Assessing safety and efficacy of a novel glucose-free amino acid oral rehydration solution for watery diarrhea management in children: a randomized, controlled, phase III trial

Pradip Kumar Bardhan,[a,e] Rina Das,[a,b,e] Baitun Nahar,[a] Md. Ahshanul Haque,[a] Rukaeya Amin Sobi,[a] Al-Afroza Sultana,[a] Mustafa Mahfuz,[a] Neil Fawkes,[c,*] Adam B. Smith,[c] Sadasivan Vidyasagar,[c,d] Olivier Fontaine,[c] and Tahmeed Ahmed[a]

[a]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka 1212, Bangladesh
[b]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta GA-30322, USA
[c]Entrinsic Bioscience, LLC., Norwood, MA, USA
[d]Department of Radiation Oncology, University of Florida Shands Cancer Center, Gainesville, FL, USA

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Summary

Background Diarrhoeal disease poses a significant global health challenge, especially in children under three years old. Despite the effectiveness of oral rehydration therapy (ORT), its adoption remains low. Glucose-based ORS (GORS) is the standard, but novel formulations like glucose-free amino acid-based VS002A have emerged as potential alternatives. This study aimed to compare the safety and efficacy of VS002A against the standard WHO-ORS in treating non-cholera acute watery diarrhoea in children.

Methods A triple-blind, randomized trial enrolled 310 male infants and children aged 6–36 months, who were assigned to receive WHO-ORS or VS002A over a 16-month period, from June 2021 to September 2022. Both groups received standard of care, including zinc supplementation. The Primary study outcome measured was the duration of diarrhoea. Secondary outcomes included stool output, treatment failure and adverse events. Exploratory endpoints included urinary output, body weight changes, blood biochemistry, stool microbiology and gut health biomarkers.

Findings Both VS002A and WHO-ORS were well-tolerated with a low adverse event rate. While not different statistically ($p = 0.10$), duration of diarrhoea was shorter in children treated with VS002A vs. WHO-ORS (65.4 h vs. 72.6 h). Similarly, stool output was also lower vs. WHO-ORS in children treated with VS002A, though not statistically different ($p = 0.40$). Serum citrulline levels, an indicator of gut health, were higher in the VS002A group at 24 h suggesting a potential protective effect ($p = 0.06$).

Interpretation The findings of this study support the non-inferiority of VS002A, a glucose-free amino acid-based ORS compared to the WHO-ORS standard of care. VS002A was shown to be safe and effective in treating non-cholera acute watery diarrhoea in young children. VS002A may offer advantages in pathogen-driven diarrhoea, supported by trends toward a lower duration of diarrhoea and stool output within the per protocol group. Furthermore, individuals with prolonged diarrhoea, severe malnutrition, environmental enteric dysfunction or have issues with obesity or insulin resistance, could benefit from a glucose-free ORS. This research contributes to addressing the persistent challenge of childhood diarrhoea by presenting an alternative glucose-free ORS formulation with potential advantages in select scenarios, offering a promising avenue for improving paediatric diarrhoea management worldwide.

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Keywords: VS002A; WHO-ORS; Dehydration

*Corresponding author.
E-mail address: nfawkes@amilyfe.com (N. Fawkes).
[e]Contributed equally.

Research in context

Evidence before this study

Before this investigation, diarrhoeal disease was recognized as a leading cause of morbidity and mortality in children under the age of five, particularly in low- and middle-income countries, imposing significant healthcare burdens worldwide. Oral rehydration therapy (ORT), endorsed by global health organizations such as WHO, UNICEF, and USAID, has been the cornerstone of fluid replacement since 1978. Despite ORT's proven efficacy and the advocacy for glucose-based oral rehydration solutions (GORS) with reduced osmolarity, surveys have shown that less than 33% of children with diarrhoea received ORT since the year 2000. In developed countries, intravenous therapy (IVT) has remained the preferred method for various reasons, including healthcare professional preferences and a notable lack of education among patients, caregivers, and healthcare professionals regarding ORT solutions. Our PubMed search, employing the string: ("diarrhoeal diseases" [Title/Abstract] OR "diarrhea" [MeSH Terms]) AND ("children" [Title/Abstract] OR "infants" [Title/Abstract]) AND ("oral rehydration therapy" [Title/Abstract] OR "ORT" [Title/Abstract] OR "oral rehydration solutions" [Title/Abstract] OR "ORS" [Title/Abstract]) AND ("low-income countries" [Title/Abstract] OR "middle-income countries" [Title/Abstract] OR "developing countries" [MeSH Terms]) AND ("morbidity" [Title/Abstract] OR "mortality" [Title/Abstract]) AND ("glucose-based" [Title/Abstract] OR "amino acid-based" [Title/Abstract] OR "glucose-free" [Title/Abstract]) AND ("efficacy" [Title/Abstract] OR "safety" [Title/Abstract] OR "use" [Title/Abstract]), was utilized to amass the pre-existing evidence base for our study.

Added value of this study

This study introduces a novel glucose-free, amino acid and electrolyte formulation, VS002A, for oral rehydration, addressing the need for innovative solutions within the ORS domain. Our research not only assesses the safety and efficacy of VS002A against the WHO-ORS formula with reduced osmolarity in a paediatric cohort but also evaluates its potential advantages over traditional ORS. Introducing a zero-sugar ORS offers an alternative therapeutic option for managing diarrhoeal disease in children globally, potentially overcoming the obstacles impeding appropriate ORT uptake worldwide. Specifically, VS002A represents a promising alternative for special patient populations, such as those with glucose intolerance, diabetes, or a preference for a low-sugar or differently flavored ORS.

Implications of all the available evidence

The insights derived from this study, alongside the existing corpus of evidence on ORT and diarrhoeal disease management, emphasize the critical need for ongoing innovation and education in rehydration therapy. The debut of VS002A aims to challenge and potentially overcome barriers to ORT adoption, providing a safe and effective alternative treatment that could be widely implemented in diverse healthcare settings, including home care. Moreover, this research suggests significant implications, proposing that a glucose-free ORS could play an essential role not only in low- and middle-income countries but also in scenarios where obesity and insulin resistance are prevalent concerns. Adopting a comprehensive approach to address diarrhoeal diseases with innovative treatments like VS002A could markedly influence public health strategies, clinical guidelines, and patient care practices on a global scale.

Introduction

Diarrhoeal disease remains a significant cause of morbidity and mortality in children under the age of five, particularly in low-and middle-income countries.[1] In 2015 alone, there were approximately 688 million cases of morbidity and 499,000 deaths attributed to diarrhoeal disease, accounting for around 12.5% of all deaths in this age group globally.[2] Developed countries also face a considerable burden, with healthcare costs exceeding two billion dollars annually in the United States for hospital and outpatient care.[3]

Oral rehydration therapy (ORT) has been the standard fluid replacement since 1978, supported by global health organizations like WHO, UNICEF, and USAID.[4–6] The WHO recommended glucose-based oral rehydration solution (GORS) with reduced osmolarity (250 mOsm/L or less) is considered the gold standard for managing mild-to-moderate watery diarrhoea in children.[7] This solution leverages the co-transport of glucose and sodium across the small intestine, countering fluid losses and electrolyte imbalances.[8,9]

Despite its proven effectiveness, GORS usage has not increased accordingly. Since 2000, surveys suggest less than 33% of children under age five with diarrhoea receive ORT.[10] In developed countries healthcare professionals also prefer Intravenous therapy (IVT).[11] IVT, while rapidly correcting fluid deficits, has drawbacks such as cost, specialized staff requirements, and potential complications, such as electrolyte imbalances and infections.[12,13] In contrast, ORT offers several advantages supporting its wider adoption. Oral administration is non-invasive and less traumatic, especially for young children, and can be conveniently administered by caregivers in various settings, including at home and primary care centers.[13] Additionally, ORT is cost-effective and associated with lower hospital admission rates and shorter stays.[11] However, barriers hinder ORT's broader use. A lack of education for patients, caregivers, and healthcare professionals contributes to

confusion between available ORT solutions and other types of drinks.[14,15] Furthermore, changes in consumer and caregiver preferences, such as taste, affect ORT acceptance and result in products with more sugar and less salt than desired for deficit therapy.[14]

Given the need for innovative solutions and considering recent economic events affecting global supply chains for both glucose-based ORS, exploring alternatives becomes crucial and has reignited interest in ORT.[16] A novel glucose-free amino acid and electrolyte formulation, VS002A, has been developed, based on evidence demonstrating the effective/successful use of amino-based ORT in cancer patients. Pre-clinical experiments have demonstrated its potential for ORT with enhanced sodium carrying capacity, reduced anion secretion, improved mucosal barrier function, and intestinal stem cell proliferation.[17]

The present study sought to evaluate the safety and efficacy of the VS002A oral rehydration solution compared to the reduced osmolarity WHO-ORS formula in infants and young children with non-cholera acute watery diarrhoea. It was hypothesized that VS002A would prove to be a safe and effective alternative in reducing the duration of infectious, non-cholera diarrhoea within a paediatric population. This investigation holds the promise of offering improved or alternative treatment options to combat the persistent burden of diarrhoeal disease in children worldwide. In addition, a glucose-free ORS also has an application in cases where children have persistent diarrhoea, which has been shown to be associated with glucose intolerance. Furthermore, a glucose-free ORS has great utility outside of the developing world, where obesity and insulin resistance are a growing issue.[18]

Methods

Trial design

The trial design and methods have been previously described.[19] This clinical trial followed a prospective, randomized, triple-blind, two-cell, parallel-arm design to compare the effectiveness of two oral rehydration solutions (ORS)—WHO-ORS and VS002A—in male infants and children aged six to 36 months presenting with acute, non-bloody, non-cholera diarrhoea. The study was conducted as a single-centre trial at the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The choice of male infants was made to facilitate the separation and collection of urine and stool during the study. The trial implemented a two-arm, four cell approach to protect the blinding of the interventions, and participants were randomly allocated in a 1:1:1:1 fashion to receive either WHO-ORS or VS002A.

Study participants

To be eligible for enrolment in the trial, male infants and children aged six to 36 months had to meet certain criteria: participants were required to have a history of diarrhoea with an onset of no more than 48 h; show signs of some dehydration (confirmed by the study physician using the "Dhaka method"), and have written informed consent provided by their parent or guardian.[20] Participants were excluded if they met any of the following criteria: severe malnutrition with weight-for-length, weight-for-height, or weight-for-age Z-scores < -3 and presence of nutritional oedema; diarrhoea caused by cholera, screened using the Cholkit™ test; presence of systemic illnesses including pneumonia, tuberculosis, enteric fever, or meningitis; bloody diarrhoea; known congenital anomalies or disorders such as diagnosed inborn errors of metabolism, congenital cardiac disease, seizure disorders, hypothyroidism, or Down syndrome; required additional intravenous fluids after being provided with an IV for 4 h upon admission; or had been treated with antibiotics and/or anti-diarrhoeal medicines within 48 h before hospitalization.

Interventions

As part of the standard emergency care followed at the Dhaka Hospital of icddr,b, participants initially received standard WHO-ORS for rehydration, with a dosage of 50–75 ml/kg administered over the first 4 h of admission. During this time, cholera cases were identified, and screening activities were conducted. Once enrolled, participants were randomly allocated into one of the two ORS groups (155 children in each group) according to a pre-determined randomization schedule at icddr,b. Both groups of children received standard care for diarrhoeal disease, which included rehydration, supplemental zinc (20 mg per day for 10–14 days), nutritional counselling, follow-up, and guidance on when to return, as per the WHO guidelines. The composition of the two ORS formulations is presented in Supplementary Table S1.

The initial treatment dosing with either WHO-ORS or VS002A was estimated based on body weight, with additional rehydration administered following five to ten ml/kg after each loose stool, in accordance with icddr,b guidelines. Regular assessments by study staff were carried out to make any necessary adjustments to hydration volumes based on measured losses. Additionally, every child was supplemented with zinc and continued to receive appropriate liquid, semi-solid, and solid foods suitable for their age. Breast-fed children also continued to receive breast milk. If stool culture revealed any pathogen requiring antibiotic treatment, these patients were excluded from the study and treated accordingly. Participants enrolled in the trial received follow-up for up to 120 h post-enrolment or until diarrhoea resolution. For those patients whose diarrhoea continued beyond 120 h, appropriate management was provided, however time point was considered the end of the study.

Outcomes

The primary outcome measure of the study was the duration of diarrhoea while the participants were in the hospital. This endpoint allowed the assessment of the effectiveness of the two ORS formulations in reducing the duration of diarrhoea. Secondary outcomes included stool output volumes, treatment failure and adverse events. Exploratory endpoints included urinary output, body weight changes, blood biochemistry, stool microbiology and gut health biomarkers.

Stool output, which was measured in grams per kilogram of body weight, was analysed in the following intervals; the first 12 h, second 12 h, first 24 h, and the total period of the study. Intake of rehydration fluid was recorded every 4 h, and any use of unscheduled intravenous fluids was also documented. The study aimed to investigate the association between these outcomes and the type of ORS received (VS002A or WHO-ORS), while adjusting for relevant covariates. Urine outputs were collected continuously, and changes in body weight between pre-randomization and post-treatment were also captured.

Stool collection and microbiology

To exclude *Vibrio cholerae*, a single, fresh stool specimen was collected from all enrolled patients at the time of enrolment and immediately assessed via the Cholkit™ test. All stool samples were routinely screened for common enteric pathogens, including bacterial pathogens (*Salmonella, Shigella, V. cholerae, Campylobacter,* and various strains of *Escherichia coli*), rotaviruses antigen, and protozoa (*Giardia intestinalis, Entamoeba histolytica*, and *Cryptosporidium* spp.) using standard laboratory methods.

Laboratory investigations

Upon admission to the trial, blood was taken from all participants and was analysed for serum electrolytes, glucose, and complete blood count. Participant stool was examined for bacterial culture and Rota viral antigen testing. These tests were performed to confirm the diagnosis of acute, non-bloody, non-cholera diarrhoea and to identify any potential pathogens responsible for the diarrhoea. In addition, gut health biomarkers, such as plasma citrulline and kynurenine-tryptophan ratio, were analysed in a subset of the first 100 enrolled children to assess changes associated with environmental enteric dysfunction. These biomarkers were measured using the enzyme-linked immunosorbent assay (ELISA) method and liquid chromatography/quadrupole time-of-flight mass spectrometry (Q-TOF LC-MS), respectively.

Sample size

The total sample size was 312 male children. In previous non-cholera studies of children comparing an anti-diarrhoeal to placebo, the effect sizes for the hourly duration of diarrhoea ranged from 0.3 to 1.0 (duration, h).[21–24] Outcome improvements ranged from 15 to 45% with treatment (vs. placebo). A clinically significant delta of 20% produced effect sizes of 0.4 to 0.7 (duration, h) using the pooled standard deviations[25,26] When applied to conventional Type-1 error (0.05) and 80% power, between 32 and 174 patients per group are required for statistical significance. The maximum anticipated accrual rate of three patients per week over 24 months results in 132 patients per group and then assuming a 15% attrition increases the sample to 156 patients per group. Therefore, the final anticipated patient group numbers are estimated to provide 68% power to detect a 20% difference in hourly diarrhoea duration. To assess if the products could be considered therapeutically equivalent, non-inferiority was likewise considered using a predefined non-inferiority delta of 20% of the reference product.[27] The study was underpowered for secondary outcomes such as stool volume, as such these outcomes were classified as exploratory outcomes.

Randomization and blinding

Eligible subjects were individually randomised into VS002A and WHO-ORS groups using a variable permuted four block procedure and a predefined allocation table. Opaque sequentially numbered envelopes containing treatment allocation were opened by the study physician on participant enrolment.

The interventions were packaged in indistinguishable Tetra Pak® cartons, except for randomized manufacturer serial numbers. The identity of the specific product was blinded to subjects, hospital staff, sponsor, and investigators due to similar colour, smell, taste, and citrus flavour.

Quality assurance

The trial adhered to good clinical practice (GCP) standards and compliance was recorded through regular data monitoring and quality checks by an independent clinical research organisation.

Statistical analysis

Data were entered using ClaimIT database software (ObvioHealth) with validation rules to prevent inconsistencies. Regular monitoring activities were performed, including data editing, range checking, and duplication checking. After completing data entry, data were transferred to Stata (Release 14. College Station, Texas 77845, USA: StataCorp LP) software to check for normality assumptions (Shapiro–Wilk test). Summary statistics such as frequency, proportion, mean, standard deviation, median, and interquartile range were used to describe the data, and outcomes and covariates were separated by study group.

For continuous variables, an independent samples t-test was used to test the mean difference between two groups of data if symmetric, otherwise, Wilcoxon rank sum test as the non-parametric test was used to compare between two median values. For categorical variables, the

Chi-squared test was used to assess the bi-variate relationship between outcomes as well as between several types of indicators and study groups. The mean difference of total stool output in the first 24 h, stool output between hours (1–12), stool output between hours (13–24), and total stool output for the study duration were also compared between the intervention and control group. Given, the absence of a normal distribution for the primary outcome measure (total stool output), the differences between the geometric means with 95% confidence intervals were calculated using the R-package emmeans (R-Studio) to estimate non-inferiority according to ICH E9 guidelines; briefly, the results were regarded as non-inferior if the 95% confidence interval of the difference fell entirely to the left of the threshold value Δ.[27] The results were regarded as statistically significant at $p < 0.05$.

Ethical conduct and institutional review board statement

The trial was approved by the Research Review Committee and Ethical Review Committee of icddr,b (DGDA/CTP-1/06/2016/56; PR-17028) and was registered on ClinicalTrials.gov under the identifiers NCT04677296 and NCT06179589. The identifier 'NCT06179589' was erroneously created as a duplicate entry within the system, however, does not reflect any change to the original study design or its intended outcomes. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with the International Council for Harmonization Good Clinical Practice (ICH GCP) guidelines and local regulatory requirements.

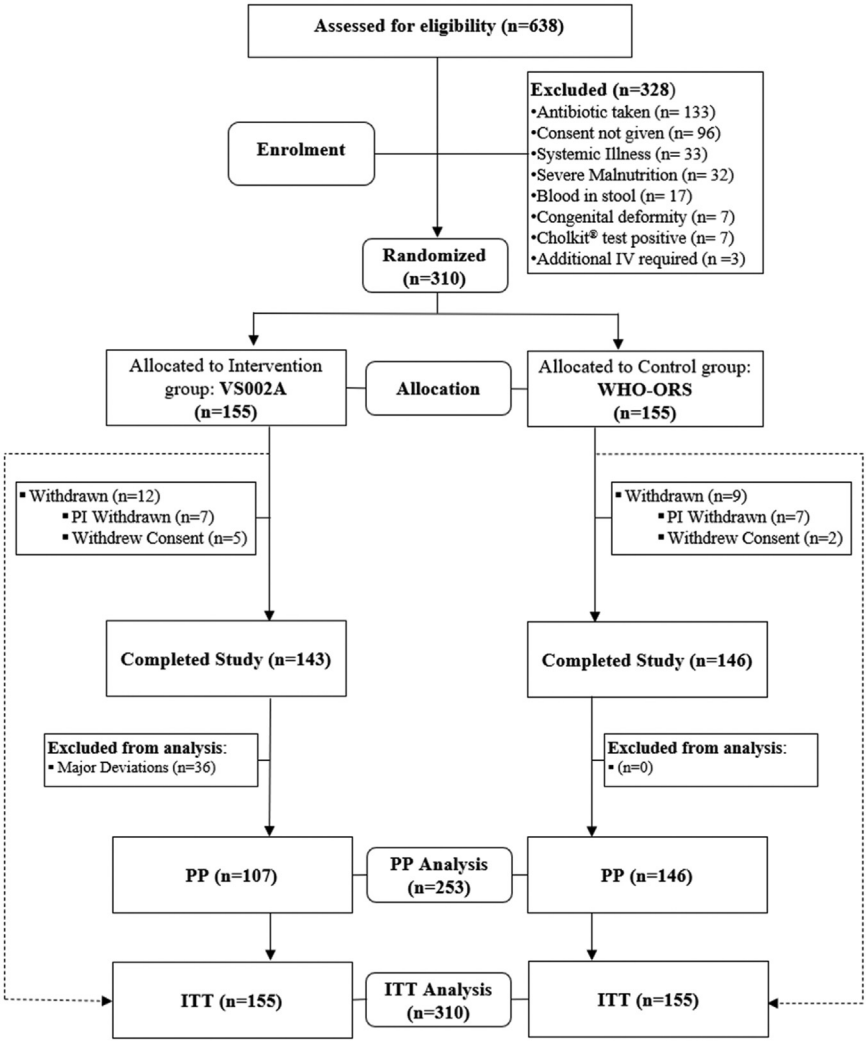

Fig. 1: Study flow diagram.

Characteristic	VS002A, (N = 155)[a]	WHO-ORS (N = 155)[a]
Age (months)	11.3 (4.6)	11.8 (4.3)
WAZ	−0.9 (1.0)	−1.1 (1.0)
WLZ	−0.7 (1.1)	−0.8 (1.0)
LAZ	−0.7 (1.1)	−0.8 (1.1)
Duration of diarrhoea (hours)	22.9 (11.0)	24.8 (10.7)
Fever		
No	117/155 (75.0%)	110/155 (71.0%)
Yes	38/155 (25.0%)	45/155 (29.0%)
Cough		
No	143/155 (92.0%)	145/155 (94.0%)
Yes	12/155 (7.7%)	10/155 (6.5%)
Vomiting		
No	22/155 (14.0%)	14/155 (9.0%)
Yes	133/155 (86.0%)	141/155 (91.0%)
Pulse rate (per minute)	108.6 (4.2)	108.5 (4.4)
Respiration rate (per minute)		
28	1/155 (0.6%)	0/155 (0.0%)
29	1/155 (0.6%)	0/155 (0.0%)
30	14/155 (9.0%)	20/155 (13.0%)
32	40/155 (26.0%)	32/155 (21.0%)
34	48/155 (31.0%)	56/155 (36.0%)
36	43/155 (28.0%)	40/155 (26.0%)
38	7/155 (4.5%)	6/155 (3.9%)
99	1/155 (0.6%)	1/155 (0.6%)
Axillary temperature (0 °C)	36.8 (5.1)	36.8 (5.1)
Breastfeeding		
No	30/155 (19.0%)	29/155 (19.0%)
Yes	125/155 (81.0%)	126/155 (81.0%)
Vaccination status		
Not vaccinated	0/155 (0.0%)	2/155 (1.3%)
Partially vaccinated	9/155 (5.8%)	11/155 (7.1%)
Vaccinated	146/155 (94.0%)	142/155 (92.0%)
Father's occupation		
Business	29/155 (19.0%)	30/155 (19.0%)
Farmer	3/155 (1.9%)	5/155 (3.2%)
Labour	6/155 (3.9%)	6/155 (3.9%)
Not applicable	1/155 (0.6%)	1/155 (0.6%)
Other	50/155 (32.0%)	54/155 (35.0%)
Rickshaw puller	4/155 (2.6%)	7/155 (4.5%)
Service	62/155 (40.0%)	51/155 (33.0%)
Unemployed	0/155 (0.0%)	1/155 (0.6%)
Mother's occupation		
Garments worker	8/155 (5.2%)	6/155 (3.9%)
Housewife	138/155 (89.0%)	138/155 (89.0%)
Labour	1/155 (0.6%)	2/155 (1.3%)
Not applicable	0/155 (0.0%)	1/155 (0.6%)
Other	3/155 (1.9%)	4/155 (2.6%)
Service	5/155 (3.2%)	4/155 (2.6%)
Family income (BDT per month)	21,262.6 (12,921.0)	21,909.7 (12,983.8)
No of members in household	4.7 (1.6)	4.9 (1.6)
No of under-five children in the family		
0	127/155 (82.0%)	125/155 (81.0%)
1	26/155 (17.0%)	26/155 (17.0%)
2	2/155 (1.3%)	4/155 (2.6%)

WAZ: weight-for age, HAZ: height-for-age, WHZ: weight-for-height; WASH: water, sanitation, and hygiene. [a]Mean (SD); n/N (%).

Table 1: **Baseline characteristics of study population (ITT).**

Characteristic	VS002A, N = 107[a]	95% CI[b]	WHO-ORS, N = 146[a]	95% CI[b]	p-value[c]
Age (months)	11.70 (4.90)	11.00, 13.00	11.70 (4.30)	11.00, 12.00	0.60
Pulse rate	107.80, (4.00)	107.00, 109.00	108.60 (4.10)	108.00, 109.00	0.10
Axillary temperature	37.00 (6.10)	36.00, 38.00	36.80 (5.30)	36.00, 38.00	0.80
Weight (kg)	8.90 (1.30)	8.60, 9.10	8.60 (1.20)	8.40, 8.80	0.08
Duration of diarrhoea before admission (h)	22.00 (10.10)	20.00, 24.00	25.20 (10.70)	23.00, 27.00	0.03
Watery stools before admission	18.00 (7.00)	17.00, 19.00	19.10 (7.10)	18.00, 20.00	0.40
Vomiting episodes before admission	5.70 (4.30)	4.90, 6.50	5.60 (3.50)	5.00, 6.20	0.90

[a]Mean (SD); n (%). [b]CI = Confidence Interval. [c]Wilcoxon rank sum test.

Table 2: **Demographics table—per protocol sample.**

Role of the funding source

Funding for this study was provided by entrinsic bioscience LLC, Boston MA. The funders are employees of entrinsic bioscience LLC., but had no role in study design, conduct of the study, data collection or management of the study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Enrolment was conducted over a 16-month period, from June 2021 to September 2022, involving a total of 638 male infants and children aged six to 36 months with diarrhoea and some dehydration, who underwent screening at icddr,b. Out of these, 310 participants were eligible for randomization, with 155 assigned to the intervention group (receiving VS002A) and 155 assigned to the reference group (receiving WHO-ORS) (Fig. 1).

Baseline characteristics of the enrolled children were similar between the two groups (Table 1, and Supplementary Table S2). The mean age in the WHO-ORS group was 11.8 ± 4.3 months, while in the VS002A group, it was 11.3 ± 4.6 months. Feeding habits, vaccination status, parental education, family income, and W.A.S.H. (Water, Sanitation, and Hygiene) practices were comparable between the treatment groups. Fig. 1 describes patient withdrawals and exclusions leading to smaller per protocol group numbers (n = 253). Faecal pathogens were identified in 240 of the study participants, with rotavirus being the most common faecal pathogen detected (n = 206).

Table 3 presents the primary outcome, total duration of diarrhoea, and exploratory analysis of stool output.

Outcome variable	ITT analysis (N = 310)				Per-protocol analysis (N = 253)			
	VS002A geometric mean (95% CI) n = 141[a]	WHO-Ors Geometric Mean (95% CI) n = 145[a]	Geometric Mean Difference (95% CI)	p- value	VS002A geometric mean (95% CI) n = 107	WHO-ORS geometric mean (95% CI) n = 146	Geometric mean difference (95% CI)	p-value
Primary outcome								
Duration of diarrhea (Hours)	40.30 (35.00, 46.40)	42.50 (36.90, 48.90)	2.20 (−10.40, 6.00)	0.50	65.40 (59.50, 71.90)	72.60 (67.00, 78.70)	−7.26 (−15.80, 1.30)	0.10

[a]Patients with values of zero were excluded from the analysis.

Table 3: **Study outcomes—duration of diarrhea.**

Outcome variable	ITT analysis (N = 310)				Per-protocol analysis (N = 253)			
	VS002A mean (95% CI) n = 155	WHO-ORS mean (95% CI) n = 155	Geometric mean difference (95% CI)	p- value	VS002A mean (95% CI) n = 107	WHO-ors Mean (95% CI) n = 146	Geometric mean difference (95% CI)	p-value
Exploratory outcomes								
Stool output (g/kg body wt.)[a]								
1st 24 h	64.3 (58.0, 71.3)	67.3 (59.8, 75.8)	–	0.3	65.4 (57.4, 74.5)	66.9 (59.2, 75.7)	–	0.6
Total stool output[b]	121.0 (103.0, 142.0)	130.0 (110.0, 155.0)	−9.5 (38.9, 19.4)	0.4	119.7 (98.2, 146.0)	131.6 (110.0, 157.0)	−11.9 (45.4, 21.6)	0.4

[a]Measurement recorded after randomization into the study. [b]Geometric mean.

Table 4: **Study outcomes—stool output.**

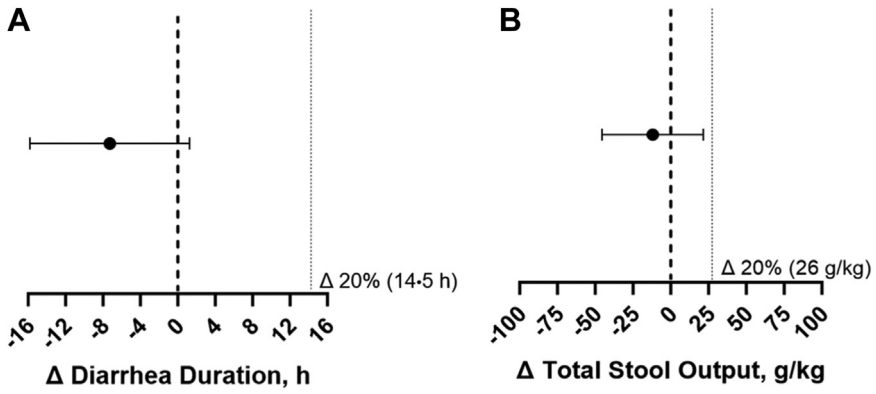

Fig. 2: **Non-inferiority (Per protocol population).** A: Duration of diarrhoea (Error bar: 95% CI's). B: Total stool output (Error bar: 95% CI's).

A heterogeneity test of the per protocol groups (Table 2, and Supplementary Table S2) revealed a significant difference in the duration of diarrhoea before hospital admission (VS002A = 22.0 h, WHO-ORS = 25.2 h; p = 0.028). Pre-and post-admission values were summed for analytical comparison. When compared to the study reference product (WHO-ORS), VS002A showed a reduction in the duration of diarrhoea (geometric mean 65.4 h vs. 72.6 h) within the per-protocol population. However, this reduction did not reach statistical significance (p = 0.1).

Table 4, compares stool output on a g/kg basis in children receiving VS002A, which also demonstrated marginal numerical improvements over WHO-ORS (geometric mean difference of –11.9 g/kg body weight (95% CI -45.4, 21.6)), (p = 0.4). Again, while not statistically significant, the non-inferiority criteria were met (Fig. 2). There were no notable differences in total urinary output between the VS002A group and WHO-ORS group (median values: 685 ml vs. 670 ml, p = 0.9) or in body weight changes (median values: 0.0 kg vs. 0.1 kg, p = 0.9).

Levels of serum citrulline, a gut-health biomarker, were shown to be preserved in the VS002A ORS group compared to the WHO-ORS group at 24 h (p = 0.059) (Supplementary Table S3). However, no notable differences were observed in plasma KT ratios.

Table 5 presented in the supplementary section shows a summary of the biochemical marker changes from baseline. Blood biochemistry parameters were comparable at baseline in both the intervention and control groups. Mean serum sodium levels at randomization and after 24 h were at the lower limits of the normal range, with only two children presenting with serum sodium <125 mmol/L, neither of whom were symptomatic for hyponatremia. There was no significant difference in the frequency of biochemical hyponatremia between the two treatment groups. The serum sodium levels decreased slightly after 24 h compared to levels at randomization for both groups (range of 1–3 mmol/L), which were not considered clinically relevant by the study investigators. The VS002A group had small decreases in serum chloride and TCO_2 levels compared to the WHO-ORS group upon study completion, however these levels were also not considered clinically significant.

VS002A and WHO-ORS were well-tolerated in the study, with both products having a demonstrably low failure rate (Table 5). A total of five adverse events were recorded during the study observation period, none of which were considered related to any of the investigational or reference products used within the study.

Discussion

This study aimed to investigate the safety and efficacy of a glucose-free amino acid-based ORS (VS002A), in reducing the duration of infectious, non-cholera diarrhoea in paediatric patients. Additionally, the

Treatment Group	Patient ID	Reason for failure
VS002A	138-495-4311	Unscheduled IV (persistent vomiting, hypokalaemia, IV correction needed)
VS002A	315-879-7571	Diarrhea continuing > 120 h
VS002A	590-613-9604	Diarrhea continuing > 120 h
VS002A	957-249-7773	Unscheduled IV (persistent vomiting, IV correction needed)
WHO-ORS	494-101-3787	Diarrhea continuing > 120 h
WHO-ORS	554-681-9824	Unscheduled IV (persistent vomiting, IV correction needed)

Table 5: **Treatment failure rates.**

performance of the amino acid-based ORS was compared to the current standard of care, WHO-ORS. The study hypothesis was based on previous observations that glucose stimulates calcium-activated chloride secretion in small intestine cells and that amino acid-based ORS may enhance intestinal epithelial proliferation and mucosal integrity, promoting nutrient absorption and intestinal health.[17,28]

This study is among the first randomized controlled trials in children to compare a non-glucose amino acid-based alternative ORS formulation to the low osmolarity WHO-ORS formula. In this randomized controlled trial of young children with acute non-cholera watery diarrhoea, VS002A proved to be safe and effective in correcting ongoing fluid and electrolyte loss. Furthermore, treatment with VS002A was found to be non-inferior to the WHO-ORS formula. In non-cholera settings, reducing the osmolality of ORS and the addition of zinc has had a positive impact on stool output and duration of illness endpoints. In this study, all participants received zinc supplementation as part of the standard care. Although treatment effects on stool output and duration of illness did not show statistically significant differences, they tended to favour the VS002A formulation. One concern with the use of a non-glucose, amino acid-based ORS was development of hypoglycaemia, however this was not observed, even in children who vomited in association with diarrhoea.

Previous studies on amino acid supplementation in childhood diarrhoea have used various amino acids combined with glucose-containing ORS, yielding mixed results.[25] Thus, direct comparisons with the present study's results are challenging. One study comparing glutamine-based glucose-free ORS with glucose-containing WHO-ORS only reported stool outputs for the time required for rehydration, limiting its applicability. The study also examined serum citrulline levels, as an exploratory biomarker of intestinal injury and repair, which indicates enterocyte integrity and absorptive capacity in small intestinal pathological conditions. The findings showed that 24 h after admission, children receiving VS002A had higher serum citrulline levels compared to those receiving WHO-ORS. The results were not statistically significant, however due to cost restraints, this biomarker was only performed in a subgroup of the study population and the results remain of clinical interest.

The study's potential limitations include a single-centre design, exclusion of children with severe diarrhoea and severe dehydration. Patient withdrawals and exclusions also reduced sample size and limited power to properly assess statistical differences within subgroups or biomarkers of clinical interests (e.g., differences between individual microorganisms). Despite the limitations, this study adds valuable insights into the efficacy and safety of VS002A, a glucose-free amino acid-based ORS, in the management of paediatric infectious diarrhoea. The non-inferiority to the WHO-ORS formula and the further pronounced findings in the per protocol analysis offer promising prospects for its potential application as an alternative treatment option for acute diarrhoea in young children. The well-tolerated nature of VS002A adds to its appeal as a viable non-glucose-based treatment option for acute diarrhoea in young children.

It was hypothesized that an amino acid-based ORS would be as safe and efficacious as the glucose-based WHO-ORS. This study provides evidence that VS002A, a non-glucose, amino acid-based ORS can be considered a safe and effective treatment for the oral correction of ongoing fluid and electrolyte losses in children with acute non-cholera, non-severe watery diarrhoea. The non-glucose-based formulation demonstrated non-inferiority compared to the WHO-ORS formula. Moreover, the amino acid ORS may offer advantages in pathogen-driven diarrhoea, as evidenced by trends toward a lower duration of diarrhoea and stool output within the per protocol group. Further research is warranted to corroborate and expand on these novel findings and to explore potential benefits of this formulation in diverse clinical settings. Individuals with compromised small intestinal mucosal integrity associated with prolonged diarrhoea, severe malnutrition, or environmental enteric dysfunction could benefit from a glucose-free ORT that has been shown to enhance sodium carrying capacity, reduce anion secretion, and improve mucosal barrier function, and intestinal stem cell proliferation. Lastly, in the developed world, a glucose-free ORS has great relevance where rates of obesity and insulin resistance continue to rise.

Contributors

T.A., P.K.B. and O.F. conceptualized the study; M.A.H. and A.B.S analysed the data and M.A.H, R.A.S., A.A.S. and A.B.S. constructed the tables and figures; R.D. and P.K.B. wrote the manuscript's initial draft. All authors have critically revised the manuscript for essential intellectual content. T.A. gave final approval for the version to be published. Every author was sufficiently involved in the research to take on public responsibility for content-related sections. All authors have read and agreed to the published version of the manuscript.

Data sharing statement

The dataset generated for the study will be available upon request, and readers may contact Ms. Armana Ahmed (aahmed@icddrb.org) of the Research Administration & Strategy of icddr,b to request the data (http://www.icddrb.org/).

Declaration of interests

Entrinsic Bioscience LLC funded the study. NF, ABS, SV, OF were either employees or paid consultants of entrinsic bioscience LLC. Dr. Sadasivan Vidyasagar has a patent Formulations and Methods for Treating Diarrhoea licensed to entrinsic bioscience LLC. No other authors have any financial interest or any other thing to declare that may affect the results of this study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102630.

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