of ETEC antigens (including CFA/I) [14], then against CFA/I fimbriae [15], and here against dscCfaE, a stable, native-like variant of the CFA/I fimbrial tip adhesin. These findings indicate that CfaE represents an antigen of intrinsic importance to this protective effect. From the standpoint of pathogenesis, our findings also provide the first piece of indirect evidence that CfaE functions as the critical small intestinal moiety of CFA/I. The blunting of serum anti-LT responses to H10407 challenge in subjects who received the active bIgG products is akin to recent observations showing that subjects undergoing rechallenge with H10407 have diminished anti-LT responses in comparison to naive challenge subjects, and suggest that antiadhesin antibodies present in the gut may play an important role in modulating the response to LT [26, 27]. The extent to which antiadhesin antibodies may modulate immune responses to other antigens was not assessed in this study.

While minor tip-localized adhesins from both gram-positive and gram-negative pili or fimbriae have been assessed as potential vaccine candidates in animal models, this trial provides the first clinical proof of principle showing the protective capacity of one such tip adhesin. Our finding that the minor fimbrial tip adhesin CfaE functions in humans as a protective immunogen builds upon previously reported observations that active (maternal) and passive vaccination with CfaE conferred protection against lethal CFA/I-ETEC infection in neonatal mice [13]. Pathogenic streptococci express pili with accessory tip adhesins, and for pneumococcus and group B Streptococcus, vaccination with the corresponding accessory protein conferred protection in mice models [28–30]. P pili and type I fimbriae

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**Table 3. Characteristics of Diarrheal Illness in Test and Control Groups**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Anti-CfaE bIgG (n = 3)</th>
<th>Anti-CFA/I bIgG (n = 1)*</th>
<th>Placebo (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to onset, h, median (range)</td>
<td>24 (9–33)</td>
<td>3</td>
<td>23 (4–34)</td>
</tr>
<tr>
<td>Duration, h, median (range)</td>
<td>3 (0–96)</td>
<td>27</td>
<td>54 (40–158)</td>
</tr>
<tr>
<td>Total No. of loose stools, median (range)</td>
<td>3 (1–8)</td>
<td>2</td>
<td>10 (5–26)</td>
</tr>
<tr>
<td>Total volume loose stools, median (range)</td>
<td>427 (318–1079)</td>
<td>220</td>
<td>1931 (269–6524)</td>
</tr>
</tbody>
</table>

Abbreviations: bIgG, bovine colostral immunoglobulin G; CFA/I, colonization factor I; CfaE, CFA/I minor tip-adhesin subunit; NA, not applicable.

*Excludes 1 subject with nonincident illness.
The findings presented here have implications for the development of both passive immunoprophylaxis against ETEC as well as an active ETEC vaccine. This demonstration of the protective effect of exogenously administered antiadhesive antibodies also lends credence to the concept of an active, adhesin-based ETEC vaccine designed to elicit endogenous, local production of such antibodies. Toward this end, clinical trials of a prototype dsc-CfaE vaccine are being conducted. Arguably, an effective, active vaccine against ETEC would serve as a widely applicable solution to prevention of disease caused by this important bacterial enteropathogen.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

**Author contributions.** S. J. S., D. R. T., C. K. P., A. O., J. H. C., and A. L. B. were involved in conception and design of the trial. S. J. S., S. T. P., C. B., and J. H. C. developed, produced, and tested the bovine vaccines and antibody preparations. R. M., D. R. T., C. K. P., J. A. C., S. A. S., B. D., C. M. W., H. K., S. L. G., and A. L. B. were involved in conduct of the trial and antibody preparations. R. M., D. R. T., C. K. P., A. O., S. A. S., and A. L. B. participated in data analysis and interpretation of data. S. J. S., C. K. P., and A. L. B. drafted the manuscript. All authors critically reviewed and approved the final manuscript. Investigators and the corresponding author had full access to data and were responsible for all aspects of preparation and submission of the manuscript.

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**Disclaimer.** The funders played no role in study design, data collection, analysis, or interpretation of the study, and did not provide any input in writing of the manuscript. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of the Army, Department of Defense, nor the US government. S. J. S., J. A. C., S. A. S., C. M. W., and C. B. were military service members and D. R. T and C. K. P. were employees of the US government.

### Table 4. Serum Immune Responses to CFA/I and LT in Volunteers Following Enterotoxigenic Escherichia coli Challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Anti-CFA/I</th>
<th></th>
<th>Anti-LT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
<td></td>
<td>IgG</td>
</tr>
<tr>
<td>Validation</td>
<td>5</td>
<td>40 (3.5)</td>
<td>0 (NA)</td>
<td>100 (20.9)</td>
<td>100 (20.9)</td>
</tr>
<tr>
<td>Anti-CfaE bIgG</td>
<td>10</td>
<td>30 (7.2)</td>
<td>20 (6.0)</td>
<td>80 (3.7)</td>
<td>40 (11.8)</td>
</tr>
<tr>
<td>Anti-CFA/I bIgG</td>
<td>10</td>
<td>0 (NA)</td>
<td>0 (NA)</td>
<td>50 (6.3)</td>
<td>30 (5.4)</td>
</tr>
<tr>
<td>Placebo</td>
<td>11</td>
<td>64 (16.2)</td>
<td>36 (8.9)</td>
<td>91 (51.0)</td>
<td>91 (17.4)</td>
</tr>
</tbody>
</table>

Abbreviations: bIgG, bovine colostral immunoglobulin G; CFA/I, colonization factor I; CfaE, CFA/I minor tip-adhesin subunit; IgA, immunoglobulin A; IgG, immunoglobulin G; LT, labile enterotoxin; NA, not applicable.

*a* A ≥2-fold increase in endpoint titer between pre- and postchallenge sera and a postchallenge reciprocal titer >10.

*b* P < .01, in comparison to placebo group seroconversion (Fisher exact test).

*c* P < .05, in comparison to placebo group seroconversion (Fisher exact test).
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References