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BENEFICIAL MICROBES (2025) DOI:10.1163/18762891-BJA00104

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RESEARCH ARTICLE

Effectiveness of a multistrain synbiotic product in children with acute diarrhoea of probable viral etiology: multicentre prospective randomised controlled study

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Received 11 June 2025 | Accepted 29 September 2025 | Published online 6 October 2025

Abstract

Acute diarrhoea in young children is very common and remains an important health problem. This study aimed to assess the effectiveness of a multistrain synbiotic compound in a drops formulation for treating acute diarrhoea of probable viral origin in children aged ≤2 years. A prospective, multicentre, randomised, open-label and controlled study was conducted in a cohort of 75 children (40 received a one-week treatment with a 7-multistrain synbiotic drops plus supportive therapy and 35 received supportive therapy alone). Based on the WHO definition of diarrhoea (≥3 loose/liquid stools/day) and the Bristol Stool Form Scale (BSFS) (stool consistency 5-7), a higher percentage of children in the synbiotic group experienced less diarrhoea (70%) vs controls (88.6%) ($P = 0.050$). This statistically significant difference was present since day two of treatment. When diarrhoea was defined as ≥3 bowel movements/day for ≥3 consecutive days, diarrhoea was absent in 20% of children in the synbiotic group, whereas none of those in the control group was free of diarrhoea ($P = 0.006$). The median days with diarrhoea was 4 (range 3-6.5) in the synbiotic group and 6 (range 5-7) in the control group ($P = 0.002$). The use of this synbiotic product allowed children's diarrhoeal process to be shortened by two days and promoted a faster recovery. These results along a very favourable safety and tolerability profile supports the use of this multistrain synbiotic product in acute diarrhoea of suspected viral origin in children two years old or younger.

Keywords

diarrhoea – synbiotics – probiotics – infants – randomised study

1 Introduction

Diarrhoea defined as loose, watery stools three or more times a day is a very common health problem in children. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) defines acute gastroenteritis (diarrhoea) as a decrease in the consistency of stools (loose or liquid) and/or an increase

in the frequency of evacuation (typically ≥3 in 24 h), with or without fever or vomiting. Despite improved hygienic conditions and effective interventions including rotavirus vaccine, young children may experience as many as 1 to 5 episodes of acute diarrhoea each year and according to the Global Burden of Disease Study in 2016, diarrhoea was the fifth leading cause of death among children younger than five years (GBD 2016 Diarrhoeal Disease Collaborators, 2018). Although most of these deaths occur in low and middle-income countries due to inequalities, lack of resources, malnutrition, unsafe water and poor sanitation, diarrhoea remains an important health problem in developed countries as it leads to frequent emergency room visits, admission to the hospital, high burden to society, families and economic costs for the healthcare systems (Elliott, 2017; Guarino *et al.*, 2020; Pinzón-Rondón *et al.*, 2015). However, implementation of an integrated approach of key interventions to effectively prevent and treat childhood diarrhoea still remains an unmet challenge in global child health (Mokomane *et al.*, 2018; World Health Organization/The United Nations Children's Fund (UNICEF), 2013).

Acute diarrhoea should be treated promptly and effectively to prevent severe dehydration, which can be life-threatening if untreated. Treatment is primarily supportive, particularly in viral gastroenteritis, based on the administration of oral rehydration solution, age-appropriate diet, limiting foods high in fat and simple sugars, etc. (Burkhart, 1999). Pharmacological treatment is not usually recommended in the management of diarrhoea in children (Burkhart, 1999). Imbalance and alterations in the composition of gut microbiota in children with diarrhoea, with significant decreases in *Lacticaseibacillus*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* in the intestine have been the rationale for the use of probiotics, which can improve the intestinal microenvironment, promote immunity and enhance resistance. Systematic reviews and meta-analyses have provided a good body of evidence to support the efficacy and tolerability of probiotics and synbiotics (a mixture of probiotics and prebiotics) in the management of pediatric acute gastroenteritis (AGE) (Applegate *et al.*, 2013; Florez *et al.*, 2018; Li *et al.*, 2021). Probiotic mixtures can include multiple strains of microorganisms, as it has been suggested that multi-species probiotic mixtures might be more beneficial for restoring the microbiome after dysbiosis has occurred. Advantages of multi-strain mixtures may include synergistic effects of different strains (increased adhesion, increased pathogen inhibition) and the combination of different mechanisms of action. Nonetheless, a systematic review concluded that multi-strain probiotics are not always more beneficial than single-strain ones. One mixture was superior for Helicobacter pylori eradication, but a single strain was superior for necrotizing enterocolitis prevention (McFarland, 2021). According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), a synbiotic is defined as a mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms that confers a health benefit on the host (Swanson, 2020). The overall benefits of synbiotics reported in clinical trials of acute diarrhoea in children include a rapid normalisation of the gastrointestinal microbiota, a reduction in the duration of diarrhoea, quicker improvement in stool frequency and consistency, lesser administration of additional medications, reduced length of hospitalisation and higher treatment satisfaction (Jog, 2019; Yang *et al.*, 2019).

In clinical practice, the diversity of probiotics/synbiotics makes choosing an appropriate probiotic product challenging. Moreover, the benefits of probiotics should be assessed in well-defined human populations, with adequate scientific methodology, in a specific disease or indication, evaluating specific strains and using the quantities of the product that is marketed (Cucalón Arenal and Blay Cortés, 2020a,b).

Some published studies on the use of synbiotics with different compositions in the treatment of AGE in children have consistently shown faster resolution of diarrhoea, with reduced daily stool outputs, better stool consistency, and less concomitant medication used, when compared with placebo or supportive treatment only (Guarino *et al.*, 2018; Gundogdu, 2013; Nocerino and Canani, 2012; Vandenplas and De Hert, 2011). Moreover, treatment with synbiotic is cost effective due to the reduction in add-on medical and extra consultations (Vandenplas and De Hert, 2012).

Previous randomised controlled trials with a multi-strain synbiotic product based on the combination of seven probiotic species (*L. casei*, *L. rhamnosus*, *Streptococcus thermophilus*, *B. breve*, *L. acidophilus*, *B. infantis*, *B. bulgaricus*) and fructooligosaccharides as prebiotic (Prodefen®) showed a reduction in the duration of diarrhoea and number of watery stools (Allahverdi *et al.*, 2010; García-Menor *et al.*, 2016). One of these studies was conducted in hospitalised Iranian children aged between 1 and 5 years (Allahverdi *et al.*, 2010) and the other in children between 6 months and 12 years of age in the outpatient setting in Spain (García-Menor *et al.*, 2016). The use of this synbiotic product increased the proportion of patients without diarrhoea at all time

points as compared with controls and reduced the duration of diarrhoea by two days in the subset of children aged 6 months to 2 years (García-Menor *et al.*, 2016). Following this line of research, the present randomised controlled trial, PRODINFANT study, was designed to assess the effectiveness of this multistrain synbiotic compound (with updated probiotic strains while maintaining its composition) in a new drops formulation for treating AGE of viral origin in a specific age group of children in the first two years of life. Maintaining the composition refers specifically to the preservation of the probiotic species and the prebiotic component (fructooligosaccharides), consistent with those used in the García-Menor *et al.* (2016) study. The term updated probiotic strains denotes the inclusion of *Lacticaseibacillus rhamnosus GG* and modifications to the remaining strains.

One of the strains included in this new formulation, *Lacticaseibacillus rhamnosus* GG, is recommended as an active treatment adjunct to rehydration therapy according to 2020 update guidelines for the management of AGE in children of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases (ES-PGHAN/ESPID) (Szajewska *et al.*, 2020). On the other hand, the strain *Streptococcus thermophilus St-21* that is also part of this drops formulation has been investigated as a treatment for irritable bowel syndrome (IBS) in adults. In a multi-centre, randomised, double-blind, placebo-controlled trial involving 202 IBS patients, treatment with a symbiotic containing this strain resulted in stool normalisation 78.1% of IBS patients with diarrhoea and 96.2% of IBS patients with constipation after 12 weeks of treatment with this *S. thermophilus St-21*-containing synbiotic (Sommermeyer *et al.*, 2024). Finally, in a study that evaluated the impact of an enteral probiotics supplementation protocol including *Bifidobacterium infantis HA-116* on the incidence of necrotizing enterocolitis (NEC) in preterm infants, the introduction of this five-strain probiotic coincided with a significant 67% reduction in the incidence of definite NEC. The mechanisms through which these probiotic strains, including *Bifidobacterium infantis HA-116*, confer benefits may involve enhancing mucosal barrier integrity, competitive exclusion of pathogens, modifying host responses to microbial products, augmenting IgA mucosal responses, improving enteral nutrition, and up-regulating immune responses (Brown *et al.*, 2022).

2 Material and methods

Design and participants

This was a multicentre, prospective, randomised, open-label and controlled clinical trial (PRODINFANT study; ISRCTN Registry identification number: ISRCTN10495 554) carried out between September 2020 and November 2021 at 10 paediatric primary care consultations or emergency services throughout Spain. The primary objective of the study was to assess the additional benefit provided by a multi-strain synbiotic formulation as compared with diet and/or oral rehydration (standard treatment) in the resolution of diarrhoea in children with AGE of viral origin aged ≤2 years. Secondary objectives included the effect of the synbiotic compound on the incidence and/or duration of other associated symptoms, and tolerability, safety and acceptability of the product.

Eligible patients were children aged two years or younger who visited the paediatrician in the outpatient setting (primary or the emergency healthcare centre), presenting an acute diarrhoea episode lasting less than 48 h, with a probable viral origin, and in whom diet and/or oral rehydration were considered the most appropriate treatment. A probable viral origin was established because the patient's history and the presenting symptoms were not correlated with clinical manifestations of gastroenteritis caused by bacterial infection or other type of gastroenteritis. Diarrhoea was defined according to criteria of the World Health Organization (WHO) as the passage of three or more loose or liquid stools per day (or more frequent passage than normal for the individual) (World Health Organization, 2023). Types 5 to 7 of the Bristol Stool Form Scale (BSFS) were used for the definition of loose or liquid (watery) stools (Lewis and Heaton, 1997). Exclusion criteria were as follows: presence of any disease that may cause diarrhoea, different from an infection such as inflammatory bowel disease, food allergy, lactose intolerance, etc., immunodeficiency or other chronic underlying diseases; severe dehydration or malnutrition; visible blood in stools; current pharmacological treatment for diarrhoea; use of antibiotics and/or probiotics within the previous seven days; milk protein allergy; and current participation in another study.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee (CEIC) of Hospital Universitario Puerta de Hierro (Majadahonda, Madrid, Spain). Written informed consent was obtained from the parents or legal tutors of children participating in the study.

Intervention

Children who met the inclusion criteria were assigned a consecutive number at each study site according to the order of arrival, and then randomised to treatment with the synbiotic product (synbiotic group; odd number) or standard supportive measures (control group; even number). All participants regardless of their assigned group received supportive standard treatment with diet and/or oral rehydration therapy. Children randomised to the synbiotic group were administered daily recommended dosage (10 drops) of the synbiotic product (Prodefen® drops, ITALFARMACO, S.A., Alcobendas, Madrid, Spain), with or without food and preferably in the morning for seven consecutive days. The synbiotic product is composed of 1×10^9 colony-forming units (CFU) per daily dose (10 drops) of the following seven probiotic strains: *Lacticaseibacillus rhamnosus* GG (formerly *Lactobacillus rhamnosus GG*), *Lacticaseibacillus casei* HA-108 (formerly *Lactobacillus casei* HA-108), *Lactobacillus acidophilus* La-14, *Lactobacillus bulgaricus* Lb-87, *Streptococcus thermophilus* St-21, *Bifidobacterium breve* BB077 and *Bifidobacterium infantis* HA-116 in combination with 3 mg of fructooligosaccharides (prebiotic). Children assigned to the control group did not receive any other active treatment besides diet recommendations and/or oral rehydration therapy (standard supportive measures).

Study procedures

The study included a 7-day treatment period with 3 visits (1 in-person visit and 2 visits by phone) and a final safety follow-up phone call at day 10. Children visited the study centre (visit 1) where the inclusion criteria were confirmed, the signed informed consent was obtained, and they were assigned to the treatment group. The first dose of the synbiotic product was administered by the investigator at the study centre (day 1) and parents were provided with a drop bottle of the product for treatment during the subsequent six days. Parents of all participants were given a patient diary card in which all information of the study variables had to be recorded on a daily basis. The parents were contacted by phone on days 4 and 7, so that the investigator could collect information on the study variables related to the course of the disease. Data of acceptability and tolerability of treatment were collected on day 7. In addition, on day 10, 3 days after the end of the treatment, adverse events occurring during that period were recorded. If scheduled phones calls were foreseen during the weekend, a 2-day window period was allowed.

Study variables

Efficacy variables assessed from day one to the end of the study included the percentage of children who suffered diarrhoea according to several definitions, the WHO criteria, the Bristol Stool Form Scale (BSFS) criteria, both WHO and BSFS criteria, and the parents' opinion regarding more stools per day or more fluid in consistency for at least 1, ≥2 or ≥3 consecutive days; number of days of diarrhoea (three or more passage of stools per day) and duration (days) of diarrhoea until the end of the study; percentage of children recovered from diarrhoea (two consecutive days without diarrhoea); characteristics of stool (frequency, consistency according to the BSFS, severity of diarrhoea); and associated symptoms (vomiting, nausea, fever, mucus in stools and abdominal pain), mean duration of symptoms and need for treatment. Other variables included number of visits to the primary care paediatrician or the emergency service as well as the need for concomitant medication. The administration of other probiotics including probiotic rich serum as a complement to the standard supportive measures (diet/oral rehydration) during the participation in the study was not allowed.

The BSFS classifies stool consistency into seven types as follows: type 1, separate hard lumps, like nuts difficult to pass; type 2, sausage-shaped but lumpy; type 3, like a sausage but with cracks on its surface; type 4, smooth and soft (average stool); type 5, soft blobs with clear cut edges; type 6, fluffy pieces with ragged edges, a mushy stool; and type 7, watery, no solid pieces entirely liquid (Lewis and Heaton, 1997). Types 1 and 2 indicate constipation, types 3 and 4 being ideal stools and types 5 to 7 indicating diarrhoea.

Tolerability of the synbiotic product and satisfaction with treatment (acceptability) were evaluated using a 5-point Likert scale, from 'very good' to 'very bad' and from 'very satisfied' to 'not at all satisfied', respectively. The impact of treatment on the quality of life and daily activities of the parents was assessed using a 5-point Likert scale ('not at all affected', 'slightly affected', 'moderately affected', 'very affected', 'extremely affected'). Parents of children in the synbiotic group were asked whether they would administer the synbiotic product again in case their child had a similar episode of diarrhoea in the future. The level of adherence to diet/oral rehydration alone (control group) or combined with the study product (synbiotic group) was categorised as poor (<60%), moderate (≥60% to <80%), good (≥80% to <90%) and very good (≥90%). Safety variables included type, frequency and duration of adverse events (AEs).

Study endpoints

Study endpoints were the evolution of diarrhoea according to the WHO definition (≥3 loose or liquid stools/daily), parents' criteria as more stools/day of a more fluid consistency than usual, and according to BSFS (types 5 to 7) during at least 1, 2, or 3 days; number of days with diarrhoea (≥3 stools for at least 1 day); rate of diarrhoea recovery; improvement in the characteristics of stools (frequency, consistency according to the BSFS, and severity of diarrhoea); disappearance of associated symptoms; the need for medical/treatment intervention (visits to pediatrician/emergency service) and the tolerability and acceptability of treatment.

Statistical analysis

The sample size was calculated based on data of a clinical trial reported by Francavilla *et al.* (2012) in which the mean duration of diarrhoea was 2.1 days in the synbiotic group and 3.3 in the placebo group. In order to detect a difference of 1.2 (0.4) days in the duration of diarrhoea between the synbiotic and the control groups, a sample of 37 children per treatment group would be required for a statistical power of 76% and a significance level of 5%, which was increased up to 40 children per group (total 80) with an estimation of 8% of children lost to follow-up.

Efficacy endpoints were analysed in the population dataset as treated i.e. children who received at least one day of the treatment prescribed and in whom some follow-up data were provided. The safety population included all randomised children who had received at least one day of the treatment prescribed.

Categorical variables are expressed as frequencies and percentages, and continuous variables as mean and SD or median and interquartile range (IQR) (25th-75th percentile). Differences in the distribution of variables in the two study groups were analysed according to normally or non-normally distribution of data sets, using the chi-square test or the Fisher's exact test for categorical data, and Student's t test (parametric test) or the Mann-Whitney U test (non-parametric test) for quantitative data. All analyses were two-tailed and statistical significance was set at $P < 0.05$. The Power Analysis & Sample Size (PASS) version 2011 (NCSS LCC) software package was used for the analysis of data.

3 Results

Baseline characteristics of children

The study population included 75 children, 40 assigned to the synbiotic group and 35 to the control group. There were 47 (62.7%) males and 28 (37.3%) females, with a mean age of 14.5 (8.1) months. As shown in Table 1, there were no significant differences between the study groups in baseline data recorded at visit 1. Only 4 patients had received previous treatments in the last 7 days, especially analgesics and vitamins. The mean time elapsed from the first loose or liquid stool to visit 1 was 1.0 (0.6) days. Associated symptoms and the characteristics of stools were also similar, except for vomiting, which was more frequent in the control group (34.3% vs 10%, $P = 0.01$).

Diarrhoea

A significantly lower percentage of children assigned to the synbiotic group suffered from diarrhoea for ≥3 consecutive days (based on WHO definition of ≥3 loose/liquid stools/day) as compared with the control group (80% vs 100%. $P = 0.006$). The superiority of the synbiotic product was also confirmed applying the strictest criteria, based on the combined WHO definition of ≥3 loose/liquid stools/day and BSFS stool consistency types 5-7 (WHO and BSFS criteria), observing 70% of children in the synbiotic group presenting diarrhoea compared with 88.6% of children in the control group ($P = 0.050$).

The median (IQR) days with diarrhoea defined as ≥3 bowel movements/day, was 4 (3-6.5) in the synbiotic group and 6 (5-7) in the control group ($P = 0.002$). Therefore, there was a reduction of 2 days in the duration of diarrhoea among children assigned to the synbiotic group.

The total duration of the diarrhoea episode was shorter in the synbiotic group (median 3 days, IQR 2-5) than in the control group (median 5, IQR 4-7) ($P = 0.0006$). Rate of full recovery from diarrhoea at the end of the study was significantly higher for children receiving synbiotic, 85.0% (34/40) compared to those in the control group, 54.3% (19/35) ($P = 0.036$). A complete list of diarrhoea-related variables can be found in Table 2, where results were generally more favourable for the synbiotic group.

The mean number of bowel movement throughout the study showed differences in favour of the active treatment since day two until the end of treatment, with differences being statistically significant since day three of therapy (Figure 1). The higher percentage of infants

TABLE 1 Baseline characteristics of 75 children included in the study

Variable	All children (n = 75)	Control group (n = 35)	Synbiotic group (n = 40)	*P*-value
Gender, n (%)				
Males	47 (62.7)	22 (62.9)	25 (62.5)	0.974
Females	28 (37.3)	13 (37.1)	15 (37.5)	
Age, months, mean (SD) (range)	14.5 (8.1) (2-34)	13.7 (7.7) (2-32)	15.2 (8.5) (4-34)	0.678
Weight, kg, mean (SD) (range)	10.3 (2.6) (4.8-17.5)	10.1 (2.7) (4.8-15.5)	10.4 (2.6) (6.5-17.5)	0.710
Height, cm, mean (SD)	77.8 (9.1)	76.9 (9.1)	78.6 (9.2)	0.520
Previous treatment (last 7 days), n (%)	4 (5.3)	3 (8.1)	1 (2.6)	0.358
Time from first loose/liquid stool to visit 1, days, mean (SD)	1.0 (0.6)	1.1 (0.7)	0.9 (0.6)	0.367
Time from diarrhoea to visit 1, days, mean (SD)	0.9 (0.7)	0.9 (0.7)	0.8 (0.7)	0.582
Associate symptoms, n (%)				
Fever	25 (33.3)	13 (37.1)	12 (30.0)	0.513
Vomiting	16 (21.3)	12 (34.3)	4 (10)	0.010
Nausea	19 (25.7)	12 (34.3)	7 (17.9)	0.108
Mucus in stools	37 (49.3)	19 (54.3)	18 (45)	0.422
Abdominal pain	32 (43.2)	14 (40)	18 (46.2)	0.593
Bowel movements in the last 24 h, mean (SD)	5.2 (2.0)	5.1 (2.3)	5.3 (1.6)	0.432
Stool consistency in the last 24 h, BSFS, n (%)				
Type 5	1 (1.3)	0	1 (2.5)	0.613
Type 6	22 (29.3)	11 (31.4)	11 (27.5)	
Type 7	52 (69.3)	24 (68.6)	28 (70.0)	
Stool consistency in the last 24 h, BSFS, mean (SD)	6.7 (0.5)	6.7 (0.5)	6.7 (0.5)	0.958

BSFS, Bristol Stool Form Scale.

without diarrhoea in the synbiotic group was already noticed on day two (Figure 2).

Stool consistency

In relation to stool consistency according to BSFS type, there were significant differences between the study groups, with higher stool consistency in the active treatment group as compared with the control group on days 4 and 6 (Figure 3). On day 4, the mean values in the synbiotic group were 5.4 (1.0) and 5.7 (0.9) in the control group ($P = 0.039$) and on day 6, the corresponding values were 4.4 (1.2) and 4.9 (1.0) ($P < 0.0001$), respectively (Figure 3).

Associated symptoms, need for medical intervention and treatment

In relation to the duration of associated symptoms, visits to the pediatrician or emergency services or need for concomitant treatment, significant differences between the synbiotic and control groups were not found (Table 3), although for every single parameter the synbiotic group scored better.

Tolerability, acceptability and adherence

Tolerability and acceptability of diarrhoea treatment were higher in the synbiotic treatment group (Table 4). The percentage of the children's parents who reported a tolerability of the study treatment as 'very good' or 'good' was significantly higher in the synbiotic group than in the control group (95% vs 69.7%). Also, the percentages of 'very satisfied' and 'quite satisfied' with the treatment were significantly higher among parents of children assigned to the active treatment (70% vs 39.4%). Ninety-five percent of parents reported the intention of using the synbiotic product in future similar diarrhoea episodes. The impact of the children's diarrhoea on the quality of life of their parents was similar, but the percentages of 'not at all affected' were higher in the synbiotic treatment group.

In relation to compliance with treatment statistically significant differences were not observed. Overall,

TABLE 2 Results of diarrhoea-related variables

Variable	All children (n = 75)	Control group (n = 35)	Synbiotic group (n = 40)	*P*-value
Diet and/or oral rehydration, days, mean (SD)	5.7 (1.9)	6.1 (1.5)	5.4 (2.2)	0.249
Diarrhoea (WHO criteria and type 5-7 BSFS), n (%)				
≥1 day	75 (100)	35 (100)	40 (100)	
≥2 days	69 (92.0)	34 (97.1)	34 (87.5)	0.206
≥3 days	59 (78.7)	31 (88.6)	28 (70.0)	0.050
Diarrhoea, ≥3 bowel movements/day, n (%)				
≥1 day	75 (100)	35 (100)	40 (100)	
≥2 days	70 (93.3)	35 (100)	35 (87.5)	0.057
≥3 days	67 (89.3)	35 (100)	32 (80)	0.006
Diarrhoea, parents' criteria*, n (%)				
≥1 day	75 (100)	35 (100)	40 (100)	
≥2 days	73 (97.3)	35 (100)	38 (95.0)	0.495
≥3 days	67 (89.3)	34 (97.1)	33 (82.5)	0.060
Diarrhoea (type 5-7 BSFS), n (%)				
≥1 day	75 (100)	35 (100)	40 (100)	
≥2 days	74 (98.7)	35 (100)	39 (97.5)	1.00
≥3 days	72 (96.0)	35 (100)	37 (92.5)	0.243
Duration of diarrhoea, days, median (IQR)				
≥3 days	5 (3-7)	6 (5-7)	4 (3-6.5)	0.002
Parents' criteria*	5 (3-6)	5 (4-7)	4 (3-6)	0.017
Until the end of treatment	4 (3-5)	5 (4-7)	3 (2.5)	0.0006
Diarrhoea recovery, n (%)	53 (70.7)	19 (54.3)	34 (85.0)	0.0036

WHO, World Health Organization; BSFS, Bristol Stool Form Scale; IQR, interquartile range.

* Parents' criteria as more stools/day of a more fluid consistency than usual.

57.3% of patients reported a very good level of compliance (≥90%) and 29.3% a poor level of compliance (<60%) regarding supportive treatment (diet and/or rehydration). In the synbiotic group, 100% adherence to the synbiotic product was recorded. The percentage of patients who followed dietary instructions from day 4 to day 6 was significantly higher in the control group (85.3%) than in the synbiotic group (62.5%) (P = 0.027). In this respect, parents who noted an improvement in their child's diarrhoea relaxed their dietary measures, which is an indirect sign of efficacy in the symbiotic product.

In all 75 children, 46 (32.0%) adverse events (AEs) were reported, 26 AEs in 12 of the 35 children (34.3%) from the control group and in 20 AEs in 12 of the 40 children (30.0%) from the synbiotic group (P = 0.691). Most AEs were of mild intensity, and the majority were related to the gastrointestinal tract, with vomiting (30.8%), mucous stools (15%), and abdominal discomfort (15%) as the most frequent. Only 5 AEs in the active treatment group were considered to have a probable or possible relationship with treatment: 1 case of loss of appetite, 2 cases of mucus in the stools and 2 cases of abdominal pain. In none of the children, treatment assigned was discontinued due to AEs.

4 Discussion

This open-label randomised controlled study carried out in children of two years of age or younger presenting diarrhoea of probable viral origin showed that a combined treatment of a multistrain synbiotic product (Prodefen® drops) with diet recommendations and/or oral rehydration was more effective to improve the evolution of diarrhoea than standard supportive measures alone. Additional benefits of the use of the synbiotic product as compared with supportive measures included experiencing less diarrhoea with improvement in stool consistency, shortening diarrhoea by two days and decreasing the total duration of the diarrhoeal episode. The rapid effect of the synbiotic product, which

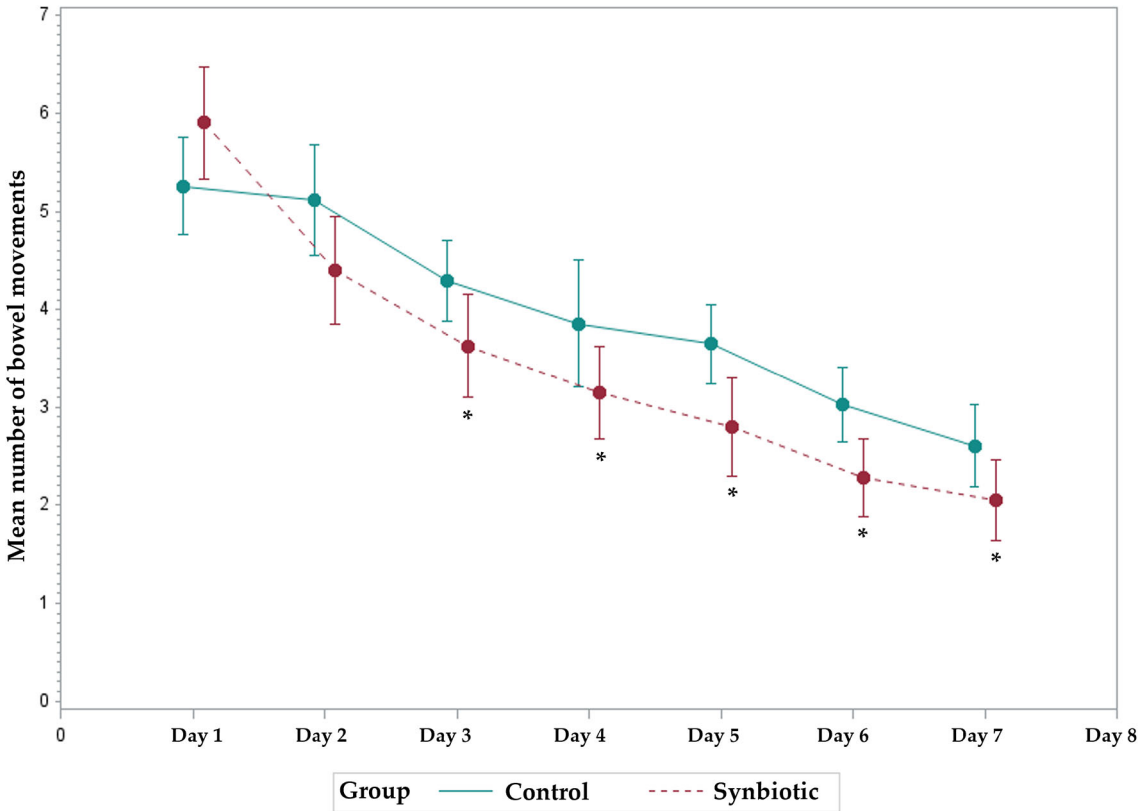
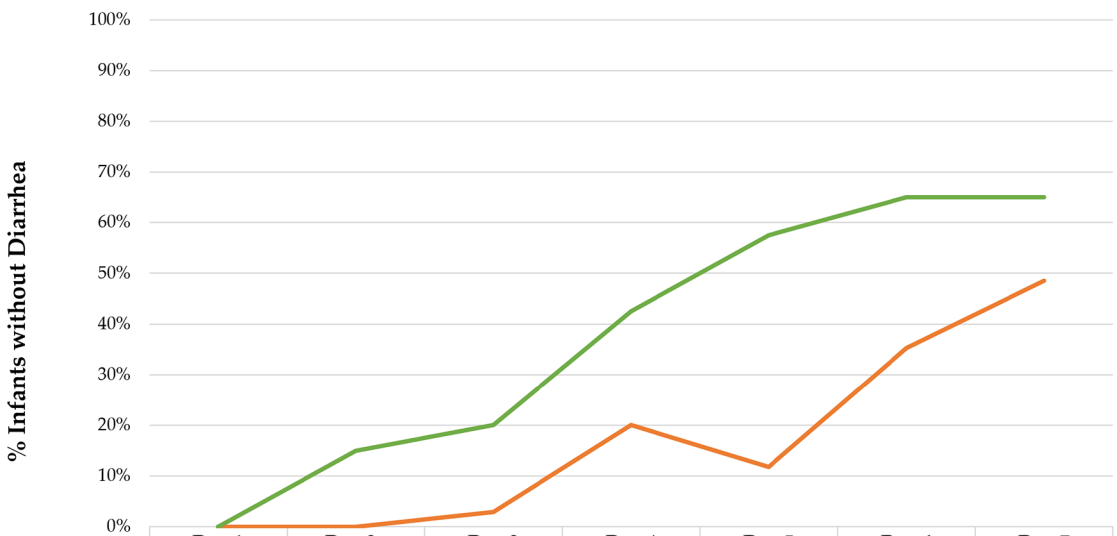


FIGURE 1 Mean number of bowel movements during the 7-day study period with significant differences from day 3 to the end of the study in favour of the active treatment group (*$P < 0.05$).



	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control group	0.0%	0.0%	2.9%	20.0%	11.8%	35.3%	48.5%
Synbiotic group	0.0%	15.0%	20.0%	42.5%	57.5%	65.0%	65.0%
p value	-	0.0271	0.0317	0.0372	< 0,0001	0.0108	0.1554

FIGURE 2 Percentage of infants without diarrhoea (≥3 stools/day) for at least one day over the study period.

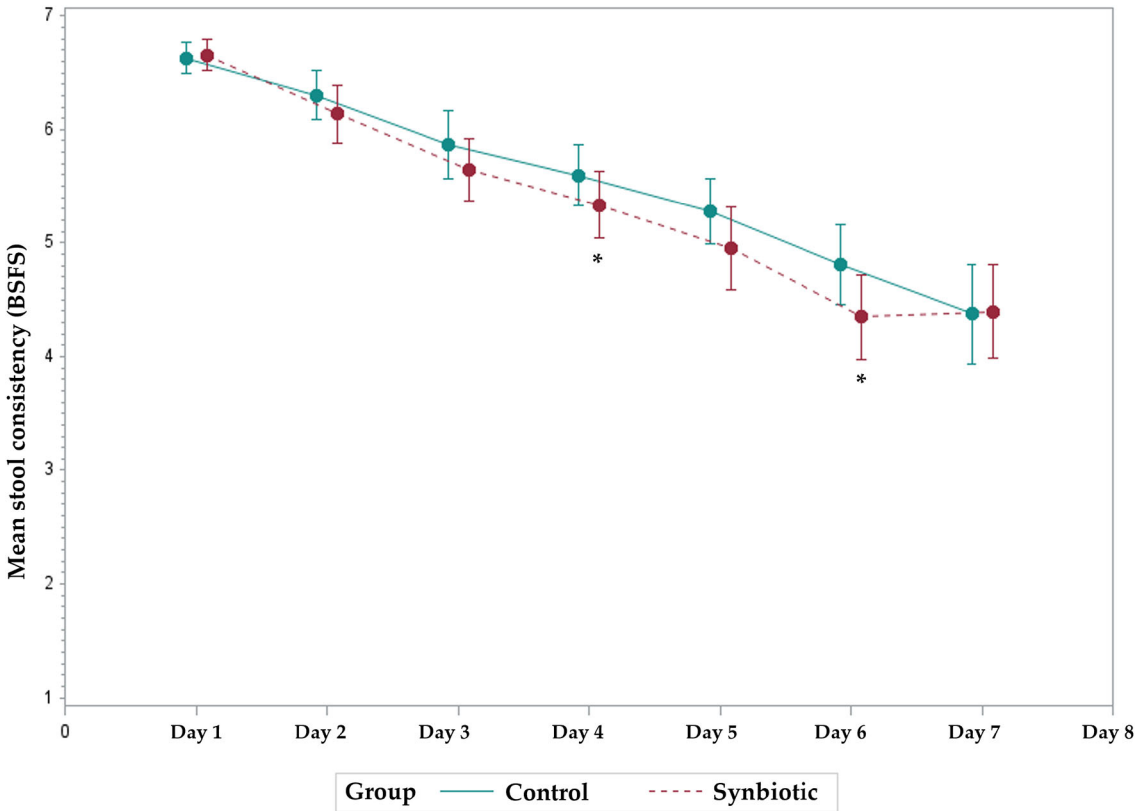


FIGURE 3 Changes in stool consistency according to the Bristol Stool Form Scale during the treatment period with significant differences on days 4 (P = 0.039) and 6 (P < 0.0001) in favour of the active treatment (lower values indicate higher consistency).

TABLE 3 Duration of associated symptoms in the study groups and need for medical intervention and concomitant treatment

Variable	All children (n = 75)	Control group (n = 35)	Synbiotic group (n = 40)	P-value
Duration of symptoms, days, mean (SD)				
Fever	0.77 (1.07)	0.83 (1.20)	0.73 (0.06)	0.948
Vomiting	0.59 (1.20)	0.91 (1.58)	0.30 (0.61)	0.107
Nausea	0.55 (0.98)	0.74 (1.20)	0.38 (0.70)	0.233
Mucus in stools	2.44 (2.61)	2.86 (2.92)	2.08 (2.28)	0.260
Abdominal pain	1.75 (2.19)	2.00 (2.51)	1.53 (1.87)	0.794
Other symptoms	0.91 (1.78)	0.94 (1.53)	0.88 (1.99)	0.515
Need for pediatrician/emergency room visit, n (%)	16 (21.3)	10 (28.6)	6 (15.0)	0.152
Concomitant treatment, n (%)	36 (48.0)	17 (48.6)	19 (47.5)	0.926

was already observed from the second day of treatment and the reduction in the duration of the episode of diarrhoea by two days are relevant findings of the study from a clinical point of view. Furthermore, the use of the synbiotic product showed a very favourable safety and tolerability profile as well as a high level of acceptability by the children's parents/tutors.

Diarrhoea remains a leading cause of morbidity and mortality among children under five years of age, responsible for killing around 525,000 children every year (World Health Organization, 2023). Moreover, diarrhoea is a leading factor causing malnutrition in children younger than five years. Because of the relevance of this preventable disease, interventions to improve sanitation, personal and food hygiene and education are essential measures in the prophylaxis of diarrhoea. Acute diarrhoea can be caused by different pathogens, particularly enteric pathogens such as Rotavirus, Adenovirus, enterotoxigenic *Escherichia coli*, *Salmonella* and Giardia (Florez *et al.*, 2020). Rotavirus is the most common aetiology and laboratory tests are not routinely required because they usually do not affect the man-

TABLE 4 Tolerability, acceptability/satisfaction with treatment and level of adherence

Variable	All children (n = 73)	Control group (n = 33)*	Synbiotic group (n = 40)	*P* value
Tolerability				
Very good	34 (46.6)	6 (18.2)	28 (70)	<0.0001
Good	27 (37.0)	17 (51.5)	10 (25.0)	
Neither good nor bad	12 (16.4)	10 (30.3)	2 (5.0)	
Bad	0	0	0	
Very bad	0	0	0	
Acceptability/satisfaction				
Very satisfied	21 (28.8)	5 (15.2)	16 (40)	0.037
Quite satisfied	20 (27.4)	8 (24.2)	12 (30.0)	
Satisfied	26 (35.6)	17 (51.5)	9 (22.5)	
Slightly satisfied	6 (8.2)	3 (9.1)	3 (7.5)	
Not at all satisfied	0	0	0	
Impact on the parents' quality of life				
Not at all affected	24 (32.9)	7 (21.2)	17 (42.5)	0.107
Slightly affected	20 (27.4)	9 (27.3)	11 (27.5)	
Moderately affected	19 (26.0)	10 (30.3)	9 (22.5)	
Extremely affected	1 (1.4)	0	1 (2.5)	
Level of adherence				
Poor (<60%)	22 (29.3)	7 (20.0)	15 (37.5)	0.389
Moderate (≥60% to <80%)	5 (6.7)	3 (8.6)	2 (5.0)	
Good (≥80% to <90%)	5 (6.7)	3 (8.6)	2 (5.0)	
Very good (≥90%)	43 (57.3)	22 (62.9)	21 (52.5)	

* In two children from the control group data at the follow-up assessment could not be recorded.

agement of the disease. Assessment of the hydration status is a key element in the approach of a child with diarrhoea, with oral rehydration therapy and diet recommendations as mainstay of treatment for paediatric diarrhoeal episodes. In a systematic review of diarrhoea duration and severity in children, it was estimated that among children under five years of age, 65% of diarrhoeal episodes were mild with a weighted mean duration of 4.3 days (95% confidence interval [CI] 4.3-4.4), whereas severe episodes showed a weighted mean duration of 8.4 days (95% CI 8.1-8.8) and caused dehydration in 85% of the cases (Lamberti *et al.*, 2012). The goals of treatment are to prevent or reverse dehydration, to shorten the duration of the illness, and to reduce the period during which a person is infectious (Guarino *et al.*, 2020).

The use of multispecies (multistrain) synbiotic mixtures in children with diarrhoea have shown beneficial effects, particularly in improving the course of the episode, reducing the duration of diarrhoea and length of hospital stay in children requiring in-patient care (Dinleyici *et al.*, 2013). In multistrain synbiotic products, the probiotic effect is improved thanks to the combination of specific properties of the strains (additive and synergistic effect) (Pandey *et al.*, 2015; Timmerman *et al.*, 2004). In the present study, the synbiotic product was a 7-strain synbiotic available as drops to be administered over 7 consecutive days. Also, it is important to note the value of the prebiotic in the composition of the synbiotic product. The prebiotic contributes to the growth of the probiotic and the beneficial bacteria that inhabit the intestinal tract (Pandey *et al.*, 2015; Timmerman *et al.*, 2004).

The target population included children ≤2 years of age presenting with acute diarrhoea and associated symptoms of less than 48 h of evolution and of a probable viral aetiology. Treatment with the synbiotic product was added to standard treatment based on oral rehydration and/or diet and was compared to supportive treatment alone. In comparison with the control group, various benefits associated to the complementary use of the synbiotic product included a significantly lower percentage of children with diarrhoea (defined as ≥3 bowel movements during ≥3 consecutive days), reduc-

tion of two days in the duration of diarrhoea, decrease in the percentage of children with diarrhoea during each day over the study period from day three until the end of treatment, decrease in the days of diarrhoea as evaluated by the parents and higher diarrhoea recovery rate. The synbiotic demonstrated a rapid onset of effect, with differences in efficacy since day two of therapy, being statistically significant from day three until the end of treatment. The results of our study are consistent with shortened duration of diarrhoea with the use of probiotics as compared with placebo reported in systematic reviews and meta-analysis (Alsabri *et al.*, 2025; Applegate *et al.*, 2023; Li *et al.*, 2021; Yang *et al.*, 2019). Also, in a previous randomised clinical study of a sachet formulation of the synbiotic product administered to patients with diarrhoea aged 6 months to 12 years, there was a significant improvement in diarrhoea-related outcomes as compared with standard supportive treatment (García-Menor *et al.*, 2016). Also, in the group of children aged 6 months to 2 years, the duration of diarrhoea was shortened by two days (García-Menor *et al.*, 2016), which is consistent with results of the present study.

All these findings are clinically relevant in children younger than two years because of the vulnerability of toddlers at this age, who are more susceptible to dehydration and related symptoms than older children. On the other hand, a 2-day reduction in the duration of diarrhoea has an important social impact for parents and caregivers due to early nursery attendance, less days of childcare, and parenteral absenteeism to work. Likewise, effective interventions capable of reducing the duration of diarrhoea (and the need for hospitalisation) in special populations such as undernourished children would have an enormous impact in resource-poor settings (Kambale *et al.*, 2021).

Compliance with the synbiotic drops was excellent and 95% of parents would recommend the product for the management of similar episodes of diarrhoea. There were no safety concerns and most common events were related to the gastrointestinal tract, such as vomiting, abdominal discomfort, and mucous stools.

The present results should be interpreted considering some limitations, such as the open-label design of the study. However, the use of a placebo instead of a synbiotic product could be an approach not easily accepted by parents at the time of providing consent. Children presented with diarrhoea of probable viral origin but the types of viral etiologies were not recorded. On the other hand, PRODINFANT study was a multicentre trial, following diarrhoea treatment aligned to real clinical practice disease management, which strengthens the generalisability of our findings. The results of PRODINFANT study are aligned to the benefits of synbiotic treatment already observed in García-Menor E *et al.* (2016).

5 Conclusions

The 7-multistrain synbiotic formulation evaluated in PRODINFANT study, administered as oral drops, provided clinically relevant benefits to supportive measures (diet and/or oral rehydration) for improving diarrhoea-related outcomes in children of two years of age or younger. The use of the synbiotic product was associated with a rapid improvement of symptoms and reduction of duration of diarrhoea by two days, which is clinically relevant for the children themselves, as well as to the parents/tutors regarding absenteeism from work and other aspects of daily life. The synbiotic product also promoted a faster complete recovery. Synbiotic therapy significantly improved the evolution of the diarrhoea in children <2 years, providing additional benefit to standard supportive measures in the management of diarrhoea. These results along a very favourable safety and tolerability profile supports the use of this synbiotic in acute diarrhoea of probable viral etiology.

Authors' contribution

Conceptualisation, EGA; methodology, EGA; formal analysis, FGM, PCT, AMA, MAMC, MJLP, LPA, EGM and EGA; investigation, FGM, PCT, AMA, MAMC, MJLP, LPA, EGM and EGA; writing (original draft preparation), EGA; writing (review and editing), FGM, PCT, AMA, MAMC, MJLP, LPA, EGM and EGA. All authors have read and agreed to the published version of the manuscript. The authors decline the use of artificial intelligence, language models, machine learning, or similar technologies to create content or assist with writing or editing of the manuscript.

Conflict of interest

EGA is a full-time employee of ITF Research Pharma SLU. The rest of authors declare no conflict of interest.

Data availability

Study data are available from the corresponding author upon request.

Funding

This study was funded by ITF Research Pharma S.L.U.

Trial registration

ISRCTN10495554.

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