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A phase 2, randomised, placebo-controlled study to evaluate once-daily oral Travelan for the prevention of Travellers' diarrhoea

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This study evaluated once-daily oral dosing of Travelan (IMM-124E), a bovine polyclonal antibody product, for prevention of Enterotoxigenic *Escherichia coli* (ETEC)-induced diarrhoea. In this randomised, double-blind, placebo-controlled trial using an ETEC-controlled human infection model, 60 healthy subjects received 1200mg Travelan or placebo once-daily for 7 days. Two days after dosing commenced, subjects were challenged with ETEC strain H10407 and monitored for diarrhoeal symptoms. Travelan was well-tolerated with a significant 50% reduction in ETEC challenge-related adverse events ($P = 0.0001$). Efficacy outcomes did not reach statistical significance, largely due to a lower-than-expected placebo attack rate (33% vs 70% anticipated); however a strong trend consistently favoured Travelan, including a 40% reduction in moderate-to-severe diarrhoea (primary endpoint), a 50% reduction in severe diarrhoea, a 44% reduction in all diarrhoea, and a 71% reduction in early antibiotic use. These findings were supported by significant reductions in intestinal inflammation markers (faecal myeloperoxidase and calprotectin) 4 days post-challenge. Significantly lower O78 lipopolysaccharide IgA and IgG titers were observed but over 80% of subjects in each group seroconverted by day 15–29, indicating Travelan did not hinder immunity to ETEC. Although underpowered and limiting definitive interpretation, these findings suggest a potential protective effect of once-daily Travelan, warranting further investigation in a larger adequately powered trial.

Keywords Enterotoxigenic *Escherichia coli*, Prophylaxis, Travelan, IMM-124E, Travellers' diarrhoea, Clinical trial

Travellers' diarrhoea (TD) is the most prevalent infectious illness encountered by travellers and military personnel deployed to endemic regions, affecting 30–70% of visitors to high-risk areas annually[1,2]. Despite this burden, no licensed TD vaccines or prophylactics are currently available in the United States (US), and the condition is typically either left untreated or managed with antibiotics[3]. Primary prevention remains the most desirable strategy, as it eliminates the need for antibiotics, reduces bacterial antimicrobial resistance (AMR), averts the onset of illness, and avoids the associated travel disruptions and productivity losses associated with diarrheal disease.

Enterotoxigenic *Escherichia coli* (ETEC) is the most common bacterial cause of TD, implicated in 44% or more of all reported cases[4,5]. ETEC also poses a substantial burden in developing countries, where it is among the most prevalent causes of childhood diarrhoea; in 2016 an estimated 223 million ETEC episodes and 51,000 deaths occurred across all age groups including approximately 75 million episodes and 19,000 deaths in children

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aged ≤ 5 years[6]. Repeated ETEC infections in paediatric populations have been associated with growth faltering and cognitive decline, with broader macroeconomic consequences for the most heavily affected regions[7].

ETEC poses a significant health threat both to travellers and to military personnel during deployment. The resulting illness, characterised by watery diarrhoea and abdominal cramping, can lead to dehydration and incapacitation, debilitating travellers and affecting a service member's ability to perform their duties. Diarrhoeal disease has historically been one of the leading causes of non-battle-related morbidity in deployed forces, with direct implications for mission readiness, personnel availability, and operational timelines[2,8]. Studies of military personnel deployed to various regions have consistently identified ETEC as a leading pathogen responsible for acute diarrhoea[8]. For deployed military personnel operating in ETEC-endemic environments and the impact of enteric illness is not only on individual health but also on the operational effectiveness of entire units. Beyond acute illness, post-infectious sequelae may result in significant long-term consequences including irritable bowel syndrome, chronic intestinal inflammation, and persistent disruption of the gut microbiome[9]. The development of preventive measures to mitigate the impact of ETEC and maintain the health and readiness of deployed forces is a priority.

The clinical spectrum of ETEC infection ranges from asymptomatic carriage and mild gastrointestinal (GI) discomfort to severe, dehydrating diarrhoea requiring aggressive medical intervention, including intravenous (iv) rehydration and rescue antimicrobial therapy. While antibiotics remain the standard of care, escalating AMR and disruption of the host microbiome underscore the urgent need for effective, non-antibiotic prophylactic strategies[10]. A safe and effective TD prophylactic would offer an attractive alternative obviating the drawbacks of AMR and microbiome disruption inherent to antimicrobial use.

Travelan® (IMM-124E) is a non-systemic, orally delivered, bovine polyclonal antibody product formulated as a tablet, designed to neutralize ETEC within the GI tract before the onset of symptomatic disease[11]. It is manufactured from colostrum of dairy cattle immunized with a proprietary blend of 13 ETEC strains representing common TD-causing serotypes, as described previously[11]. Briefly, raw colostrum undergoes pasteurisation, centrifugal fat removal, diafiltration to reduce lactose, ultrafiltration concentration, and freeze or spray drying to form a powder. IMM-124E is further processed into a tablet form, whilst preserving the immunoglobulin integrity and potency. Travelan acts by disrupting bacterial attachment and adhesion to the intestinal epithelium, reducing motility, preventing colonisation, and impairing disease progression. Importantly, Travelan also exhibits broad-spectrum antibacterial activity against other gram-negative enteric pathogens[12], including *Shigella spp*, where it demonstrated 75% protective efficacy in a non-human primate challenge study[13].

Travelan has been marketed as a listed medicine and an over-the-counter product in Australia since 2004, indicated to reduce the risk of TD (AUST L 106709). It was launched in Canada in 2013 as a licensed natural health product (NPN 80046016) indicated to reduce the risk of TD, and in the US, Travelan® has been marketed as a dietary supplement for digestive tract protection since 2015. Ongoing development activities are directed towards advancing Travelan through the US Food and Drug Administration (FDA) regulatory processes via a Biologics License Application (BLA), with a proposed indication for the prevention of TD. Travelan has been administered to > 500 subjects in clinical studies with minimal safety concerns[11,14]. Reported adverse events were rare, and limited to mild GI symptoms consistent with other bovine milk-derived products. Immuron's ongoing pharmacovigilance program, spanning more than 10 years of post-market surveillance, has consistently demonstrated a strong safety profile for the marketed product. Each Travelan tablet contains 200 mg of hyperimmune bovine colostrum (HBC; drug substance), manufactured in accordance with current Good Manufacturing Practice (cGMP) guidelines.

Controlled human infection models (CHIMs) are historically a useful tool to assess preliminary protective efficacy of prophylactics or vaccine candidates[15–18]. By exposing a cohort of healthy volunteers to the same bacterial inoculum this allows for monitoring of the whole cycle of disease, duration of symptoms and clearance of the pathogen. This double-blinded study allows for an unbiased assessment of the efficacy of a once daily dose of Travelan prophylaxis compared with an inactive placebo in this CHIM study.

Two previous CHIM studies have been performed in which Travelan was administered at the total daily dose of 1200 mg, delivered as three divided doses[11]. In the first study only 1 of 15 subjects (6.6%) receiving Travelan developed diarrhoea compared to 11 of 15 subjects (73%) in the placebo group, corresponding to 90.9% protective efficacy ($P = 0.0005$). In a second CHIM, involving 60 subjects, protective efficacy ranged from 76.7 to 83.3% at 400 mg three times daily ($p = 0.0004$) and 58.7% at 200 mg three times daily ($P = 0.04$). In both clinical studies Travelan was safely administered and demonstrated clear and reproducible clinical efficacy[11].

A three-times-daily regimen may however be impractical for deployed military personnel and for some travellers as less frequent dosing could improve treatment adherence. This is consistent with evidence from malaria prophylaxis compliance studies, which have established a strong correlation between simpler dosing schedules and higher rates of patient compliance[19]. Accordingly, the present study was designed to evaluate whether an equivalent total daily dose of 1200 mg, delivered as a once-daily regimen, could provide prophylactic benefit.

Results

Subjects

A total of 191 healthy volunteers were screened for participation over a 2 month period, 113 were screen failures and 78 met the eligibility criteria and pre-defined clinical characteristics (Fig. 1). A number of eligible subjects could not be enrolled, due to study capacity constraints. In total, 63 subjects were enrolled and admitted to the inpatient facility on study day -3 (safety analysis set) and randomised to receive either Travelan (32 subjects) or placebo (31 subjects). Three subjects were discharged prior to challenge; one left the study due to illness and two were dismissed per investigator discretion as the cohort was full.

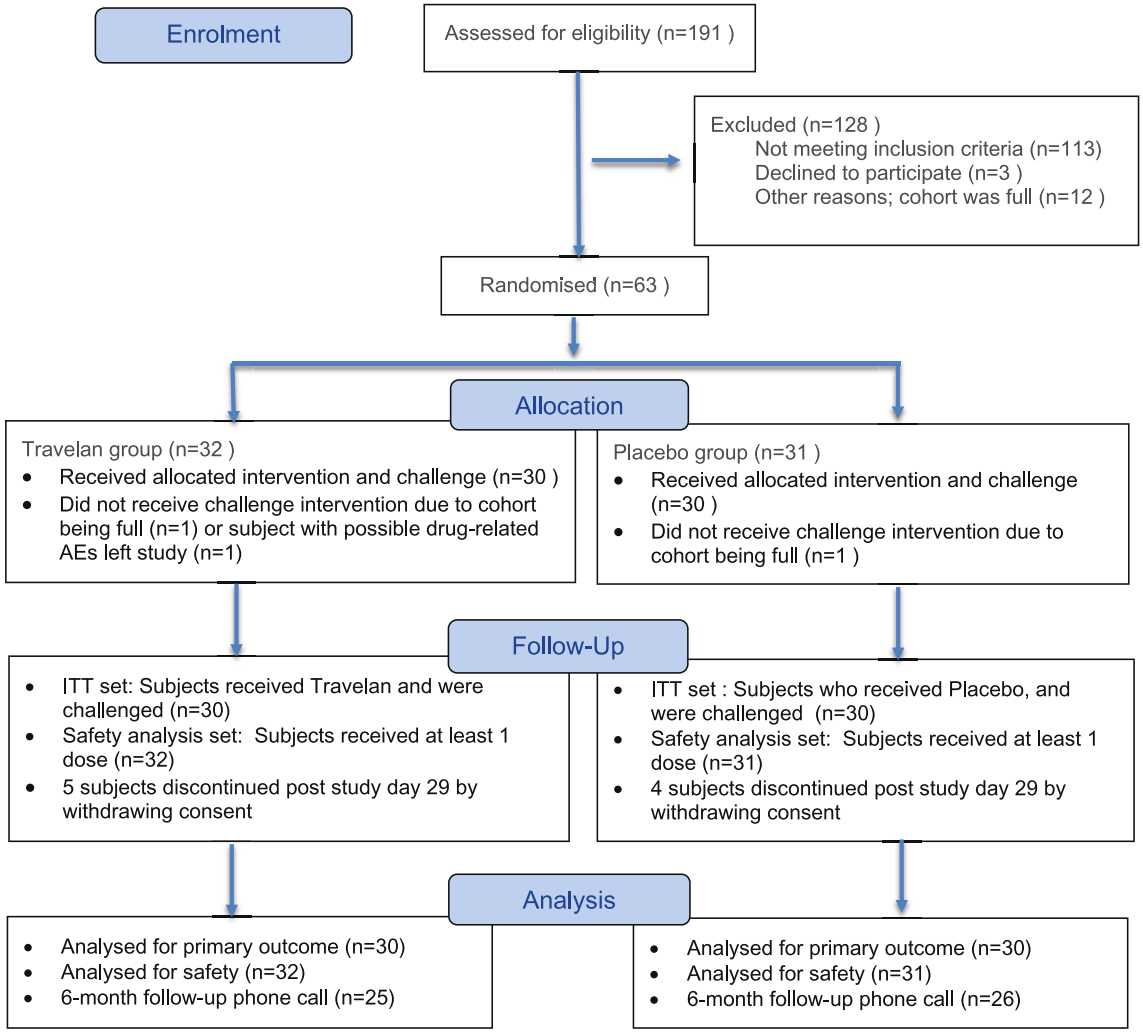


Fig. 1. Flow diagram of the process through the phases of the CHIM study: enrolment, intervention allocation, follow-up, and data analysis (CONSORT 2025 guidelines[20]).

Sixty subjects evenly distributed between the Travelan and the placebo groups completed the challenge on study day 1 with 1.045×10^8 CFU of ETEC H10407 (mean value); this dose was confirmed by identity test and culture. All subjects were compliant with per-protocol dosing and administration of the challenge strain, and no subjects were excluded from the Intent-to-Treat (ITT) set for assessment of the primary outcome.

Nine subjects withdrew consent after completing the day 29 outpatient follow-up, prior to the final 6-month post-challenge phone call for reasons unrelated to any AE. Fifty-one subjects, 25 in the Travelan cohort and 26 in the placebo cohort completed all protocol-defined procedures.

Demographic characteristics including age, sex, and ethnicity were broadly similar between treatment groups (Table 1). The majority of enrolled subjects were male (n = 34, 54%), compared with (n = 29, 46%) females.

Safety and efficacy

Assessment of Travelan product safety was limited to the two days prior to receipt of the ETEC-challenge dose (study day -2 and -1). One subject in the Travelan arm developed abdominal cramps, chills, and headache approximately 90 min after the first dose. Symptoms largely resolved without medication vital signs remained normal and the event was not confirmed but classified as a possible treatment-related AE. No other treatment-related AEs were recorded during the pre-challenge period.

Concomitant medications were provided only as required during the trial, for treatment of symptoms including rehydration and abdominal pain. There were no serious adverse events (SAEs) or deaths reported in the study.

Post-challenge inoculation AEs were solicited and events not temporally associated with receipt of Travelan, were considered likely related to the ETEC H10407 challenge strain. The vast majority of AEs (94%) for both groups were mild or moderate in intensity and resolved. The total number of Post-challenge AEs was significantly lower (50%) in the Travelan group (55 events) compared to the placebo group (109 events; $P = 0.0001$; Table 2). GI disorders were the most commonly reported system organ class (SOC) AEs in both groups, occurring in

	Travelan	Placebo	Total
Number of subjects (N)	32	31	63
Mean age at consent (years)	33.1	35.1	34.1
Gender N			
Male	18	16	34
Female	14	15	29
Race N (%)			
African-American	23 (72)	22 (71)	45 (71)
Caucasian	8 (25)	5 (16)	13 (21)
Other	1 (3)	4 (13)	5 (8)
Ethnicity N (%)			
Hispanic/Latino	5 (16)	10 (32)	15 (24)
Not Hispanic/Latino	27	21	48 (76)

Table 1. Demographic characteristics of enrolled subjects (Safety analysis set).

System organ class (SOC) preferred term	Travelan (N = 32)		Placebo (N = 31)		Reduction for Travelan Group %m
	Subjects n	Events m	Subjects n	Events m	
Total Subjects with at least 1 AE	24	55	28	109	50% $P = 0.0001$
Gastrointestinal (GI) disorders (diarrhoea, abdominal pain & distension, nausea, vomiting, constipation)	22	42	28	66	36%
Nervous system disorders (headache, dizziness, hyper anaesthesia)	6	6	10	13	54%
General disorders (chills, pyrexia, other abnormal tests)	2	2	6	10	80%
Musculoskeletal and connective tissue (back pain, myalgia, arthralgia)	1	1	7	7	86%
Metabolic and Nutrition disorders (decreased appetite)	1	1	4	4	75%
Skin and tissue (rash, itch, redness)	1	1	2	3	67%
Cardiac disorders (tachycardia)	0	0	3	3	100%
Infections (viral, respiratory)	2	2	3	3	33%

Table 2. Summary of post-challenge AEs by System Organ Class (SOC) and preferred term for the safety analysis set. A) Subjects with at least 1 AE per SOC and % reduction in the number of events for the Travelan group for study period day 1 to day 29. Subjects with multiple occurrences of AEs in the same SOC were counted only once within that SOC. Severity was graded by CTCAE v5.0. P value reported for all AEs, Chi squared analysis. AE = Adverse event AE coded with MedDRA version 25.1; N = Number of subjects in the respective treatment arm in the safety analysis set, n = Number of subjects with event, m = Number of events.

22 Travelan subjects (42 events) and 28 placebo subjects (66 events). The majority of GI AEs were classified as unrelated to study drug in both groups, with diarrhoea being the most frequently reported individual AE. The incidence of GI-related AEs was lower across every GI symptom category for the Travelan group, with an overall reduction of 36% relative to placebo.

At the 6-month follow-up, there were no long-term AEs reported for any subjects that could be attributed to the challenge inoculum or study drug administration.

Based on previously reported attack rates for the same ETEC H10407 strain[19], the expected placebo attack rate for moderate-to-severe diarrhoea was approximately 70% (Fig. 2a). In this study following ETEC H10407 challenge with 1×10^8 CFUs, the observed attack rates in the placebo group were substantially lower, with only 33% (10/30) of subjects developing moderate-to-severe diarrhoea. In comparison, 20% (6/30) of subjects receiving once-daily Travelan met the primary endpoint of moderate-to-severe diarrhoea (Fig. 2a). This corresponded to a 40% relative reduction in moderate-to-severe diarrhoea (95% CI – 82.2, 82.1; Table 3 & Fig. 2b). However, the lower than anticipated attack rate resulted in the study being underpowered to detect a statistically significant difference between groups for this primary endpoint and the wide confidence intervals limit definitive interpretation of the observed trend (Fig. 2a).

Similarly, there was a non-significant trend indicating that Travelan consistently reduced the frequency and severity of all secondary outcome measurements with reductions ranging from 28 to 100% (Table 3 & Fig. 2b). This included a 50% reduction in the proportion of subjects experiencing severe diarrhoea (4/30 compared to 8/30), and a 44% reduction in diarrhoea of any severity (9/30 compared to 16/30; Table 3 & Fig. 2b). Stool output measurements followed a similar trend, with Travelan subjects showing a 29–30% reduction in both the maximum number and total number of loose stools per day (Table 3 & Fig. 2b). The mean maximum number of loose stools in any 24-h period was lower in the Travelan group (2.8) compared to the placebo group (3.9). Mean values for both the total number and total weight of loose stools for the Travelan group were also reduced (4.2; 504 g) when compared to the placebo group (5.9; 715 g). When the daily totals of loose stools were

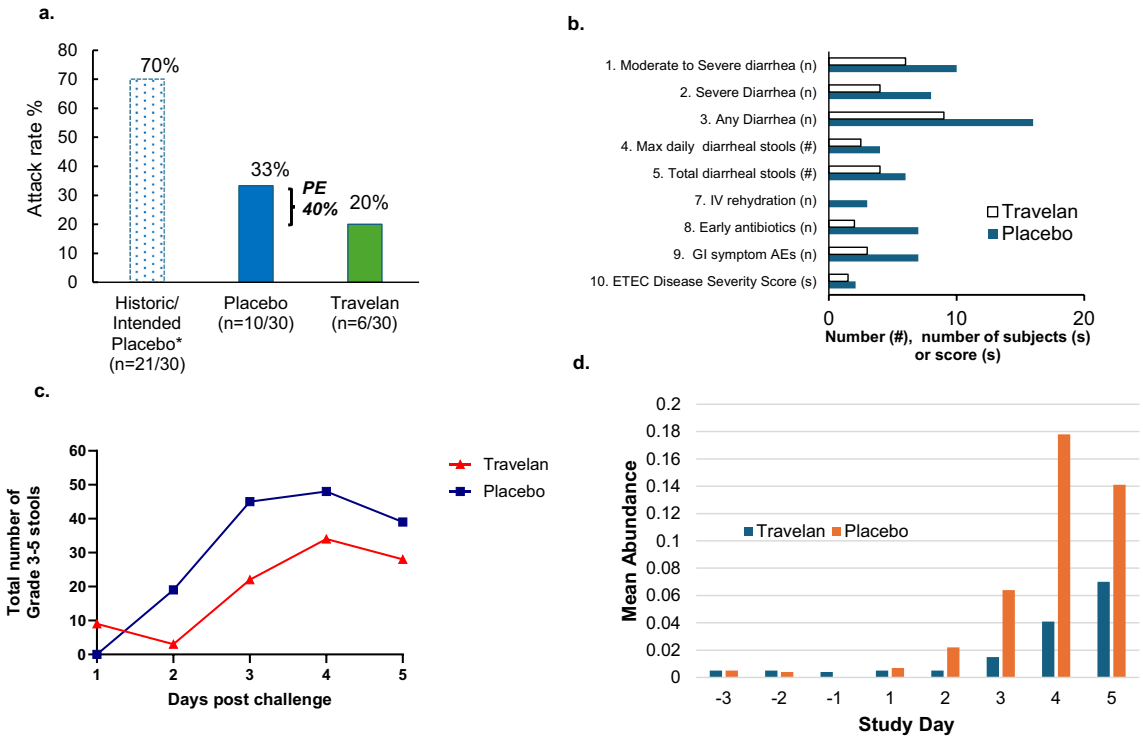


Fig. 2. Attack rates, efficacy, diarrhoea severity and outcome measurements within the 5-day period of Travelan or placebo dosing post challenge inclusive of those who met early antibiotic criteria for the ITT analysis set. (**a**) Intended attack rate for a sample size of 30 subjects per arm of 70%, as predicted from a historic study[19], and actual attack rates of the placebo and the Travelan groups and PE. (**b**) Diarrhoeal severity and endpoint measurements (refer to Table 3 1–10) illustrated as a bar graph comparing the % reduction in incidence for Travelan and placebo groups. (**c**) Total number of Grade 3–5 diarrhoeal stools per day post-challenge for the Travelan and the placebo groups. (**d**) Relative abundance of *Escherichia -Shigella* over time in Travelan and placebo groups defined as the percent composition of a specific organism relative to the total number of organisms identified in a sample. The mean relative abundance of the pathogenic genus *Escherichia-Shigella* in each group at baseline (day -3), pre-challenge (day -2 and-1), and post-challenge (days 1–5) was between 0 and 0.2 (20%) of the total community.

compared across all subjects, the Travelan group recorded lower daily totals on every post-challenge day with the exception of challenge day 1 (Fig. 2c). Although these differences were not statistically significant, the consistent direction of effect suggests a potential reduction in stool frequency and output that warrants further evaluation in adequately powered studies.

The trend toward reduction in diarrhoeal severity in Travelan-treated subjects compared with the placebo group was accompanied by fewer diarrhoeal-related symptoms. No subjects in the Travelan group required iv fluid administration, compared to 3/30 in the placebo group, representing a 100% relative risk reduction (Table 3 & Fig. 2b). Early rescue antibiotic use prior to day 6 was also lower in the Travelan group, with only 2/30 subjects requiring treatment compared with 7/30 in the placebo group a 71% reduction (Table 3 & Fig. 2b). The incidence of key moderate to severe symptoms including nausea, vomiting, anorexia, and/or abdominal cramps/discomfort, was 66% lower in the Travelan group with only 3 subjects affected compared to 7 in the placebo group; no fever was reported for either group. Nausea was the most commonly reported moderate-to-severe symptom occurring in 5 subjects who received placebo and 2 who received Travelan (Table 3 & Fig. 2b).

ETEC disease severity scores (ETEC scores) are a combined standardized measurement of diarrhoeal output and symptom severity[21], where a higher score indicates more severe disease. ETEC scores were low across all subjects in both treatment groups, with mean scores of 1.5 for Travelan and 2.1 for placebo groups, respectively, consistent with the lower than anticipated attack rates (Table 3 & Fig. 2b). Among subjects who met the primary endpoint, ETEC scores were notably higher than average, ranging from 2–6. Subjects requiring early rescue antibiotic therapy (2 in the Travelan and 7 in the placebo groups, respectively) showed the greatest disease burden with ETEC scores of 3–6. The 3 placebo subjects who required iv fluid replacement represented the most severe diarrhoeal cases overall, as all required early antibiotic therapy and carried the highest diarrhoeal-associated symptom burden with ETEC severity scores of 5–6.

Clearance of the challenge strain was assessed 48 h post-challenge, with viable ETEC detected in 90% Travelan and 97% placebo subjects. Substantial variability in stool CFU counts was observed between individuals at this single time point and no clear correlation between increased CFU level and more severe diarrhoea. At this time point, the mean number of viable CFUs of the challenge strain per gram of stool was significantly higher

Outcome/Endpoint measurement	Travelan N = 30	Placebo N = 30	Travelan efficacy or relative risk reduction to placebo (%)	*P*-value 95% 2-Sided Confidence Interval
Diarrhoeal severity and output				
1. Subjects with moderate-to-severe diarrhoea[a]	6	10	40%[b]	$P = 0.382$[c] (−82.2%, 82.1%)
2. Subjects with severe diarrhoea[d]	4	8	50%	$P = 0.197$[e] (−6.6%, 33.3%)
3. Subjects with diarrhoea of any severity[f]	9	16	44%	$P = 0.668$[e] (−0.9%, 47.6%)
4. Max number of diarrhoeal stools in any 24-h period Mean (s.e.m)	2.8 (0.56)	3.9 (0.65)	29%	$P = 0.221$[g] (−2.00,0.00)
5. Total number of diarrhoeal stools Mean (s.e.m)	4.2 (1.09)	5.9 (1.04)	29%	$P = 0.218$[g] (−5.0,1.0)
6. Total cumulative weight of diarrhoeal stools (grams) Mean (s.e.m)	504 (134)	715 (146)	30%	$P = 0.208$[g]
Diarrhoea-related symptoms and interventions				
7. Subjects requiring intravenous rehydration	0	3	100%	$P = 0.075$[e] (−0.7%, 20.7%)
8. Subjects requiring early antibiotics	2	7	71%	$P = 0.0706$[e] (−0.9%, 34.2%)
9. Subjects with GI AEs—fever, nausea, vomiting, anorexia, abdominal pain (Grades 2–4)	3	7	66%	$P = 0.165$[e] (−5.2%, 31.9%)
10. ETEC disease severity score[h] Mean (s.e.m)	1.5 (0.3)	2.1 (0.34)	29%	$P = 0.111$[g] (−1.0, 0.00)
Clearance of the challenge strain				
11. CFUs 48-h post challenge Mean	2.13×10^8	1.53×10^8	28%	$P = 0.0121$[g]

Table 3. Efficacy outcome measurements within the 5-day period of Travelan or placebo dosing post-challenge, inclusive of those who met early antibiotic criteria for the ITT analysis set. [a]Moderate-to-severe diarrhoea, defined as > 4 Grade 3–5 stools or > 401 g of Grade 3–5 stools in any 24-h period over the 5-day period post-challenge. [b]Protective efficacy (primary endpoint), defined as PE $(\%) = \frac{\text{incidence (placebo)} - \text{incidence (Travelan}^*\text{)}}{\text{incidence (placebo)}} \times 100\%$; [c]Fisher's 2-sided exact test for difference in proportions and binomial distribution; [d]Severe diarrhoea defined as > 6 Grade 3–5 stools or totalling > 800 g in any 24-h period; [e]Chi-squared test; [f]Any diarrhoea defined as all subjects who experienced > 2 loose/liquid grade 3–5 stools; [g]Wilcoxon rank-sum test log-normal-transformed values; [h]ETEC disease severity is a combined measure of diarrhoeal output and diarrhoeal symptoms, where a higher score indicates more severe disease (further defined in Porter et al.[21]); s.e.m, standard deviation of the mean; Max, maximum; CFUs, Colony-forming Units; GI, gastrointestinal; AEs, adverse events; ETEC, enterotoxigenic *Escherichia coli*.

($P = 0.0121$) in Travelan-treated subjects (2.13×10^8, standard error = 1.77×10^8) than those treated with placebo (1.53×10^8, standard error = 4.47×10^7; Table 3). These findings indicate differences in early post-challenge bacterial recovery between groups but do not demonstrate reduced bacterial load or accelerated clearance in Travelan-treated subjects.

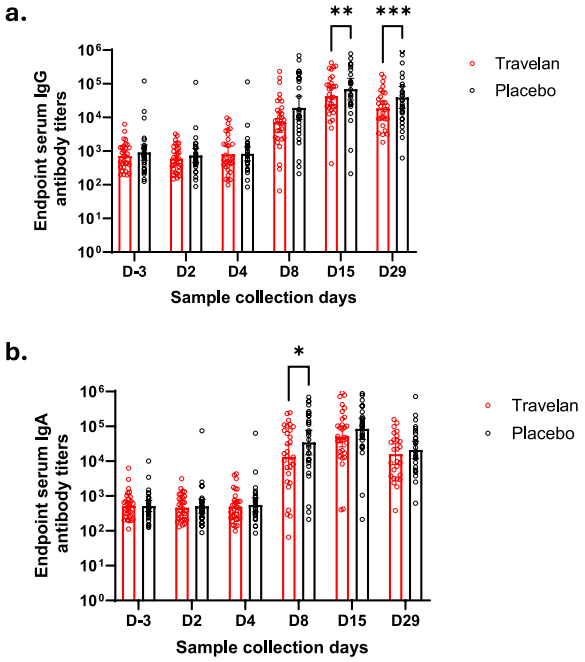
Travelan administration had measurable effects on the relative abundances of key gut bacteria after ETEC infection. At the genus level, the Travelan group consistently showed lower relative abundance of *Escherichia-Shigella* species compared to the placebo group. The placebo group experienced a pronounced increase in *Escherichia-Shigella* abundance during the acute infection (days 1–5) whereas the Travelan group maintained only minimal levels of *Escherichia-Shigella* throughout these stages (Fig. 2d).

Serological responses

Regardless of treatment allocation, the majority of subjects mounted an immune response to the ETEC strain following challenge. Baseline anti-O78 Lipopolysaccharide (LPS) titers in IgA or IgG were observed at day -3 up to day 4 post-challenge (Fig. 3). Between days 8 and 29, however, the placebo group exhibited significantly higher endpoint titers than the Travelan group, specifically for IgG at days 15 and 29 (Fig. 3a) and IgA at day 8 (Fig. 3b). Notably, despite these differences in endpoint titer magnitude, seroconversion rates were consistent across both groups. At 15 days post-challenge, 87% of placebo and 90% of Travelan subjects seroconverted O78 IgG and seroconversion was retained at 87% for placebo and 83% for Travelan at day 29. For IgA 93% of Travelan subjects and 90% of placebo subjects seroconverted at day 15, with rates remaining at 83% for both groups at day 29 (Fig. 3c).

Inflammatory markers

The inflammatory changes induced by challenge with ETEC H10407 were assessed by measuring two key faecal biomarkers of gut inflammation; myeloperoxidase (MPO) an enzyme released during the inflammatory response to bacterial infection, and calprotectin a protein marker of intestinal inflammation[22]. At four days



c.

	Study Day	Travelan n=30	Placebo n=30
Serology -IgG	D8	21 (70%)	23 (77%)
	D15	27 (90%)	26 (87%)
	D29	25 (83%)	26 (87%)
Serology –IgA	D8	24 (80%)	27 (90%)
	D15	28 (93%)	27 (90%)
	D29	25 (83%)	25 (83%)

Fig. 3. Serum antibody titers to O78 LPS. (**a**) Endpoint titers graphic representation of IgG antibodies specific for O78 LPS, *P*-values **0.0011, ***0.0002; (**b**) Endpoint titers graphic representation of IgA antibodies specific for O78 LPS, *P*-value *0.0169. Two-way ANOVA was performed and considered significant when $P < 0.05$. (**c**) Proportion of subjects demonstrating an ETEC O78 LPS IgA or IgG seroconversion, defined as a ≥ 4-fold increase of antibody titre compared to baseline level (day -3). The number of subjects and the percentage of subjects with a response are shown for post-challenge days (D) 8, 15, and 29, grouped into Travelan and placebo seroconversion level.

post-challenge, levels of both MPO and calprotectin were significantly lower in the Travelan group compared to placebo, indicating a reduction in the level of gut inflammation (Fig. 4). This effect was transient; following antibiotic administration on days 6–8 and subsequent clearance of the challenge strain both biomarkers returned to baseline levels with no significant difference observed between the groups thereafter.

Discussion

The primary objective of this randomized double-blind placebo-controlled study was to evaluate the clinical and biological prophylactic effects of Travelan (IMM-124E) once-daily dosing following challenge with ETEC H10407. Prior clinical evidence has established that the same daily dose of 1,200 mg of Travelan administered as 3 individual doses provided up to 90% protective efficacy against ETEC infection[11]. However, Travelan's broader impact on systemic inflammation, bacterial shedding, microbiome protection and the host's capacity to mount a natural immune response had not been comprehensively characterised. We designed this study as a controlled, hypothesis-generating evaluation of Travelan prophylaxis as a single daily dose in a human challenge model, integrating clinical, microbiological and immunological endpoints.

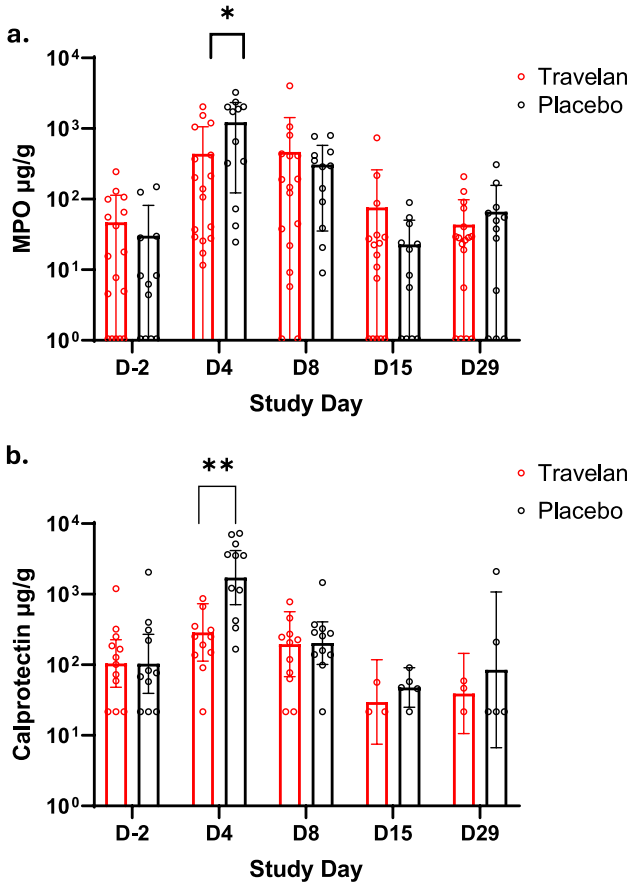


Fig. 4. Analysis of inflammatory markers in faecal samples. (**a**) Myeloperoxidase (MPO) levels in faecal samples from 30 subjects across both the Travelan and placebo groups. (**b**) Calprotectin levels in faecal samples from 24 subjects across both Travelan and placebo groups were measured at baseline, 2 days prior to ETEC challenge (D-2) and 4, 8,15, and 29 days post-challenge. *$P = 0.04$; **$P = 0.006$.

The observed safety and tolerability profile of Travelan was consistent with findings from prior published clinical studies[11] and post-market data accumulated across more than 30 million doses. During the pre-challenge period, only one subject in the Travelan arm developed minor gastrointestinal symptoms (abdominal cramps, chills, and headache) following the first dose, though not confirmed, was classified as a possible treatment-related AE. No other treatment-related AEs were recorded during the pre-challenge period.

It should be noted that, by design, AEs in this study were solicited and largely reflect symptoms induced by the challenge strain. Compared with placebo, subjects receiving Travelan reported 50% fewer total AEs and 36% fewer GI challenge-related AEs ($P = 0.0001$) indicating that Travelan reduced both the onset and severity of challenge-associated adverse events.

Travelan has previously been administered at higher doses and for longer durations in multiple clinical studies without any safety concerns. Notably, a Phase 2 randomised, double-blind, placebo-controlled study in 133 participants evaluated doses of up to 1,200 mg 3 times daily (3,600 mg/day) for 24 weeks, with no SAEs and only minor AEs reported (unpublished data; ClinicalTrials.gov: NCT02316717). To date, more than 500 clinical trial participants have received Travelan at doses up to 10.65 g/day for durations ranging from 3 days to 24 weeks in clinical studies conducted under FDA IND applications as well as and other clinical studies conducted in Europe under Good Clinical Practice, with no SAEs and very few AEs reported. The commercial product, recommended dosing three times daily, has also demonstrated safe use in the US, Canada, and Australia, with no SAEs or suspected unexpected serious adverse reactions (SUSARs) identified through Immuron's ongoing pharmacovigilance program.

In this study, Travelan demonstrated a non-significant 40% reduction in moderate-to-severe diarrhoea (primary endpoint), accompanied by non-significant reductions across multiple secondary outcomes including a 50% reduction in severe diarrhoea, elimination of intravenous rehydration requirements, a 44% reduction in diarrhoea of any severity, and a 71% reduction in early rescue antibiotic use. Although none of these differences reached statistical significance, the consistent direction of effect across several independent clinical measures suggests a reproducible trend favouring a single daily dose of Travelan prophylaxis. These findings, while preliminary, support the biological plausibility of a treatment effect, and the wide confidence intervals and limited statistical power of the study necessitate cautious interpretation and underscore the need for confirmation in larger, adequately powered trials.

Interpretation of efficacy outcomes in this study should be considered in the context of the substantially lower-than-anticipated placebo attack rate (33%), compared with the intended rate of approximately 70% derived from historical H10407 challenge studies[19]. This reduction directly limited statistical power for the primary endpoint and resulted in wide confidence intervals across clinical outcomes. In addition the data was fully inclusive of all subjects and there was no adjustment for multiplicity of the secondary and exploratory outcomes. As such, protective efficacy estimates and outcomes from this study should viewed as supportive trends rather than definitive evidence of clinical efficacy.

The reduced placebo attack rate observed in this study is most likely attributable to differences in the challenge strain and inoculum. An inoculum of 1×10^8 CFU was used here, compared with the 1×10^9 CFU dose employed in prior studies that achieved attack rates exceeding 70%[11,17,23,24]. The relationship between inoculum dose and attack rate is not, however, straightforward. Two previous CHIM studies using as few as 2×10^7 CFU of the H10407 strain achieved attack rates of approximately 70%, indicating that inoculum dose alone does not fully explain the observed variability[15,25]. Variation in H10407 challenge strain bacterial preparation methods at different facilities may also have contributed. In the present study, the challenge material was derived from frozen cGMP cell banks thawed on the day of challenge whereas earlier studies used freshly cultured organisms with conditions that may influence pathogen viability and infectivity[19]. Measures have been implemented to standardize CHIM methodologies, including efforts to minimize the challenge dose[15,26] to avoid overwhelming participants and potentially masking protective effects. These findings highlight the need for more rigorous standardization of challenge strain preparation across institutions and study designs, including careful control of cell bank derivation and culture conditions, to ensure that future CHIM studies reliably achieve their intended attack rates.

Beyond clinical outcomes, several biological findings provided additional mechanistic evidence of Travelan's activity. Although a statistically significant increase in faecal bacterial load was observed 48-h post-challenge in Travelan subjects relative to placebo exploratory microbiome analyses demonstrated a reduction in relative abundance of faecal *E. coli* (Fig. 2d) alongside a statistically significant increase in beneficial bacterial populations in Travelan-treated participants following challenge (manuscript in preparation). These findings could reflect Travelan's mechanism of action: neutralization of ETEC at the luminal surface to facilitate pathogen clearance while supporting a favorable microbial environment. This is biologically plausible given that the active component of Travelan (IMM-124E) hyperimmune bovine colostrum, contains not only anti-ETEC antibodies but also bioactive components such as lactoferrin, cytokines, and growth factors known to selectively promote beneficial microbiota and support microbial homeostasis[27,28].

Prophylactic protection conferred by Travelan did not compromise the host's ability to develop natural immunity. Serological analysis confirmed seroconversion in over 90% of Travelan subjects by day 15, with robust IgG and IgA antibody responses to lipopolysaccharide O78, an abundant challenge-strain antigen albeit significantly lower in magnitude to those observed in the placebo group at day 8 for IgA and day 15 and 29 for IgG. This pattern is consistent with findings from other ETEC challenge studies in which protection against diarrhoea correlated positively with a preserved but attenuated immune response[18,23,24]. The reduced endpoint titers among Travelan recipients could reflect diminished intestinal bacterial load resulting from pathogen clearance, which would limit the antigen exposure driving systemic immune priming. These data suggest that while Travelan attenuates ETEC colonisation at the mucosal surface, it does not substantially impair the host's capacity to mount a systemic adaptive immune response.

Importantly there were measurable reductions in downstream gut inflammation in the subjects who received Travelan. Faecal concentrations of MPO and calprotectin, established markers of GI inflammation, were significantly lower in Travelan subjects on day-4 post-challenge, highlighting the potential utility of such biomarkers and other inflammatory markers as exploratory outcome measures in future TD prophylaxis studies.

In conclusion, although this controlled human infection study was underpowered to demonstrate definitive efficacy, it revealed consistent, biologically plausible trends; indicating that once-daily administration of Travelan reduces the severity of ETEC-associated illness, attenuates intestinal inflammation, and promotes clearance of the ETEC pathogen without impairing the development of natural immunity.

Previous CHIM studies administering Travelan at a total daily dose of 1,200 mg in three divided doses demonstrated clear and statistically significant protective efficacy, ranging from 76.7% to 90.9% across dosing regimens. In contrast, the present study evaluated whether the same total daily dose delivered once daily could provide comparable prophylactic benefit. Once-daily dosing resulted in a non-significant 40% reduction in moderate-to-severe diarrhoea, alongside non-significant reductions in several secondary outcomes, including severe diarrhoea, intravenous rehydration requirements, diarrhoea of any severity, and early rescue antibiotic use. Although these trends consistently favoured Travelan, the wide confidence intervals and limited statistical power prevent conclusions regarding equivalence between once-daily and three-times-daily dosing. The current formulation delivers 200 mg of active IMM-124E per immediate-release tablet and given the practical advantages of simplified dosing for deployed military personnel and travellers, an enteric-coated capsule formulation designed for small-intestinal release is under investigation. This next generation formulation may enhance bioavailability and support a viable once-daily prophylactic strategy, but confirmation will require adequately powered clinical studies.

Collectively the clinical, safety, microbiological, and immunological findings presented here provide a strong rationale for larger, adequately powered confirmatory trials to evaluate the prophylactic effects of once-daily Travelan.

Methods

Study design and participants

The clinical trial was a randomised, double-blind and placebo-controlled CHIM with the ETEC challenge strain H10407[19]. The trial was registered on clinicaltrials.gov on 26 May 2023, ID: NCT05933525. The clinical protocol (IMM-124E-2002) followed a similar design to previous ETEC-CHIM studies for IMM-124E[11] and other similar CHIMs[17,18,21,22]. There were no major changes to the trial protocol after commencement of the study. This study was conducted under a Food and Drug Administration (FDA) Investigational New Drug (IND) Application 029087 at Pharmaron Clinical Pharmacology Center (Baltimore, MA, US).

Travelan and placebo composition was described in our previous published CHIM studies[11]; briefly Travelan is a tableted powder made from the first milking (colostrum) of cattle that have been immunized in the weeks before calving with a vaccine that contains concentrated surface antigens of human Enterotoxigenic *E. coli* (ETEC) bacteria.

Healthy volunteers between 18 and 50 years of age were recruited and screened for eligibility, for full details refer to clinicaltrials.gov ID: NCT05933525. Informed consent was received from all eligible subjects and/or their legal guardians. Consented subjects were admitted to the Pharmaron clinical research unit on study day -3 to complete the 9-day inpatient component of the study. Randomisation was performed at Pharmaron by assigning a 5-digit code to all participants that was blinded to all investigators and participants until completion of the study. Subjects were randomly assigned 1:1 to receive either 1,200 mg of Travelan or matched placebo (skim milk powder formulated into tablets to match the Travelan presentation) in a double-blinded manner, administered orally once daily 30 min before food. Dosing commenced two days prior to challenge on study day -2 and continued for a total of 7 days to study day 5. The challenge was administered on study day 1 (see "Challenge procedures" section below). Following the dosing period, all subjects received a three-day course of ciprofloxacin antibiotic (500 mg twice daily) on study days 6–9 and were discharged from the in-patient facility after two consecutive culture-negative stools. Subjects had the right to withdraw from the study at any time and if severe symptoms were met; for example rescue antibiotic treatment was initiated if subjects met pre-specified criteria for severe diarrhoea exceeding 24 h, fever, or diarrhoea accompanied by two or more severe symptoms, and prophylaxis was discontinued.

Subjects were monitored daily for vital signs and adverse events (AEs), graded according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0). All stools were collected and weighed throughout the inpatient phase, with stool consistency graded on a five-point scale: Grade 1 (fully formed), Grade 2 (soft), Grade 3 (thick liquid, unformed), Grade 4 (opaque watery, unformed), and Grade 5 (rice-water, unformed). Subjects producing Grade 3–5 stools, also defined as loose or diarrhoeal stools, were offered oral rehydration solutions matching their stool output volume and iv fluids were provided in severe cases. Safety follow-up was conducted at outpatient visits on days 15 and 29 post-challenge, with a six-month telephone follow-up to assess long-term safety. Only concomitant medications approved by the study physician were used during the study and medications provided during the study were recorded.

Challenge procedures

The challenge organism ETEC strain H10407 master cell bank (Lot #15ETEC201-02M) was prepared under Good Manufacturing Practices (GMP) by MassBiologics for human administration[19] under IND 15900. Following an overnight fast, on the day of challenge, (study day 1), the requisite number of vials were thawed, pooled, and diluted in phosphate-buffered saline to a final concentration of 1×10^8 colony-forming units (CFU) per mL; the final concentration was confirmed by identity testing and CFU plate count. Subjects consumed 120 mL of sodium bicarbonate buffer to neutralize gastric acidity, then ingested the challenge preparation suspended in 30 mL of sodium bicarbonate buffer.

Safety and efficacy measurements

Subjects who received the challenge were included in the ITT and all subjects who received treatment (Travelan or placebo) irrespective of the number of doses or receipt of the challenge were included in the safety analysis set. Assessment of Travelan product safety was limited to the 2 days prior to receipt of the challenge dose (study days -2 and -1). Treatment-related AEs were classified as definite, probable, possible, or unrelated. Unless AEs were temporally related to receipt of Travelan, post-inoculation solicited AEs were generally attributed to the inoculum ETEC H10407 challenge strain. All AEs were assessed for severity by the investigator using the CTCAE v5.0 grading scale. Diarrhoeal outcomes were assessed over the 5-day post-challenge period (study days 1–5), prior to scheduled antibiotic administration on day 6.

The primary efficacy endpoint was the incidence of moderate-to-severe diarrhoea, defined as ≥ 4 Grade 3–5 stools or ≥ 401 g (g) of Grade 3–5 stool output in any 24-h period within 5 days of challenge. Secondary endpoint measurements included incidence of severe diarrhoea (> 6 Grade 3–5 stools or > 800 g in any 24-h period), any diarrhoea (includes mild diarrhoea of > 2 Grade 3–5 stools), the maximum number of diarrhoeal or loose stools in any 24-h period, the total number and weight of Grade 3–5 stools, and the number of subjects with moderate to severe symptoms of fever, nausea, vomiting, anorexia, abdominal pain or discomfort. ETEC disease severity scores were calculated according to previously defined criteria[21].

Challenge strain shedding was quantified by CFU count from a stool sample collected approximately 48 h post-challenge. The number of CFUs was determined, by preparing 1g of stool resuspended in 10 ml of PBS and preparing a 10-fold dilution series cultured in duplicate using standardised previously established methods for H10407[28] For measurements of relative abundance, briefly the total DNA was extracted from faecal samples using the QIAGEN DNeasy PowerSoil Pro Kit according to the manufacturer's protocol and subjected to 16s rRNA gene sequencing and analysis as previously described[29]. The abundance of *Escherichia coli-Shigella* genus

in the entire genomic library in relation to the total genome was quantitated as previously described[29] and defined as relative abundance.

Immunological analysis

Venous blood was collected for immunological analysis on study days -3, 2, 4, 8, 15, and 29. Serum was separated, aliquoted, and frozen until analysis. Systemic antibody responses to the ETEC H10407 challenge strain were assessed by enzyme-linked immunosorbent assay (ELISA), measuring IgG and IgA reactivity to the O78 lipopolysaccharide antigen, as previously described[13]. Seroconversion was defined as a ≥ 4-fold rise in endpoint antibody titres relative to baseline.

To assess the levels of acute inflammation in the gut biomarkers myeloperoxidase (MPO) and calprotectin[30] were measured in a sub-set of faecal samples. To minimize the risk of bias, samples were analysed from an even population of subjects per group who had no diarrhoea, mild, moderate and severe diarrhoea collected on study days -2, 4, 8, 15, and 29 analysed using commercial ELISA kits (Epitope Diagnostics) according to the manufacturer's recommended procedure.

Statistical analysis

Analyses were performed on the ITT population. Proportions were evaluated using chi-square analysis or Fisher's 2-sided exact tests, while non-parametric data were analyzed using Wilcoxon rank-sum tests. Confidence intervals (CI) were estimated under the assumption that the number of diarrhoea cases in each treatment group followed a Poisson distribution, with each group having its own mean. Under these assumptions, the lower and upper bounds of a 95% exact confidence interval for protective efficacy (PE) were based on the Beta distribution. The sample size of 30 subjects per arm was determined based on a prior H10407 ETEC challenge study reporting an approximate 70% diarrhoeal attack rate[19]. For the primary endpoint, Fisher's exact test with a two-sided alpha = 0.05 assumed an approximate 80% power to detect a significant difference in the incidence of moderate-to-severe ETEC-attributable diarrhoea between groups, assuming an attack rate of 70% in the placebo group. All subjects who received the challenge were included in outcome measurements inclusive of those who had received early antibiotics and there was no adjustment for multiplicity of secondary endpoints. More details of the statistical analysis plan can be found at clinicaltrials.gov ID: NCT05933525.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Author contributions

All authors contributed to the design of the clinical protocol; RML, FP, DI contributed to immunological analyses; CKP, SDI, KT, JLC, JK and VC contributed to ethics applications; AS and AEF performed the microbiome analysis; ST and WHC contributed to the challenge strain management and microbiology; KL project managed the clinical study; MAI was the clinical PI supported by clinician CB; JK was the sponsor PI responsible for the entire project and MTEC grant PI awardee; JLC wrote the manuscript and all authors reviewed, the revised versions. Some of the authors are federal or contracted employees of the United States Government. This work was prepared as part of their official duties. Title 17 U.S.C. 105 provides that 'copyright protection under this title is not available for any work of the United States Government'. Title 17 U.S.C. 101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The views expressed in this article reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., the Department of the Navy, the Department of the Army, the Department of Defense, or the United States Government.

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Declarations

Competing interests

JK and JLC are employees and shareholders of Immuron Limited. All other authors declare no competing interests.

Ethical approval

This clinical study was ethically-approved by the Advarra Institutional Review Board. The study was performed in accordance with the relevant guidelines and regulations in compliance with all federal regulations by the US Department of Defense Office of Human Research Oversight governing the protection of human participants.

Additional information

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