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Efficacy of an Encapsulated Probiotic *Bifidobacterium infantis* 35624 in Women with Irritable Bowel Syndrome

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BACKGROUND: Probiotic bacteria exhibit a variety of properties, including immunomodulatory activity, which are unique to a particular strain. Thus, not all species will necessarily have the same therapeutic potential in a particular condition. We have preliminary evidence that *Bifidobacterium infantis* 35624 may have utility in irritable bowel syndrome (IBS).

OBJECTIVES: This study was designed to confirm the efficacy of the probiotic bacteria *B. infantis* 35624 in a large-scale, multicenter, clinical trial of women with IBS. A second objective of the study was to determine the optimal dosage of probiotic for administration in an encapsulated formulation.

METHODS: After a 2-wk baseline, 362 primary care IBS patients, with any bowel habit subtype, were randomized to either placebo or freeze-dried, encapsulated *B. infantis* at a dose of 1×10^6, 1×10^8, or 1×10^{10}, cfu/mL for 4 wk. IBS symptoms were monitored daily and scored on to a 6-point Likert scale with the primary outcome variable being abdominal pain or discomfort. A composite symptom score, the subject's global assessment of IBS symptom relief, and measures of quality of life (using the IBS-QOL instrument) were also recorded.

RESULTS: *B. infantis* 35624 at a dose of 1×10^8 cfu was significantly superior to placebo and all other bifidobacterium doses for the primary efficacy variable of abdominal pain as well as the composite score and scores for bloating, bowel dysfunction, incomplete evacuation, straining, and the passage of gas at the end of the 4-wk study. The improvement in global symptom assessment exceeded placebo by more than 20% ($p < 0.02$). Two other doses of probiotic (1×10^6 and 1×10^{10}) were not significantly different from placebo; of these, the 1×10^{10} dose was associated with significant formulation problems. No significant adverse events were recorded.

CONCLUSIONS: *B. infantis* 35624 is a probiotic that specifically relieves many of the symptoms of IBS. At a dosage level of 1×10^8 cfu, it can be delivered by a capsule making it stable, convenient to administer, and amenable to widespread use. The lack of benefits observed with the other dosage levels of the probiotic highlight the need for clinical data in the final dosage form and dose of probiotic before these products should be used in practice.

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INTRODUCTION

After many years without therapeutic innovation, new medications have recently become available for irritable bowel syndrome (IBS). A number of potential targets for manipulation such as cholecystokinin (1), neurokinin (2), corticotrophin (3), and serotonin (4) have been identified, with the latter being the focus of particular attention. One of the drawbacks associated with the advent of some of the newer drugs is that it has become essential, in many instances, to select patients for a specific treatment according to bowel habit subtype (diarrhea- or constipation-predominant), rather than necessarily being able to give a single medication to any IBS patient, as has been traditional in the past (5, 6).

Another more recent development in the understanding of the pathogenesis of IBS is the recognition that in a proportion of patients, there might be an inflammatory component (7). Up to 25% of patients date the onset of their problem from a gastrointestinal infection (8), and evidence accumulates to indicate the presence of an inflammatory response in the gastrointestinal mucosa in IBS (9–12). In addition, the observation that IBS may be exacerbated by antibiotics (13),

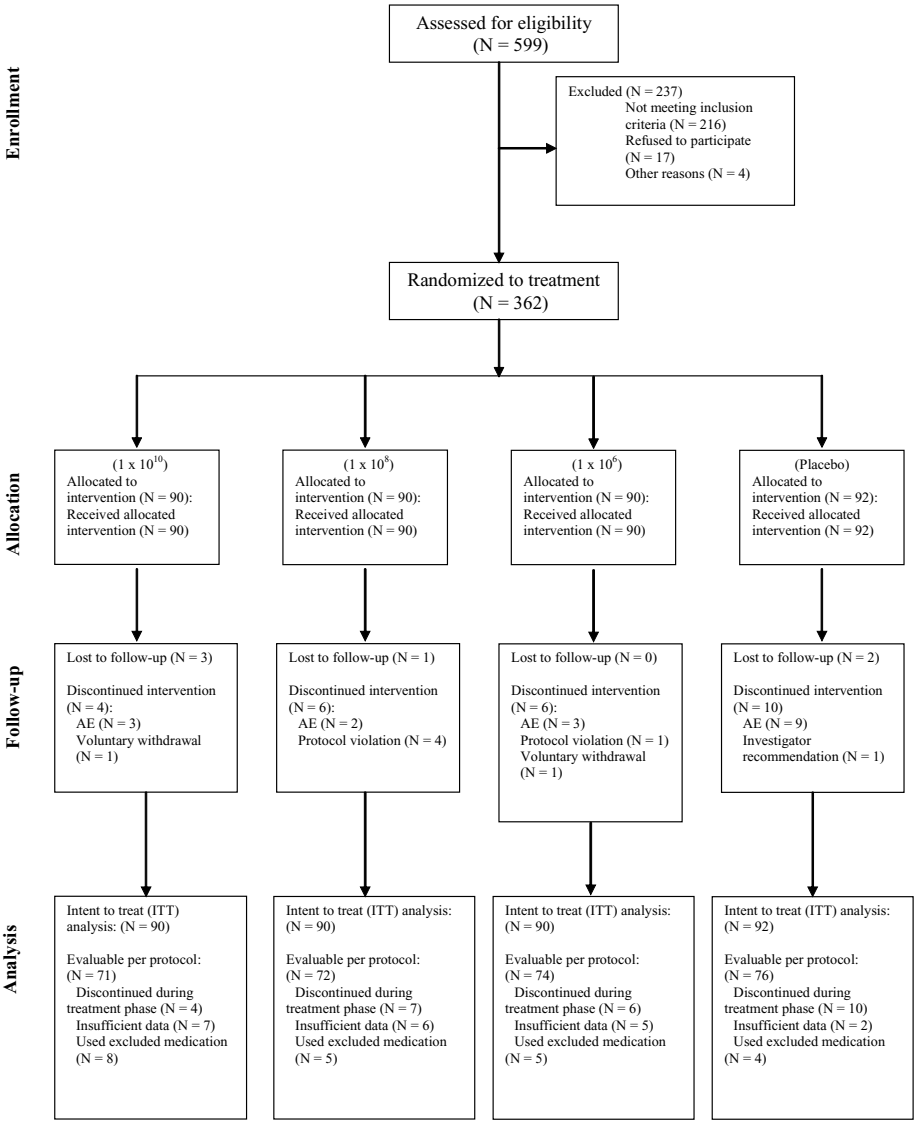

Figure 1. Diagram of flow of subjects through the study protocol.

coupled with reports of abnormal colonization of the small bowel in some patients (14, 15), suggests that it is possible that alterations in the bacterial flora of the gut may be of relevance.

The therapeutic potential of probiotic bacteria, especially lactobacilli and bifidobacteria, is the center of considerable interest in a number of fields and gastroenterology is no exception (16). Some of these organisms appear to have properties that might be advantageous, especially in conditions in which there maybe an infectious trigger or an inflammatory component, such as IBS. We have demonstrated that one specific strain, *Bifidobacterium infantis* 35624, stimulates an antiinflammatory response within the host (17, 18), inhibits the growth of pathogenic organisms such as salmonella, and prevents bacterial translocation (19). These properties are not shared by every strain and while combinations of probiotics may have theoretical appeal, such synergies are not always borne out when two or more strains are formally tested together, being found, in some instances, to be antagonistic (18). These observations might explain why, despite their apparent promise, previous trials using other species and strains have been disappointing (20).

In contrast, we recently reported that *B. infantis* 35624 appears to have considerable promise in the treatment of IBS (21). However, the organism was delivered in milk, a formulation which is not optimal or practical for use in clinical practice. The objectives of this study were, therefore, first, to confirm our observations in the pilot study, second, to evaluate a more convenient encapsulated formulation of *B. infantis* 35624 and, third, to establish the optimal dosage for this formulation.

PATIENTS AND METHODS

Study Population

Patients were recruited from 20 primary care centers across the United Kingdom. The study protocol was approved by MREC (Multi Research Ethics Committee), and all subjects

Table 1. Demographic Characteristics (Intent-to-Treat Population)

Demographic	Statistic	Group BIFIDO10 (N = 90)	BIFIDO8 (N = 90)	BIFIDO6 (N = 90)	Placebo (N = 92)	Overall (N = 362)	*p* Value*
Age	Mean (SE)	41.8 (1.10)	42.7 (1.10)	40.8 (1.10)	42.4 (1.09)	41.9 (0.55)	0.6206
	Min–max	22–63	20–62	20–65	19–69	19–69	
Alcohol (units/wk)	Mean (SE)	4.42 (0.54)	3.77 (0.54)	3.59 (0.54)	5.32 (0.53)	4.28 (0.27)	0.0915
	Min–max	0–28	0–20	0–18	0–21	0–28	
Height (cm)	Mean (SE)	163 (0.79)	163 (0.79)	163 (0.79)	164 (0.78)	163 (0.39)	0.8992
	Min–max	147–176	149–180	101–179	145–179	101–180	
Weight (kg)	Mean (SE)	69.2 (1.57)	71.6 (1.57)	71.7 (1.57)	71.4 (1.55)	71.0 (0.78)	0.6317
	Min–max	46–118	48–155	48–130	50–118	46–155	
Smoker?–N (%)	No	73 (81.1)	67 (74.4)	74 (82.2)	70 (76.1)	284 (78.5)	0.5139
	Yes	17 (18.9)	23 (25.6)	16 (17.8)	22 (23.9)	78 (21.5)	
Race–N (%)	Caucasian	89 (98.9)	89 (98.9)	89 (98.9)	90 (97.8)	357 (98.6)	0.5469
	Asian Indian	0 (0.0)	1 (1.1)	1 (1.1)	2 (2.2)	4 (1.1)	
	Multiracial	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	
Sex–N (%)	Female	90 (100)	90 (100)	90 (100)	92 (100)	362 (100)	N/A

*p values were obtained by using analysis of variance or Pearson's χ^2 test.

provided informed consent. Women aged between 18 and 65 yr of age who met Rome II criteria (22) for the diagnosis of IBS and in whom organic diseases, including inflammatory bowel disease, and significant systemic diseases had been excluded were eligible to participate. Subjects were also excluded if they were over 55 yr of age and had not had a sigmoidoscopy or colonoscopy performed in the previous 5 yr, had used antipsychotic medications within the prior 3 months or systemic steroids within the prior month, had suffered from a major psychiatric disorder (DSM-III-R or DSM-IV) (23) within the past 2 years, were pregnant, had known lactose intolerance or immunodeficiency, or had undergone any abdominal surgery, with the exception of hernia repair or appendectomy.

Study Protocol

This was a randomized, double-blind, placebo-controlled, multicenter, dose-ranging study. Each potentially eligible

Table 2. LS Mean Scores for Efficacy Variables at Wk 4: *B. infantis* Compared with Placebo

	LS Mean Scores ± SE (Change from Baseline)—*B. infantis versus* Placebo						
	Placebo	Bifido 1×10^6	*p versus* placebo	Bifido 1×10^8	*p versus* placebo	Bifido 1×10^{10}	*p versus* placebo
Base size	92	90		90		90	
Abdominal pain/discomfort*	1.73 ± 0.10 (−0.58)	1.89 ± 0.10 (−0.42)	0.24	1.43 ± 0.10 (−0.89)	<0.03	1.84 ± 0.10 (−0.47)	0.44
Bloating/distention*	1.96 ± 0.10 (−0.44)	2.04 ± 0.10 (−0.36)	0.54	1.70 ± 0.10 (−0.71)	<0.05	2.07 ± 0.10 (−0.34)	0.43
Urgency*	1.68 ± 0.09 (−0.34)	1.81 ± 0.09 (−0.21)	0.29	1.48 ± 0.09 (−0.54)	0.09	1.63 ± 0.09 (−0.38)	0.71
Incomplete evacuation*	1.73 ± 0.10 (−0.25)	1.67 ± 0.10 (−0.30)	0.69	1.43 ± 0.10 (−0.54)	<0.04	1.86 ± 0.10 (−0.12)	0.34
Straining*	1.63 ± 0.09 (−0.07)	1.46 ± 0.09 (−0.24)	0.19	1.32 ± 0.09 (−0.38)	<0.02	1.65 ± 0.09 (−0.05)	0.89
Passage of gas*	2.04 ± 0.09 (−0.30)	2.13 ± 0.09 (−0.21)	0.47	1.80 ± 0.09 (−0.54)	<0.04	2.10 ± 0.09 (−0.24)	0.63
Bowel habit satisfaction†	2.21 ± 0.09 (−0.26)	2.17 ± 0.09 (−0.29)	0.75	1.92 ± 0.09 (−0.55)	<0.02	2.32 ± 0.09 (−0.15)	0.39
Overall assessment of IBS symptoms*	2.09 ± 0.10 (−0.42)	2.15 ± 0.09 (−0.36)	0.63	1.76 ± 0.09 (−0.76)	<0.01	2.13 ± 0.09 (−0.38)	0.76
Composite score‡	5.91 ± 0.26 (−1.27)	6.09 ± 0.25 (−1.09)	0.59	5.06 ± 0.25 (−2.12)	<0.02	6.22 ± 0.26 (−0.96)	0.36
Global assessment of pain relief§	51.6 ± 6.1	43.2 ± 6.1	0.29	58.8 ± 6.0	0.36	39.1 ± 6.0	0.11
Global assessment of IBS relief§	42.0 ± 6.4	44.0 ± 6.4	0.80	62.3 ± 6.2	<0.02	36.9 ± 6.1	0.51

Wherever available, baseline values are provided.
*Assessed using a 6-point scale where 0 = none and 5 = very severe.
†Assessed using a 6-point scale where 0 = very satisfied and 5 = very dissatisfied.
‡Combined variable of scores for abdominal pain/discomfort, bloating, and bowel habit satisfaction.
§Yes or No answer assessed at end of each treatment wk. No baseline value is available. Percent reported is percent of subjects responding "yes."

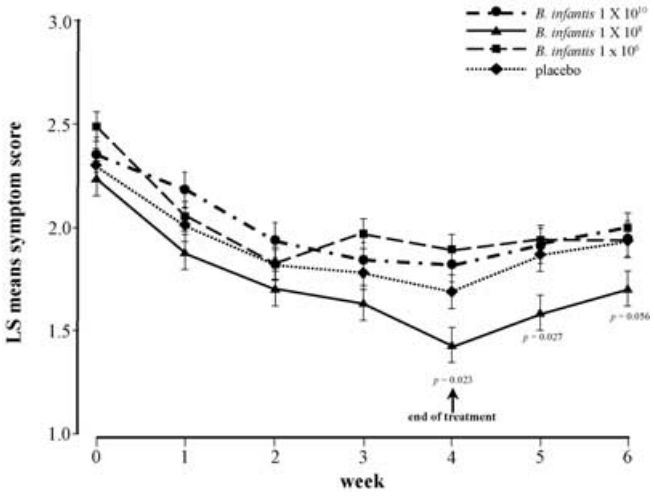

Figure 2. Comparison of effects of placebo and *Bifidobacterium infantis* 35624 on abdominal pain/discomfort. Recorded on a six-point scale from 0 (none) to 5 (severe). Treatment commenced at wk 0 and continued to wk 4.

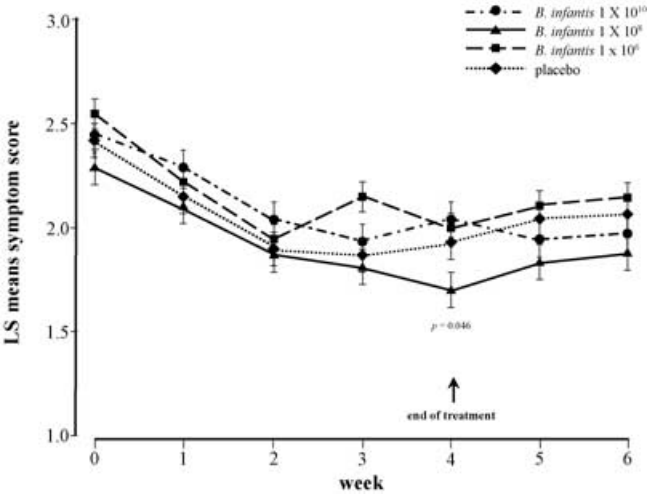

Figure 3. Comparison of effects of placebo and *Bifidobacterium infantis* 35624 on bloating/distension.

patient was evaluated by a full review of clinical history and physical examination as well as full blood count and serum chemistry. Clinically significant abnormalities in any of the latter tests resulted in exclusion of the patient from the study. All clinical assessments were standardized across sites.

Eligible patients entered a 2-wk run-in phase during which symptoms were recorded on a daily basis using a telephone-based interactive voice recording system (IVRS). The second wk of this run-in phase was considered as the baseline period for statistical analysis.

At the end of the baseline period, diary data obtained from the IVRS over the last 7 days were reviewed and subjects with at least 5 days of complete symptom data, at least one bowel movement in that wk, an average abdominal pain/discomfort score of at least one but not exceeding four, and an average Bristol Stool Form score (24) for that wk exceeding two but less than seven, were considered for randomization.

Subjects eligible for the treatment phase were stratified by study center and average Bristol Stool Form scale (scores <4 and ≥ 4) during the last wk of the baseline phase. Subjects were randomly assigned to receive one of four treatments: *B. infantis* 35624 at treatment concentrations of 1×10^6 live bacterial cells, *B. infantis* 35624 1×10^8, *B. infantis* 35624 1×10^{10}, or placebo, in a 1:1:1:1 ratio. Each treatment was provided in an identical capsule and taken once daily for 4 wk during the treatment phase.

On completion of the treatment phase subjects were followed, off all therapy, for a further 2 wk: the follow-up (wash-out) phase. At the end of the study, a full review of clinical history, physical examination, and analysis of full blood count and serum chemistry were performed.

Probiotic Preparation

Clinical product was prepared for this study by The Procter & Gamble Company under good manufacturing process (GMP) conditions. Probiotic bacteria were grown in a protein-rich liquid growth medium by an internationally

Table 3. LS Mean Scores for Efficacy Variables by IBS Subtype—Change from Baseline

	Change in LS Mean Scores—*B. infantis* 1×10^8 *versus* Placebo					
	IBS-D	*p* Value	IBS-C	*p* Value	IBS-A	*p* Value
Base size: Bifido	49		18		18	
Placebo	56		18		23	
Abdominal pain/discomfort	−0.29	0.099	−0.58	**0.036**	−0.24	0.403
Bloating/distention	−0.33	0.056	−0.15	0.622	−0.25	0.362
Urgency	−0.29	0.075	0.01	0.978	−0.12	0.623
Incomplete evacuation	−0.45	**0.008**	−0.07	0.834	−0.11	0.730
Straining	−0.39	**0.007**	0.08	0.799	−0.34	0.263
Passage of gas	−0.28	0.073	−0.00	0.995	−0.35	0.169
Bowel habit satisfaction	−0.37	**0.014**	−0.59	**0.047**	0.24	0.355
Overall assessment of IBS symptoms	−0.36	**0.028**	−0.48	0.078	−0.21	0.456
Composite score	−0.99	**0.027**	−1.32	0.074	−0.15	0.838

Values in bold are significant.

recognized fermentation specialist. Probiotic bacteria were harvested through centrifugation, stabilized, freeze-dried, milled, and sieved. The dry powder bacteria were mixed with an excipient and packed into capsules. For this study, the blend of bacteria to excipient was adjusted to achieve the desired dosing concentrations for the active probiotic capsules. Because of the hygroscopic nature of the product, containers used for the clinical product contained an integrated desiccant. Placebo capsules were identical in all aspects, but contained excipient only.

Data Analysis

Throughout the study, subjects recorded symptoms on a daily basis using the IVRS, whereby patients telephoned a voice-prompted recording system and recorded scores for the primary symptoms of IBS (abdominal pain/discomfort, bloating/distension, sense of incomplete evacuation, straining at stool, urgency of bowel movement, passage of gas and mucus, and bowel habit satisfaction). Each was recorded on a six-point scale, which ranged from 0 (none) to 5 (very severe). They also recorded the frequency (number per day) and consistency (Bristol Stool Form (24); seven-point scale) of bowel movements. Rescue medication (bisacodyl 5 mg for constipation, if no bowel movement on four consecutive days or loperamide 2 mg for troublesome diarrhea) usage was also recorded. During the treatment phase, subjects also recorded, at the end of each wk, their global assessment (SGA) of relief for both abdominal pain/discomfort and IBS symptoms. This assessment was obtained by defining the response (Yes or No) to the following question. "Please consider how you felt in the past week in regard to your IBS-related abdominal pain and discomfort (or, your IBS, in particular your overall well-being, and symptoms of abdominal discomfort or pain, bloating or distension and altered bowel habit). Compared to the way you usually felt before beginning the study medication, have you had adequate relief of your IBS-related abdominal pain and discomfort (or IBS symptoms)?" At these same intervals they were also asked to give an opinion as to whether they were receiving an active treatment or a placebo.

At baseline, at the end of the treatment phase and at the end of the study each subject completed an IBS-specific quality of life questionnaire (IBS-QOL) (25) and the Hospital Anxiety and Depression Scale (26).

Statistical Analyses

All data were collected and analyzed independently of the investigators, who did not have access to the data until the study had been completed. Thereafter, investigators had full access to all data. Wk 0 (the end of the second wk of the run-in phase) was considered as baseline in all statistical analyses.

As designated by the protocol, the primary comparison for efficacy was the daily abdominal pain/discomfort score, with the last wk of the treatment period (wk 4) considered as primary and the other wk of the treatment and follow-up

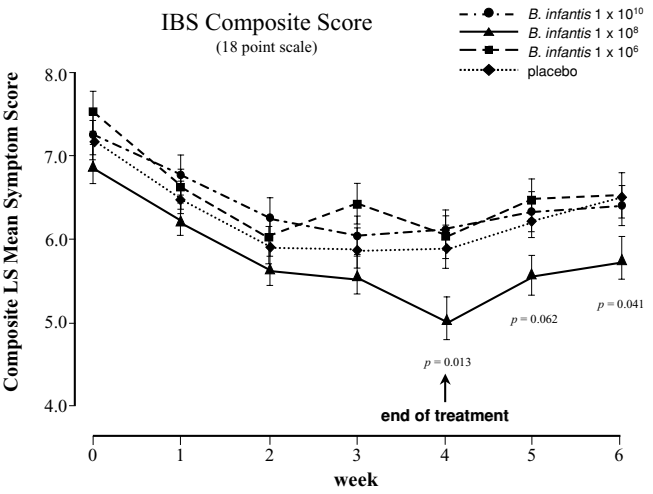

Figure 4. Comparison of effects of placebo and *Bifidobacterium infantis* 35624 on IBS Composite Score. Treatment commenced at wk 0 and continued to wk 4. The Composite Score represents the sum of individual scores for abdominal pain/discomfort, bloating/distension, and bowel movement difficulty (straining at stool or urgency of bowel movement). Recorded on a 0–15 scale.

period considered as secondary. All other efficacy variables were considered as secondary.

Two sets of populations were identified for the purpose of assessing efficacy, namely, the "intent-to-treat" population and the "per-protocol" (PP) population. Efficacy results were similar on both populations, therefore only the results based on the "intent-to-treat" population are reported because this analysis is more conservative. All the statistical comparisons were two-sided using the 0.05 significance level. After adjusting by baseline and taking into account the different sources of variability, the daily IVRS symptom scores were analyzed on a weekly basis using a linear mixed model so that LS means are interpreted as weekly averages. Because an analysis of covariance was performed at each wk, symptom scores and changes in symptom scores are presented as adjusted (least-squares [LS]) means. In the mixed models, the fixed effects were BASELINE and TREATMENTS. The baseline was the average of the repeated measurements during the second wk of the screening phase. The random effects of the linear mixed model were CENTERS, SUBJECTS WITHIN CENTERS, and RESIDUAL. Weekly global assessments were analyzed using a generalized linear mixed model with logistic link and binomial distribution. The fixed effect was TREATMENTS and the random CENTERS.

Based on the data from our prior probiotic study (21)*, we determined that a sample size of 90 women per treatment group would provide at least 95% power to detect a difference of 0.7 unit in the wk 4 abdominal pain/discomfort score on a 0 to 5 point scale (previous probiotic IBS study* used a 0–7 scale) assuming a variance of 1.13 and a two-sided type I error rate of 0.05. Because the study was sized and powered based on a primary efficacy end point at a specific time point, no adjustments for multiple comparisons were made.

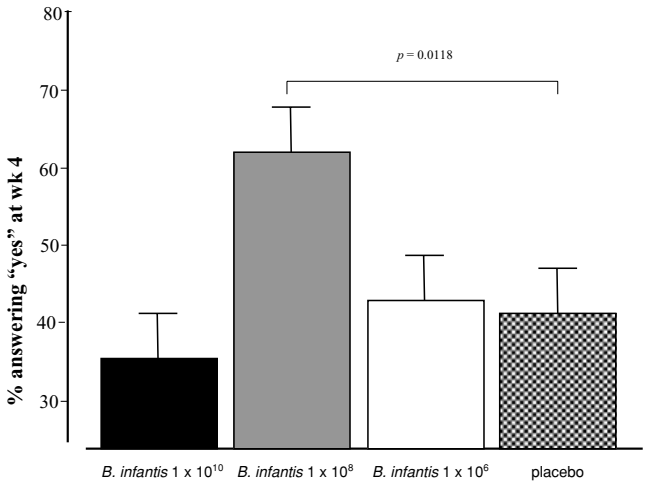

Figure 5. Comparison of effects of placebo and *Bifidobacterium infantis* 35624 on Subjects' Global Assessment (SGA) of IBS symptoms. Positive response rates recorded at wk 4 at the end of therapy. Subjects responded "yes" or "no" to the following question: "Please consider how you felt in the past week in regard to your IBS, in particular your general well-being, and symptoms of abdominal discomfort or pain, bloating or distension and altered bowel habit. Compared to the way you felt before beginning the medication, have you had adequate relief of your IBS symptoms?"

RESULTS

Subjects

Figure 1 describes the flow of subjects through the protocol. A total of 362 subjects were randomized to treatments; 32 discontinued and 330 completed the study. Prior to unblinding of the data, a further 37 subjects were deemed nonevaluable thus providing an intention-to-treat (ITT) population of 362 and a PP population of 293.

Baseline Characteristics

No significant differences were evident between the groups in terms of baseline characteristics (Table 1). Using Rome II criteria and a previously described scoring algorithm (27), 55.5% subjects were classified as diarrhea-predominant IBS (D-IBS), 20.7% as constipation-predominant IBS (C-IBS), and 23.8% of subjects as alternators (A-IBS); at baseline, a similar distribution of IBS subgroups was evident in all treatment groups ($p = 0.55$). No significant differences were found between the *bifidobacterium* 1×10^8 group and the placebo group in terms of the mean scores for the symptom efficacy variables at baseline.

Response to Treatment

PRIMARY EFFICACY VARIABLE—WK 4 ABDOMINAL PAIN/DISCOMFORT. As stated in the methods, wk 4 was declared as the *a priori* time point to be considered the primary end point. At wk 4, the LS mean values for each dose of the probiotic capsule were compared with placebo. Only one of the three dosages studied, *Bifidobacterium* in a dose of 1×10^8, was associated with a significant improvement in the primary variable of abdominal pain/discomfort (change from baseline of -0.89 *vs* -0.58, $p = 0.023$): a therapeutic gain of 0.31 (Table 2, Fig. 2). The other dosages were not significantly different from placebo (1×10^6, change from baseline -0.42 *vs* -0.58, $p = 0.24$; 1×10^{10} change from baseline -0.47 *vs* -0.58, $p = 0.44$).

SECONDARY EFFICACY VARIABLES—WK 4 RESULTS *VERSUS* PLACEBO. Table 3 provides the wk 4 LS mean scores and the change from baseline symptoms scores for the probiotic doses compared with placebo. For the 1×10^8 capsule dose, the secondary variables of bloating/distension (Fig. 3), sense of incomplete evacuation, passage of gas, straining, and bowel habit satisfaction were all significant in comparison to placebo. The magnitude of the therapeutic gain for the other symptoms in which 1×10^8 had a significant benefit was similar to the gain for the primary efficacy variable (0.25–0.31). There was no significant change in the quality of life or HAD scores with any of the probiotic dosages in comparison to placebo.

Comparisons of the effects of the various doses on a composite score (the sum of the individual scores for the three cardinal IBS symptoms: abdominal pain/discomfort, bloating/distension, and bowel movement satisfaction) also demonstrated a significant benefit for the *Bifidobacterium* 1×10^8 capsule over placebo (Table 3, Fig. 4). At wk 4, the reduction in composite score was 2.12 for 1×10^8 and 1.27 for placebo, a therapeutic gain of 0.85 ($p < 0.02$).

Figure 5 illustrates the response rates for the SGA of IBS symptoms. Response rates for *Bifidobacterium* in a dose of 1×10^8 was again superior to placebo, being over 20% greater than placebo ($p < 0.02$).

Subjects were asked each wk to guess whether they were on active or placebo therapy. Among subjects randomized to *Bifidobacterium* in doses of 1×10^6, 1×10^8, 1×10^{10}, and placebo, 48%, 70%, 42%, and 46%, respectively, stated that they were on an active medication when questioned at wk 4; $p < 0.003$ for the 1×10^8 capsule group *versus* placebo.

SECONDARY EFFICACY VARIABLES—RESULTS AT OTHER TIME POINTS *VERSUS* PLACEBO. The probiotic legs were compared with placebo at other time points as part of the secondary analysis. The 1×10^8 capsule continued to show efficacy for abdominal pain/discomfort during wk 5 when compared with placebo ($p < 0.03$). This capsule dosage was also significant *versus* placebo for urgency at wk 3 ($p < 0.04$), reduction in passage of gas at wk 1 ($p < 0.02$) and wk 6 ($p < 0.01$), increased bowel habit satisfaction at wk 6 ($p < 0.02$), and composite score at wk 6 ($p < 0.05$). There were two time points for which the 1×10^6 capsule was superior to placebo for softer stool form using the Bristol Stool Form scale—wk 4 ($p < 0.01$) and wk 5 ($p < 0.02$). There were no time points at which the 1×10^{10} capsule was significantly different from placebo, and additionally, there were no time points for which the placebo capsule was significantly better than any of the probiotic doses.

Table 4. Analysis by Baseline BM Frequency

		Week 4			
		Change from Baseline: Number of Daily BMs			
Baseline Percentile	Average BM/Day	*B. infantis* 35624 1×10^8 capsule	Placebo	Difference	*p* Value
10th	0.71	+0.57	+0.31	+0.25	0.037
15th	0.80	+0.51	+0.27	+0.23	0.049
25th	1.00	+0.36	+0.18	+0.18	0.098
50th	1.43	+0.04	−0.03	+0.07	0.457
75th	2.29	−0.58	−0.44	−0.15	0.145
81st	2.57	−0.79	−0.58	−0.22	0.049
88th	3.00	−1.11	−0.78	−0.33	0.010
90th	3.14	−1.21	−0.85	−0.36	0.007

Stool frequencies at baseline arranged in percentiles and changes from baseline for each percentile calculated and compared between probiotic and placebo groups. Note significant increase in frequency noted for those treated with *Bifidobacterium infantis* 35624, who had lower frequencies at baseline (10th and 15th percentiles), and reduced frequency for those who had higher frequencies at baseline (81st, 88th, 90th percentiles).

ANALYSIS BY IBS SUBTYPE AND BASELINE BM FREQUENCY. To determine the impact of the probiotic on each IBS subtype, two additional *post hoc* analyses were performed. For the first analysis, subjects were categorized based on the Rome II criteria and symptom efficacy and LS mean scores for the effective *Bifidobacterium* dosage of 1×10^8 were compared with placebo within each subtype for each of the efficacy variables. Additionally, the effects of the probiotic on those with different frequencies of bowel movement was evaluated by grouping subjects, at baseline, into percentiles based on BM frequency, and then comparing the change in BM frequency, from baseline, within each percentile group at wk 4 between *Bifidobacterium* 1×10^8 and placebo groups.

The data for each IBS subtype are provided in Table 3. Even with the very small baseline sizes in this subanalysis, there were some significant results. Of interest, first, is that the improvement in scores for bowel habit satisfaction were significant for both the IBS-D and the IBS-C subtypes, and, second, that the assessment of overall IBS relief and the IBS composite score were both significantly improved in the larger IBS-D group and approached significance in the IBS-C group.

The median BM frequency at baseline was 1.43 BM/day, with an interquartile range of 1 BM/day to 2.39 BM/day. The distribution of BM frequencies over the entire range of percentiles is illustrated in Table 4. While there were no statistical differences in the change in BM frequency from baseline between placebo and *Bifidobacterium* at the midpoint of the distribution frequency, significant differences ($p < 0.05$) were noted at both ends (*i.e.,* below the 15th percentile and above the 81st percentile) of the frequency distribution, with the *Bifidobacterium*-treated group experiencing a normalization of bowel habit in each instance (Table 4).

Use of Rescue Medication

Use of rescue medication was low in all treatment groups; no significant treatment effect was noted.

ADVERSE EVENTS. Only 17 (<5%) of all subjects withdrew from the study because of an adverse event. The overall prevalence of adverse events was not different between placebo and active treatment groups.

DISCUSSION

This study provides additional evidence, in a much larger cohort of patients than our pilot study, and in a more convenient formulation, that a specific probiotic strain *B. infantis* 35624 at a dosage level of 1×10^8 cfu, is effective, albeit in a short-term, 4-wk study, in reducing the symptoms of IBS irrespective of bowel habit subtype (IBS-C or IBS-D) and independent of an effect on stool form or frequency. Indeed, subjects at the extremes of bowel habit frequency at baseline experienced a normalization of stool frequency and therapy, with the probiotic demonstrating efficacy among subjects who, at baseline, were defined as constipation- and diarrhea-predominant according to Rome II criteria. We acknowledge, however, that the numbers in some of these subgroups were small and that there were trends toward more efficacy for the IBS-D subgroup.

The effective dosage level of 1×10^8 cfu of *B. infantis* 35624 was effective across a range of IBS symptoms. In this study, we chose pain as the primary outcome measure, in line with the recently proposed points-to-consider for IBS trials developed by the European Agency for the Evaluation of Medicinal Products (EMEA) (CPMP/EWP/785/97: www.emea.eu) (28). We emphasize that although pain or discomfort was the primary outcome measure, other parameters showed significant improvement. In particular, there was a positive effect on bloating, which is a notoriously difficult symptom to treat (29). This more generalized beneficial effect on symptoms is important as some of the traditional medications for IBS only improve one feature, such as pain or bowel habit. Furthermore, when the outcome was evaluated by an instrument which determined the SGA of improvement of IBS symptoms, taken as a whole, it was striking that the therapeutic gain for the 1×10^8 dose was in excess of 20% over placebo, whereas the effects of this dose on individual symptoms was more modest. This suggests that a given SGA of all of their symptoms captures elements of symptomatology,

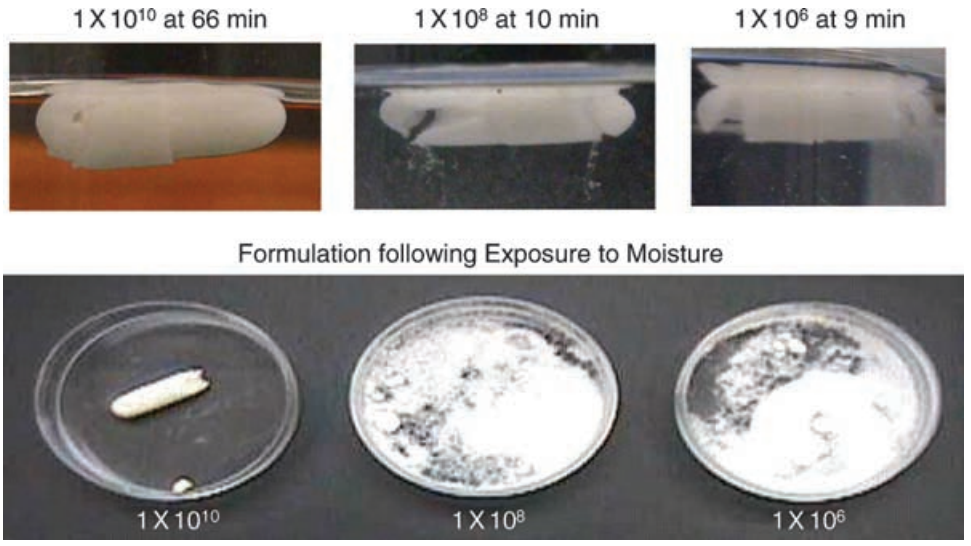

Figure 6. Photographs of results of experiments with study product. The first series of photographs (upper panels) shows the dissolution experiment and time to capsule rupture. Note that for the high-dose capsule, not only is the time significantly longer but also the capsule breaks apart in a different manner. The second series of photographs (lower panels) shows the formulation response to exposure to moisture. Capsules were exposed to ambient temperatures and humidity overnight and then opened. The high dose capsule solidified while, under the same conditions, the lower dosage capsules continued to disperse in a normal fashion when opened.

positively influenced by probiotic therapy, and of considerable clinical significance to the patient, which are not captured by conventional scores of "classical" IBS symptoms. This is not surprising, given the description of the frequent occurrence, in IBS, of other symptoms, both gastrointestinal (30, 31) and extra-intestinal (32–34), which may impact considerably on quality of life. We suggest that some form of global assessment of total IBS symptomatology be an integral part of future studies. This global effect of the probiotic, producing improvement in symptoms other than those usually regarded as primary outcomes in IBS studies, might also explain why, in this study, patients on the effective active treatment had a striking ability to correctly conclude that they were not on placebo. The validity of this observation is supported by the fact that this effect became more marked over the duration of the trial rather than being apparent right from the beginning.

We deliberately chose to study IBS in a primary care setting and to select patients who would be regarded as being at the mild-to-moderate end of the spectrum. We felt that it was important to render the study population as homogeneous as possible and to avoid the various issues that arise when one studies a tertiary referral population. Furthermore, should this product become available, as other probiotic products have, over-the-counter, it is most likely to be sought as an initial therapy by patients with such symptomatology.

This trial demonstrated that *B. infantis* 35624, in a dose of 1×10^8 cfu, provided greatest benefit. Given that the 1×10^{10} dosage level of the same probiotic strain had been demonstrated effective when provided in a milk-based formulation (21), the lack of efficacy with the capsule formulation in this current study was surprising. A series of *post hoc* dissolution experiments found that the dissolution characteristics of the three different strength capsules clearly showed that the highest dose formulation, 1×10^{10} cfu, "coagulated" into a firm glue-like mass which was resistant to acid and intense, prolonged agitation, a phenomenon that can be explained by the intensely hygroscopic nature of this organism (Fig. 6). The formation of such a coagulum would impact the growth characteristics of the organism, especially in the proximal gut, a site which probably contributes to the symptoms of IBS just as much as the large bowel. These findings highlight the need for the development of rigorous quality control standards for this industry and preferably, for clinical trials to be conducted with probiotic products, in the final formulation, prior to their use in clinical practice. Furthermore, it needs to be reiterated that a milk vehicle was used in the pilot study and a capsule in this study.

When compared with the milk-based 1×10^{10} dose used in the previous study, it appears that the encapsulated probiotic formulation may be associated with some delay in the onset of noticeable benefits (21). It seems reasonable to assume that with sustained use, the bacteria would replicate within the gut, eventually reaching the concentrations that were attained following administration of the milk-based formulation, and efficacy would continue to improve with longer use. This notion is supported by the observation that in this trial the slope of the therapeutic response curve had not flattened out by the end of the treatment phase. It is also noteworthy that the beneficial effect was gradually lost after the cessation of treatment, suggesting that more prolonged dosing may be required if an improvement in symptoms is to be sustained. We readily acknowledge that this was a short-term study of only 4 wk duration in a condition that is, by definition, chronic and often intermittent and that further long-term studies will

be required before one can truly assess the impact of this promising therapy in IBS.

The treatment was remarkably well tolerated. While the quality of life and hospital anxiety and depression scores did not improve, this would not necessarily be expected in a trial only lasting 4 wk as these parameters are known to change in either direction slowly. This view is supported by the fact that in the previous trial using a milk vehicle (21), which was of longer (8 wk) duration, quality of life did improve.

B. infantis 35624 has been shown to be superior to *Lactobacillus salivarius* UCC4331 (21) in a head-to-head comparison in IBS, and the results presented here are superior to those achieved previously with other probiotic strains (20, 35–39) suggesting that this particular organism may possess unique properties that are especially important in the management of IBS. Given the increasing evidence that some patients with IBS may have dysregulation of pro- and antiinflammatory cytokines and the demonstration that *B. infantis* 35624 has been shown to restore the balance of these cytokines to normal in such individuals (21), the antiinflammatory effects of this organism may be of particular relevance.

In conclusion, this study has shown that *B. infantis* 35624, delivered in a formulation that is convenient for patient use and when administered for a short, 4-wk period, is a safe and effective treatment for patients with mild to moderate IBS symptoms and has the distinct advantage that it can be given to patients whose IBS is characterized by either diarrhea or constipation.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Irritable bowel syndrome (IBS) is a common disabling disorder.
- Evidence accumulates to implicate mucosal inflammation and abnormalities in the enteric flora.
- Preliminary data suggest a possible role for probiotics.

What Is New Here

- The probiotic *Bifidobacterium infantis* 35624 can be delivered to the human intestine in an encapsulated form in doses of 10^6 and 10^8 cfu.
- *Bifidobacterium infantis* 35624 relieves the primary symptoms of IBS in female subjects.
- This therapy is well tolerated.

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CONFLICT OF INTEREST

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Potential conflicts: Peter J. Whorwell

Linda Altringer, Jorge Morel, Yvonne Bond, and Duane Charbonnean are employees of Procter and Gamble.

Liam O'Mahony is an employee of Alimentary Health and the Alimentary Pharmabiotic Centre.

Barry Kiely is an employee of Alimentary Health.

Fergus Shanahan and Eamonn M.M. Quigley are affiliated with a multidepartmental university campus company (Alimentary Health), which investigates host-flora interactions and the therapeutic manipulation of these interactions in various human and animal disorders. The content of this article was neither influenced nor constrained by this fact.