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ORIGINAL ARTICLE

Assessment of a Multispecies Probiotic Supplement for Relief of Seasonal Allergic Rhinitis: A Randomized Double-Blind Placebo-Controlled Trial

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Abstract

Background: Early phase clinical research provided initial support for the use of a multispecies probiotic supplement to improve quality of life (QoL) in adults with seasonal allergic rhinitis (AR) and reduce the use of AR symptom relieving medication. This study aimed to confirm these early phase findings in a double-blind randomized placebo-controlled trial.

Methods: Individuals, aged 18–65 years, with a minimum 2-year history of AR, moderate-to-severe AR symptoms, and a positive radio-allergosorbent test to Bermuda (Couch) Grass were randomized to receive either a multispecies probiotic supplement (total colony-forming units 4×10^9/day) or placebo twice daily for 8 weeks. A mini-rhinoconjunctivitis quality of life questionnaire (mRQLQ) scale was administered at screening, days 0, 28, and 56. The proportion of participants with a >0.7 improvement in mRQLQ was the primary outcome. Participants also completed a daily symptom and medication diary during the supplementation period.

Results: There were 165 participants randomized, with 142 included in the primary outcome analysis. The percentage of participants meeting the threshold for a clinically meaningful reduction in the mRQLQ from days 0 to 56 was not significantly different between groups (61% vs. 62%, $p = 0.90$). However, 76 participants had a clinically meaningful improvement in QoL (decrease in mRQLQ >0.7) prior to the start of supplementation (screening to day 0).

Conclusion: Changes in self-reported QoL and other disease severity metrics between screening and the start of supplementation limited the ability to discern an effect of supplementation and highlight the need for adaptive clinical trial designs in allergy research.

Clinical Trial Registration: The trial was registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12619001319167).

Keywords: allergic rhinitis, probiotics, clinical trial design, mRQLQ

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Introduction

Allergic rhinitis (AR) affects up to 30% of the general population with symptoms, including, but not limited to, rhinorrhea, sneezing, and nasal congestion. The prevalence of AR is increasing[1] with a significant burden on quality of life (QoL) and well-being, impaired performance, loss of productivity, and health care costs.[2] Early phase studies suggest that probiotics can improve symptoms and QoL in individuals suffering from AR.[3,4] A recent meta-analysis that included 21 double-blind randomized controlled trials and two cross-over trials with supplementation periods ranging from 3 weeks to 6 months concluded that probiotics were associated with improved QoL and that over two-thirds of studies examining the effect of probiotics on AR had found significant clinical benefits.[4–6] Given evidence indicates that not all probiotics exert a beneficial effect on AR symptoms; there is a continued requirement for clinical research to determine the effects of specific strains to guide the use of these supplements.

AR is considered a chronic inflammatory disease driven by T helper 2 (Th2) inflammatory mediators. There are several potential mechanisms of action of probiotics for AR. Probiotic bacteria may reduce Th2 related antigen-specific Immunoglobulin E (IgE) and change the balance of inflammation. Animal models of probiotics and allergy suggest that probiotics may alter inflammation through regulatory T cells (Foxp3+).[7] The effect of probiotics on the immune system may also be mediated through effects on the gut microbiome and intestinal epithelial integrity or ''leaky gut.''[8] A breakdown in integrity of the epithelial barrier is implicated in several health conditions by allowing the translocation of bacterial products into the systemic circulation that activates a range of inflammatory and immune-signaling pathways, including allergic disease.[9] Evidence from *in vitro* and animal models[10] indicates that probiotic bacteria may have beneficial health outcomes by improving intestinal epithelial integrity. Modulation of the intestinal immune system and improving intestinal epithelial integrity are two avenues by which probiotic bacteria may reduce the severity of AR symptoms.

The authors have shown in a previous Phase II single-arm open-label study that supplementation with Ecologic® AllergyCare (Winclove Probiotics B.V., The Netherlands) improved QoL scores measured using the mini-rhinoconjunctivitis quality of life questionnaire (mRQLQ) and reduced the severity of symptoms and the use of medication in seasonal AR.[11] These findings support previous preclinical[12] and clinical research that demonstrates modulation of Th2 mechanisms and a beneficial effect on the development of atopic disease in children.[13] The overall objective of the current study was to confirm the effects of Ecologic® AllergyCare on QoL and the severity of symptoms over 8 weeks in individuals with AR in a larger double-blind randomized placebo-controlled trial.

Materials and Methods

Study design

The randomized double-blind placebo-controlled trial recruited community dwelling Australian adults in the (southern hemisphere) Spring/Summer months of September through March (2019–2020; 2020–2021) when AR symptoms are most severe (Fig. 1). Participants underwent screening as described below, before eligible participants were randomized to receive either a probiotic supplement or placebo twice daily for 56 days. At days 0, 28, and 56 participants completed the mRQLQ.[14] During the 56-day supplementation period participants were asked to complete a daily symptom and medication diary (SMD). The study protocol was reviewed and approved by the Griffith University Human Research Ethics Committee (Ref. No. 2019/474) and registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12619001319167). All subjects provided written and informed consent prior to participation.

Participants

Participants were both male and female, aged between 18 and 65 years, and required to have a minimum 2-year history of moderate-to-severe persistent AR based on the Allergic Rhinitis and its Impact on Asthma criteria.[15] For inclusion in the study participants were also required to return a positive radio-allergosorbent test (RAST) to Bermuda (Couch) grass. Those returning a positive RAST result were randomized to either probiotic or placebo. Individuals returning a negative RAST were excluded from further participation. Other exclusion criteria included: non-AR, reported history of nasal polyposis or chronic obstructive pulmonary disease, reported treatment with systemic corticosteroids in the prior 6 months, reported treatment with

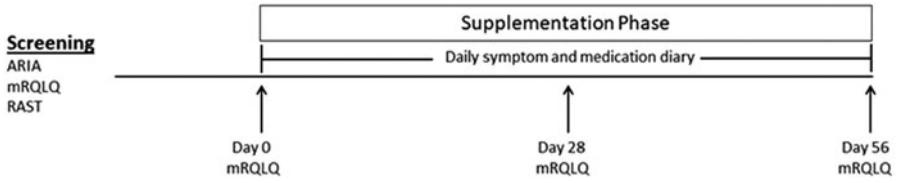

FIG. 1. Representation of the study design, including an initial screening assessment (incorporating assessment of AR history and severity and RAST-confirmed sensitivity to Bermuda grass) and 56-day supplementation phase with daily assessment of symptom severity and periodic assessment of quality of life using the mRQLQ. AR, allergic rhinitis; ARIA, allergic rhinitis and its impact on asthma; mRQLQ, mini-rhinoconjunctivitis quality of life questionnaire; RAST, radio-allergosorbent test.

antibiotics in the prior 30 days, current use of a probiotic/prebiotic supplement, symptoms consistent with current respiratory tract infection at the time of randomization, pregnant/actively trying to become pregnant, or history of sensitivity/intolerance to ingredients of either supplement.

Intervention

Participants were randomized to receive either a probiotic supplement or a placebo twice daily. Supplements were provided as 2 g sealed sachets to be dissolved in 100 mL of water and consumed orally. The probiotic supplement contained six bacterial strains, including *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Lactobacillus acidophilus* W55, *Lacticaseibacillus casei* W56 (formerly known as *Lactobacillus casei* W56), *Ligilactobacillus salivarius* W57 (formerly known as *Lactobacillus salivarius* W57), and *Lactococcus lactis* W58 (total colony-forming units: 4×10^9/day), as well as vitamin B2 (35 mg/100 g), biotin (750 μg/100 g), maize starch, and maltodextrins. The placebo supplement contained maize starch, maltodextrins, and tartrazine (E102) coloring. The supplements were identical in color, taste, and mouthfeel. Compliance was assessed by counting the number of sachets remaining at the end of the intervention period and reporting of missed doses using a daily SMD.

Participants were permitted to use over the counter products/medicines for symptomatic relief if considered necessary during the intervention period, including: saline nasal irrigation, topical steroid (e.g., Mometasone furoate, Budesonide, or Fluticasone propionate) or antihistamine nasal sprays, nonsedating oral antihistamines (e.g., Cetirizine hydrochloride, Fexofenadine hydrochloride, or Loratadine), or oral decongestants (e.g., Phenylephrine). Participants were instructed to record details of the use of any medications in the daily SMD. Use of symptom-relieving medication was discouraged in the 48 h prior to the assessments at days 0, 28, and 56.

Outcome measures

The mRQLQ is a validated survey consisting of 14 items across 5 domains (activity limitations $n=3$; practical problems $n=2$; nose symptoms $n=3$, eye symptoms $n=3$, other symptoms $n=3$) with a minimum score of 0 (no impacts on QoL) and a maximum possible score of 6, designed to assess the degree to which AR symptoms impact on an individuals' QoL.[14] The mRQLQ was administered on days 0, 28, and 56. The primary outcome was a change in the proportion of participants reporting a >0.7 decrease in mRQLQ; a decrease of 0.7 or more has been previously defined as a clinically meaningful change.[14]

Secondary outcomes included the proportion of patients whose numeric score on the mRQLQ dropped from >3.0 at day 0 to <2.5 at day 56 and change in mean mRQLQ score. Further secondary outcomes were assessed using the SMD, which included questions related to severity of overall and specific symptoms (nasal itch, eye itch, sneezing, runny nose, postnasal drip, unrefreshed sleep, and sinus pain). Participants were asked to score the severity of each symptom on a 10-point visual analog scale (VAS) where 0 = "no distress" and 10 = "unbearable distress." The weekly average of the overall symptom severity score was calculated to monitor the change in overall severity for each of the 8 weeks of supplementation. The severity scores for the individual symptom domains were totaled for each SMD entry and the average weekly total severity score calculated. The number of days participants reported use of allergy medications was tallied for the first half and the second half of the supplementation period and expressed as a percentage of the completed daily diary entries over the same period.

Randomization and allocation concealment

Participants were allocated to intervention or placebo in a 1:1 ratio. Randomization was stratified by mRQLQ at screening (3/4/5/6) and occurred in blocks of four. The randomization schedule was constructed using computer generated random numbers. Randomization was performed by the study biostatistician (R.S.W.), using blinded group allocations only. The investigators responsible for the day-to-day conduct of the trial remained unaware of the blinded group allocations until all participants had completed the supplementation phase. Between-group comparisons were initially performed using the blinded group allocations. Treatment groups were unblinded by Winclove Probiotics B.V. upon completion of the data analysis.

Sample size estimate

The sample size for the study was estimated based on assumptions from our prior Phase II open label trial in which the authors observed a 60% response rate (based on the 0.7 mRQLQ change threshold) in response to 56 days of probiotic supplementation.[11] A sample size of 150 participants ($n=75$ per group) was determined to be sufficient to detect a difference in the primary outcome from 30% in the placebo group to 52% in the supplement group with >80% power and $\alpha=0.05$. To account for loss to follow-up and protocol deviations, a total of 160 participants were recruited to the study.

Statistical analysis

For demographic and baseline characteristics, the categorical data were tabulated by frequency and percentage and continuous data reported as mean and standard deviation. For participants with follow-up mRQLQ scores, the proportions of participants with a >0.7 change in mRQLQ were compared between groups using a two-sided Chi-squared test as the primary outcome. A Chi-squared test was also used to compare the proportion of participants with mRQLQ scores changing from >3 (day 0) to <2.5 (day 56) and reporting of allergy medication use. mRQLQ and VAS scores were compared between groups using independent sample *t* tests. Differences in patterns of change in SMD data between groups were compared using a two-factor (week × group) analysis of variance. Analyses were completed using IBM SPSS Statistics version 26 (IBM Corporation, Armonk, NY). Statistical significance was accepted at $p<0.05$.

Results

Participant flow, tolerability, and compliance

Of the 160 individuals randomized to the intended treatment in the study, primary endpoint data were available for 142 (88.8%). Participant flow is detailed in Figure 2. Fourteen participants withdrew during the supplementation period due to noncompliance with the protocol ($n=9$), adverse reactions ($n=3$, all involving self-reported gastrointestinal distress), or unrelated adverse events ($n=2$). A further four participants completed the intervention period, but did not complete the day 56 mRQLQ questionnaire. Overall compliance was estimated to be >96% based on remaining supplement sachets and reported consumption in the daily SMD data.

Baseline characteristics

Baseline characteristics of participants at recruitment are presented in Table 1. The cohort was largely composed of middle-aged adults with more females than males. Individuals who failed to complete the primary endpoint were less likely to have a family history of AR and had lower concentrations of total IgE and Bermuda grass specific IgE (Supplementary Table S1). For completing participants, the Bermuda grass-specific IgE was significantly higher in the probiotic group ($p=0.04$); otherwise the groups were well matched on key attributes at recruitment (Table 1).

QoL change

Overall, 61% of the participants reported a >0.7 decrease in mRQLQ, the primary outcome, between days 0 and 56. The proportion of participants with a >0.7 decrease in mRQLQ was not significantly different between the two groups at either day 28 or day 56 (Table 2). However, 76 participants had a clinically meaningful improvement in QoL (decrease in mRQLQ >0.7) prior to the start of supplementation (screening to day 0). The proportion of participants with a clinically meaningful improvement (reduction >0.7 on mRQLQ) when stratified by day 0 mRQLQ score is included in Table 3.

Symptom severity and medication use

Assessment of the change in the overall daily symptom severity score indicated that the responses did not differ between groups [Interaction: $F(7,762)=0.15$, $p=0.99$];

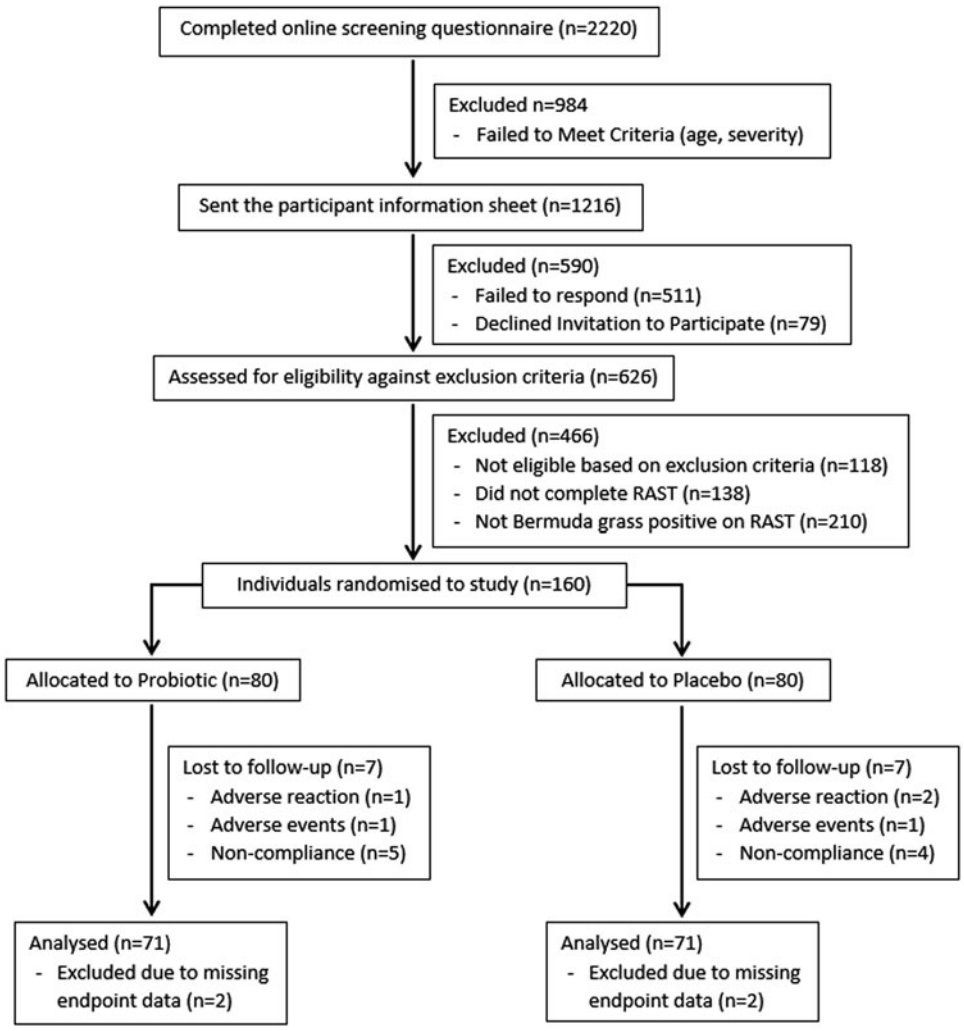

FIG. 2. Study flow diagram indicating participant progression and retention for an 8-week randomized, double-blind placebo-controlled trial of a multispecies probiotic supplement. RAST, radioallergosorbent test.

Table 1. Demographic Characteristics, Disease History, and Baseline Blood Measures for All Recruited Participants

	Randomized			*Primary outcome available*		
	Probiotic (N = 80), n *(%)*	*Placebo (N = 80),* n *(%)*	p	*Probiotic (N = 71),* n *(%)*	*Placebo (N = 71),* n *(%)*	p
Age (years)	42.4 ± 13.1	42.4 ± 12.8	0.99	43.1 ± 12.9	42.8 ± 12.9	0.87
Female:Male (% female)	51:29 (64)	51:29 (64)	1.0	44:27 (62)	45:26 (63)	0.86
BMI (kg/m^2)	27.4 ± 4.7	26.4 ± 5.0	0.21	27.1 ± 4.3	26.5 ± 5.2	0.42
Allergic disease history						
Eczema	26 (32.5)	28 (35.0)	0.74	21 (29.6)	25 (35.2)	0.47
Urticaria	21 (26.3)	18 (22.5)	0.58	16 (22.5)	17 (23.9)	0.84
Food allergies	28 (35.0)	21 (26.3)	0.23	22 (31.0)	18 (25.4)	0.46
Family history of allergic rhinitis	43 (53.8)	49 (61.3)	0.34	40 (56.3)	46 (64.8)	0.30
Blood measures						
RCC ($\times 10^{12}$/L)	4.70 ± 0.50	4.70 ± 0.40	0.98	4.68 ± 0.50	4.69 ± 0.40	0.89
WCC ($\times 10^9$/L)	6.82 ± 1.64	6.91 ± 2.02	0.75	6.74 ± 1.61	6.92 ± 2.00	0.56
Neutrophils ($\times 10^9$/L)	3.86 ± 1.31	3.81 ± 1.52	0.83	3.79 ± 1.28	3.80 ± 1.46	0.98
Lymphocytes ($\times 10^9$/L)	2.10 ± 0.65	2.23 ± 0.56	0.17	2.09 ± 0.66	2.25 ± 0.57	0.11
Monocytes ($\times 10^9$/L)	0.52 ± 0.14	0.52 ± 0.14	0.93	0.53 ± 0.14	0.52 ± 0.14	0.81
Eosinophils ($\times 10^9$/L)	0.28 ± 0.18	0.30 ± 0.31	0.93	0.28 ± 0.18	0.30 ± 0.33	0.61
Basophils ($\times 10^9$/L)	0.06 ± 0.04	0.07 ± 0.07	0.51	0.06 ± 0.04	0.07 ± 0.07	0.42
CRP (mg/L)	2.5 ± 2.8	2.9 ± 3.9	0.47	2.54 ± 2.86	2.92 ± 4.02	0.52
Total IgE (kIU/L)	362 ± 618	317 ± 436	0.59	393 ± 649	343 ± 455	0.59
Bermuda grass (kU/L)	6.4 ± 9.8	3.9 ± 5.0	**0.04**	6.9 ± 10.3	4.1 ± 5.1	**0.04**

Significant *p*-values at $p < 0.05$ are in bold.
Data are presented as mean ± SD or count (percentage).
BMI, body mass index; CRP, C-reactive protein; RCC, red cell count; SD, standard deviation; WCC, white cell count.

however a general reduction in severity over time was noted [Week: $F(7,762) = 4.80$, $p < 0.001$] (Fig. 3). Similarly, the pattern of change in the total symptom severity score did not differ between groups [Interaction: $F(7,763) = 0.15$, $p = 0.99$], but collectively trended downwards over the supplementation period [Week: $F(7,759) = 6.03$, $p < 0.001$] with scores generally higher in the placebo group relative to the probiotic group [Group: $F(1,763) = 12.07$, $p = 0.001$] (Fig. 3). Reported use of allergy medications for symptom relief was largely consistent between the first (33%–37%) and second (32%–34%) half of the supplementation period and not significantly different between the groups (Table 2).

Discussion

In early phase clinical research, supplementation with a multispecies probiotic supplement had been associated with

Table 2. Quality of Life and Symptom Severity Metrics in Response to Supplementation

	Probiotic (n = *71*)	*Placebo* (n = *71*)	p
mRQLQ			
Proportion with >0.7 change			
Days 0–28	52%	52%	1.0
Days 0–56	62%	61%	0.86
mRQLQ score			
Day 0	3.1 ± 1.3	3.2 ± 1.2	0.45
Day 28	2.1 ± 1.3	2.3 ± 1.3	0.43
Day 56	1.9 ± 1.5	2.0 ± 1.4	0.64
Proportion moving from >3 at day 0 to <2.5 at day 56	28%	27%	0.85
VAS (scored 0–10)			
Day 0	2.9 ± 2.2	3.4 ± 2.4	0.23
Day 56	2.3 ± 2.3	2.5 ± 2.3	0.50
Allergy medication use (% SMD entries)			
Days 0–28	33 ± 37	37 ± 39	0.55
Days 29–56	32 ± 39	34 ± 40	0.79

Allergy medication use was calculated as the proportion of completed SMD entries in which the participant reported use of allergy medications. Data are expressed as mean ± SD or count (percentage).
mRQLQ, mini-rhinoconjunctivitis quality of life questionnaire; SD, standard deviation; SMD, symptom and medication diary; VAS, visual analog scale.

Table 3. Number and Proportion of Participants Reporting a >0.7 Mini-Rhinoconjunctivitis Quality of Life Questionnaire (mRQLQ) Change at Day 56 (Relative to Day 0), Stratified by Day 0 mRQLQ Score

	Probiotic (n = *71*)	*Placebo* (n = *71*)	p
Day 0 mRQLQ	Change >0.7/total (%)		
>0	44/71 (62)	43/71 (61)	0.86
>1.50	43/65 (66)	36/62 (58)	0.35
>2.50	30/46 (65)	33/54 (61)	0.67
>3.50	20/27 (74)	22/34 (65)	0.43
≥4.50	10/11 (91)	4/8 (50)	0.14

mRQLQ, mini-rhinoconjunctivitis quality of life questionnaire, score ranges from 0 to 6.

improvement in seasonal AR QoL metrics that warranted continued investigation through a phase 3 clinical trial.[4,11] Furthermore, the Phase II study provided key information to underpin the strategy for recruitment, the timing for initiation of supplementation, the outcome measures, and the assumptions for sample size.[11]

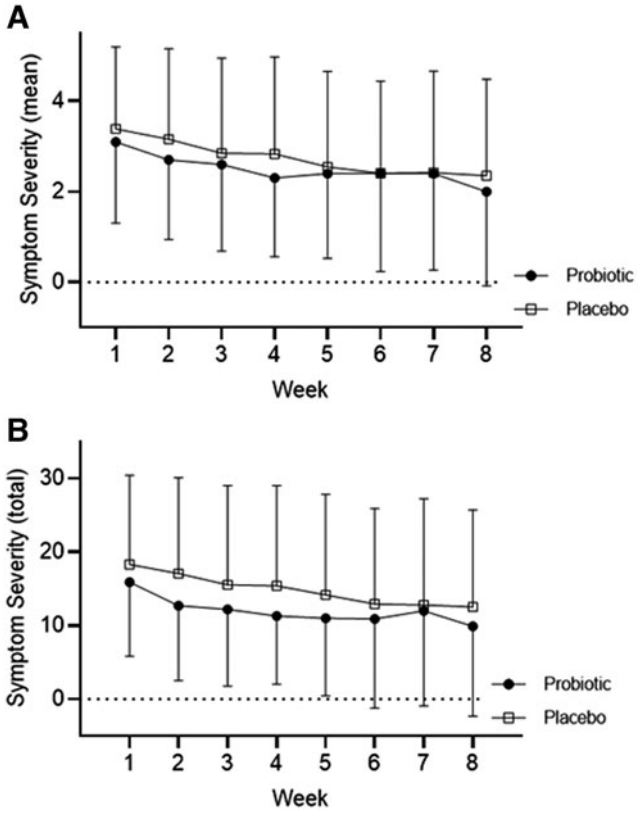

FIG. 3. **(A)** Weekly average overall symptom severity score reported using 10-point VAS and **(B)** total symptom severity score (calculated from the VAS scores for nasal itch, eye itch, sneezing, runny nose, postnasal drip, unrefreshed sleep, and sinus pain) collected using a daily symptom and medication diary completed during the supplementation period. VAS, visual analog scale.

Consistent with our early phase trial, participants in this study were screened, recruited, and commenced supplementation on a rolling basis throughout the grass pollen season. The authors also recruited individuals who may have been sensitized to other aeroallergens, in addition to Bermuda Grass, although did not test for this. This approach means that participants were likely to encounter peaks in local pollen levels at different points during the supplementation phase. Such fluctuations in allergen exposure may also be one explanation for the degree of variation in symptom severity noted prior to the start of our supplementation period.

Probiotics have been shown to reduce the overall burden of allergic disease.[4,5] In addition to assessing changes in QoL, incorporation of additional tools to adequately capture changes in the number or severity of flares over the supplementation period could have been considered. Likewise, use of specific instruments for collection of information on particular environmental exposures (e.g., garden work, localized weather events) may have been beneficial to understanding the high heterogeneity in individual patterns of symptoms throughout the study. Appropriate recognition of such experiences and challenges is crucial to both improving design aspects of future clinical trials utilizing supplementation strategies to alleviate AR and critically evaluating studies reporting positive clinical outcomes, or otherwise.

For chronic conditions such as AR with flaring symptoms, the timing of any intervention in relation to disease pathogenesis is challenging, particularly in relation to allergen exposure. In relation to probiotics, one study in a cohort of 250 children was initiated 3 months prior to the onset of seasonal AR symptoms and reported an ~60% reduction in symptom severity and reductions in medication use.[16] However, a similar approach taken in an investigation of *Escherichia coli* strain Nissle 1917, in which probiotic supplementation was initiated 2 months before the onset of the grass pollen season, was not associated with any clinical effect.[17] In contrast, and similar to our design, delivery of a probiotic mixture during a grass pollen season in 425 individuals significantly improved rhinitis QoL 5 weeks after supplementation began.[18] Also of note, the composition of the supplements differed between these studies and the current investigation which could be a further contributor to the variable clinical outcomes; indeed species-specific effects of probiotic supplements have been recognized for over a decade in consensus statements from experts in probiotic science.[19]

Finally, while the authors have previously shown that fecal recovery of ingested probiotic bacteria can continue to increase for 14 days following initiation of probiotic supplementation,[20] the authors did not assess the extent of colonization in this study and so are unable to relate changes in symptomatology with peak colonization. As a preventive strategy that aims to alleviate chronic and acute symptoms of seasonal AR, initiating probiotic supplementation with sufficient time for the ingested species to exert immune modulatory effects that may be necessary for perceived improvements in symptoms remains an important consideration.

Limitations

The present study is an extension of prior work that has included assessment of six probiotic strains in preclinical

models that demonstrated effects on aspects of inflammatory dysregulation involved in atopy,[12,21] children with atopic dermatitis,[22] and our own early phase clinical trial.[11] Despite this base and the adoption of previously successful study design elements, the challenges of the inherent variability between individuals' perceptions when utilizing self-reported instruments to detect a clear effect at a predefined threshold are considerable. That said, the authors do note outcomes from a prior study, assessing supplementation with a three-strain probiotic (*Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *Bifidobacterium longum* MM-2) for 8 weeks in 173 individuals. Although not meeting the criteria set by Juniper et al for a clinically significant effect of 0.7 of a change,[14] a significant 0.68 reduction in the mRQLQ compared to a nonsignificant reduction of 0.19 in the mRQLQ in the placebo group was noted, with both groups beginning the supplementation period with mRQLQ scores below 2.[23]

The complexity and impact of AR symptom variability on the outcomes of clinical trials is well recognized and is the basis for ongoing development of online tools to better define AR symptom profiles that can be used to underpin assumptions for powering clinical trials.[24] An ongoing study that incorporates four study groups: a blinded probiotic and placebo arm, an open label placebo arm, and a no treatment arm,[25] will also provide a better understanding of the natural variation of seasonal AR to further inform study design considerations. This natural variation was evident in the current study, where the authors observed large interindividual variation in mRQLQ reporting, notably in the period ($n = 42$ days) between screening to the trial and the start of supplementation, with QoL scores in almost a third of participants indicating that their AR symptoms were only modest. While the screening and enrolment approaches across the allergy season meant that the study had a high ecological validity, the change in mRQLQ score from initial screening to the start of supplementation may have negated the likelihood that supplementation would be associated with a clear treatment benefit. This variability may have also contributed to the lack of clear effect in the stratified analysis (Table 3), with only a small proportion of the cohort having mRQLQ scores >4.5 at day 0. Trial designs that can be adapted should the assumptions underpinning the trial change during implementation will also provide greater opportunity to meet the prespecified power and sample size estimates.

The use of probiotic supplements as a complementary strategy for alleviation of AR symptoms remains a promising area. Based on this study, the effectiveness of the multispecies probiotic supplement for seasonal AR in this research is inconclusive and based on previous early phase clinical evidence[11] remains a promising strategy for AR.

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Authors' Contributions

A.J.C., N.P.W., A.W.C., R.S.W., and I.B.v.d.V. contributed to the experimental design. A.J.C., R.R., and N.P.W. contributed to the study conduct and data collection and data analysis. A.J.C., R.R., N.P.W., and R.S.W. contributed to the data analysis. A.J.C., R.R., and N.P.W. contributed to the data analysis. All authors contributed to the interpretation of data and the article preparation.

Author Disclosure Statement

I.B.v.d.V. is employed at Winclove Probiotics B.V. This does not alter this authors' adherence to all publication policies on sharing data and materials. All other authors have no conflicts of interest to declare.

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Supplementary Material

Supplementary Table S1

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