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RESEARCH LETTER

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Probiotics-impregnated bedding covers for house dust mite allergic rhinitis: A pilot randomized clinical trial

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1 | INTRODUCTION

Allergic rhinitis (AR) affects up to one-third of the adult population and causes illness and disability world-wide.[1] Main symptoms include sneezing, rhinorrhea and nasal obstruction. AR is often accompanied with conjunctivitis, characterized by watery, itchy and sometimes red or swollen eyes. House dust mite (HDM) allergens are one of the most relevant indoor allergy triggers.[2] Treatment of AR consists of symptomatic medications (mainly antihistamines and nasal corticosteroids) and allergen-specific immunotherapy (in more severe cases).[1] Although HDM avoidance is generally recommended, there is little evidence of beneficial effects on allergy symptoms. Several studies evaluated the effect of HDM avoidance in patients with AR, however, to date only nine studies fulfil the criteria of randomized clinical trials (RCTs).[3] Five of these studies evaluated the use of impermeable bedding covers, alone or in combination with other avoidance measures. Based on these studies, especially the study by Terreehorst et al.[4], it is concluded that the isolated use of impermeable bedding is unlikely to be effective.[3] The use of high efficiency particulate air (HEPA) filters is poorly investigated (two small studies), and results are not convincing.[3] Two small RCTs in the early 1990s suggested some beneficial effects of acaricides as a HDM avoidance measure, but to date, no larger, well-designed RCTs have confirmed the results.[3]

As current evidence for existing HDM avoidance measures is low, new methods are being developed. Purotex® is a probiotics-based textile treatment, which contains five different probiotic and natural bacterial strains of Bacillus species (strains of *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus pumilus*). In laboratory setting, Purotex®-treated fabric showed a reduction of 89.3% of Der p 1 levels compared with untreated fabric (BMA-Labor GbR, Bochum, Germany). However, up to now, the effect of these probiotics-impregnated covers had not been tested in a clinical trial. Therefore, we set out to perform the first pilot RCT to evaluate the effectiveness of Purotex® covers to reduce HDM allergen levels in bedding, and to improve allergy symptoms and quality of life (QoL) of patients with AR to HDM.

2 | METHODS

All patients were recruited via the Department of Otorhinolaryngology in Ghent University Hospital (Belgium). Prior to patient

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enrolment, the trial was registered at *clinical.trials.gov* (NCT01997606).

Patients aged 18-65 years were considered eligible if they had a clinical history consistent with AR to HDM for at least 1 year, a positive skin prick test to *D.Pteronyssinus* (D.*pt*), a positive nasal allergen provocation test (NAPT) to HDM extract (Alyostal, Stallergenes) and sIgE to D.*pt* of at least 0.7 kU/L (ImmunoCAP; Phadia, Uppsala, Sweden) at screening. Moreover, subjects were randomized only when Der p 1 was detectable (≥0.488 ng/mL dust extract, corresponding to ≥9.76 ng/g dust) in dust samples collected from their mattress and/or pillow at screening. This trial was designed as an explorative pilot study with an intended sample size of 20 patients.

Purotex® textile treatment contains five different probiotic and natural (not genetically modified) bacterial strains of Bacillus species. The probiotic spores are encapsulated in microcapsules (2-3 μm), which are diffusely inserted in the textile. Upon friction forces between the cover and the sleeper's body, a small number of the microcapsules rupture and release their probiotic bacteria.

The probiotics-impregnated covers and the untreated (placebo) covers were indistinguishable from one another: they were identical in colour, feeling and smell.

The study was designed as a pilot double-blind, randomized, placebo-controlled, crossover trial (see Figure S1). The study consisted of four periods: (i) a *run-in period* of 4 weeks (without study cover), (ii) a *first study period* of 8 weeks (with probiotics-impregnated covers, resp. placebo covers), (iii) a *washout period* of at least 4 weeks (without study cover) and (iv) a *second study period* of 8 weeks (with placebo covers, resp. probiotics-impregnated covers).

Der p 1 levels in mattress and pillow dust samples were the primary outcome measures. Dust samples were collected by the patients every 2 weeks (day 0, 14, 28, 42, 56).

Symptoms, QoL and use of reliever medication were secondary outcome measures. Questionnaires (Visual Analogue Scales, VAS[5]; Rhinoconjunctivitis Quality of Life Questionnaire, RQLQ[6]; Nocturnal Rhinoconjunctivitis Quality of Life Questionnaire, NRQLQ[7]) were completed weekly.

Additional descriptions of the methods can be found in Appendix S1.

3 | RESULTS

Of 34 screened subjects, 24 subjects met all inclusion and exclusion criteria and were randomized. Four randomized subjects discontinued the trial, and finally, 20 patients were retained for analysis (patient enrolment and dropout are summarized in Figure S2). Patient characteristics at screening are shown in Table S1.

Der p 1 levels, symptom and QoL scores at baseline of the Purotex period and the placebo period were comparable (see Table 1).

A significant and comparable decrease in Der p 1 levels was observed with the placebo covers and the probiotics-impregnated covers (both for mattress and pillow dust samples) (see Figure 1).

TABLE 1 Outcomes: baseline levels

	Purotex period (n=20)	Placebo period (n=20)	*P*-value*
VAS for AR symptoms control, score/100	51.8 (39.5-64.2)	50.8 (38.4-63.1)	.86
VAS for global discomfort, score/100	52.8 (40.5-65.0)	53.0 (40.8-65.3)	.97
VAS for nasal symptoms, score/100	42.8 (32.0-53.6)	40.8 (30.0-51.6)	.67
VAS for eye symptoms, score/100	35.2 (24.2-46.2)	28.6 (17.6-39.5)	.19
VAS for interference with sleep, score/100	43.8 (31.0-56.6)	43.0 (30.1-55.8)	.89
VAS for lower airway symptoms, score/100	15.7 (8.2-23.2)	17.3 (9.8-24.8)	.64
RQLQ total score, score/6	2.3 (1.8-2.7)	2.3 (1.8-2.8)	.88
RQLQ nose symptoms, score/6	2.7 (2.0-3.3)	2.6 (2.0-3.2)	.81
RQLQ eye symptoms, score/6	2.0 (1.4-2.5)	1.7 (1.2-2.2)	.37
NRQLQ total score, score/6	2.2 (1.6-2.7)	2.2 (1.7-2.8)	.77
NRQLQ sleep, score/6	2.0 (1.4-2.7)	2.0 (1.6-2.9)	.50
NRQLQ sleeptime symptoms, score/6	2.1 (1.5 -2.7)	2.0 (1.5-2.6)	.73
Der p 1 in Mattress dust samples, ng/g	336 (128-884)	386 (146-1016)	.60
Der p 1 in Pillow dust samples, ng/g	126 (48-332)	186 (70-496)	.26

Variables are presented as means with 95% Confidence Intervals.
*P-value for difference between Purotex and Placebo period (analysed with Mixed models).
AR, Allergic Rhinitis; NRQLQ, Nocturnal Rhinoconjunctivitis Quality of Life Questionnaire; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; VAS, Visual Analogue Scale.

With the probiotics-impregnated covers, there was a significant overall improvement compared to baseline of several symptoms and QoL scores (see Figure 1 and Figure S3): VAS for subjective control of AR symptoms (*P*=.001), VAS for global discomfort (*P*=.008), VAS for nose symptoms (*P*<.001), VAS for eye symptoms (*P*=.03), RQLQ total score (*P*=.01), RQLQ nose symptoms (*P*=.02), RQLQ eye symptoms (*P*=.02) and NRQLQ total score (*P*=.02). Improvement of symptoms and QoL with the probiotics-impregnated covers occurred mainly during the second half of the study period (week 5-8). By contrast, there was not any significant overall change from baseline with the placebo covers. However, the differences in effect between the probiotics-impregnated covers and placebo were not significant. The only significant *P*-value (*P*=.048 for *NRQLQ sleeptime*) for difference in effect between both study covers was no longer significant after correction for multiple comparisons (Benjamini-Hochberg procedure).

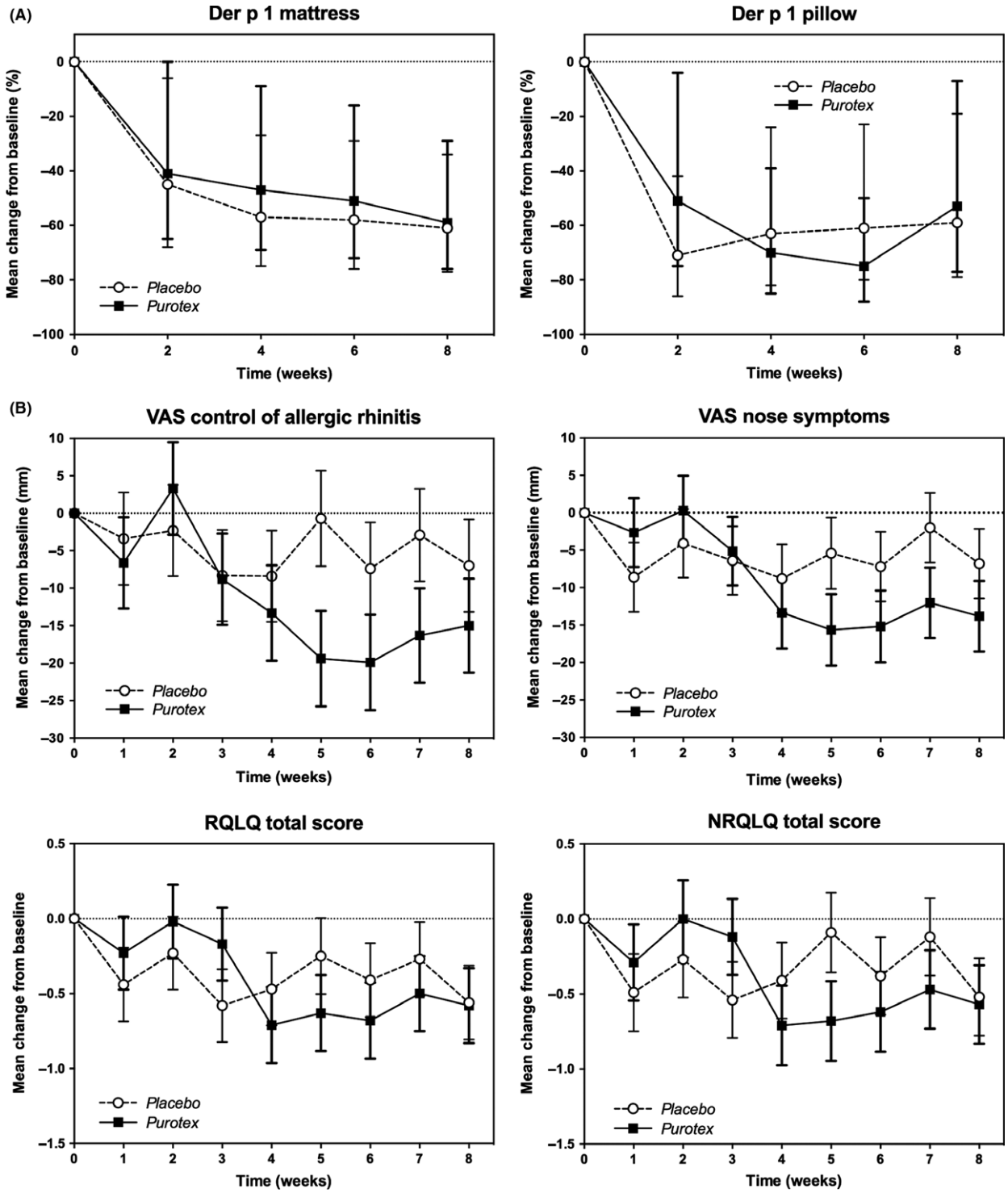

FIGURE 1 (A) Relative (%) change from baseline of Der p 1 concentration in mattress and pillow dust samples, illustrated with line charts (error bars represent 95% CI). *Mattress Der p 1:* significant decrease with both the probiotics-impregnated covers (P=.02) and the placebo covers (P=.003); no difference in effect between both study covers (P=.98). *Pillow Der p 1:* significant decrease with both the probiotics-impregnated covers (P=.001) and the placebo covers (P=.007); no difference in effect between both study covers (P=.36). (B) Change from baseline of VAS scores and QoL scores (RQLQ, NRQLQ), illustrated with line charts (error bars represent Standard Errors of the Mean). *VAS for control of AR*: significant improvement with probiotics (P=.001) and not with placebo (P=.81); no significant difference in effect between both study covers (P=.17). *VAS for nose symptoms:* significant improvement with probiotics (P<.001) and not with placebo (P=.36); no significant difference in effect between both study covers (P=.09). *RQLQ total score:* significant improvement with probiotics (P=.01) and not with placebo (P=.32); no significant difference in effect between both study covers (P=.33). *NRQLQ total score:* significant improvement with probiotics (P=.02) and not with placebo (P=.30); no significant difference in effect between both study covers (P=.14).AR, Allergic Rhinitis; NRQLQ, Nocturnal Rhinoconjunctivitis Quality of Life Questionnaire; RQLQ, Rhinoconjunctivitis Quality of Life Questionnaire; VAS, Visual Analogue Scale

Only minor changes of medication intake were observed, and changes compared to baseline were not significant in both study periods (P=.83 for placebo; P=.45 for probiotics; P=.71 for difference between both periods).

4 | DISCUSSION

We conducted the first pilot, double-blind, randomized, placebo-controlled clinical trial to investigate the effect of probiotics-impregnated covers on (i) HDM allergen levels in bedding, and (ii) allergy symptoms and QoL of patients with AR to HDM.

Although no difference in effect was observed on Der p 1 levels between the probiotics-impregnated covers and the placebo covers, only the probiotics-impregnated covers were associated with a significant improvement of symptoms and QoL compared to baseline.

This observation may raise the hypothesis that the potential effect of the probiotics on symptoms and QoL may not be (solely) attributable to an effect on HDM allergen levels in bedding, but may be related to direct effects of the probiotics on the patient. Although evidence is rather limited, some studies have suggested beneficial effects of probiotics for treatment or prevention of atopic dermatitis[8] and AR.[9]

However, in this trial, the measured Der p 1 levels were highly variable (both between-subject and within-subject observations) and may not always have reflected the real levels in bedding. Dust sampling is known to be inherently variable,[10] and additional variation in this trial may have resulted from possible improper dust sampling by the patient. It should be noted that the Der p 1 levels in this trial were markedly lower than those previously reported, which may explain some of the lack of effect seen in this study. In addition, the Der p 1 levels measured in the pillow dust samples should be considered less reliable, as the pillow dust samples were often inadequate in mass (19% of samples weighed <10 mg, see Appendix S1). At last, it should be emphasized that both the placebo and the probiotics-impregnated covers were new. Indeed, new bedding material tends to show a lower HDM concentration, which may explain the decrease in HDM allergen levels with both covers.

To our knowledge, this study is the first clinical trial to evaluate any probiotics-based method aiming to reduce HDM allergen exposure. Other studies have investigated the effect of physical (heating, ventilation, freezing, washing, barrier methods, air filtration, vacuuming and ionizers) and/or chemical (acaricides) methods.[3] Terreehorst et al.[4] reported a significant reduction in HDM levels in bedding with impermeable covers compared to non-impermeable covers. Nevertheless, this large study did not reveal a clinical effect on rhinitis symptoms of the impermeable covers compared to the non-impermeable covers (even not a trend).

In the study by Terreehorst et al.[4], patients were encouraged to carry out additional avoidance measures (including weekly washing and cleaning of bedding in water at 60°C, and ventilating the homes according to regular schedules). Hence, the observed improvement of rhinitis symptoms in this study may be attributed to these additional avoidance measures, irrespective of the use of impermeable or non-impermeable bedding covers.

In our study, no additional avoidance measures were applied. Moreover, patients were urged to use their allergy medication not as maintenance treatment, but only when needed. Hence, the observed improvements of symptoms and QoL are unlikely to be attributable to either additional environmental control measures or increased intake of allergy medication.

The use of the bedding covers in our study was generally well tolerated, and no serious adverse events were considered related to Purotex®. Three subjects had an airway infection during the Purotex® period (see Appendix S1); however, it is not possible to speculate on a possible relation with Purotex® based on this small pilot study.

The small sample size of this pilot trial entails a restricted statistical power to show significant differences in effect between the probiotics-impregnated covers and the placebo covers. A power analysis (in Appendix S1) revealed that a potential future large-scale study should include 262 patients to have 90% chance that the currently observed effect of probiotics-impregnated covers on QoL (evaluated with RQLQ total score) would be significant compared to the placebo covers. Other limitations of the current trial include the rather short study period, the possibility of carry-over effects in this cross-over design, the possible influence of seasonal fluctuations in HDM exposure and the lack of control on dust sample collection.

In conclusion, even though the probiotics-impregnated covers compared to untreated covers did not show an effect on the primary outcome measure Der p 1 levels, there was significant improvement compared to baseline of several symptoms and allergy-related quality-of-life scores (secondary outcomes) with the probiotics-impregnated covers and not with the placebo covers. Although the effects of probiotics-impregnated covers were not significant compared to untreated covers in this pilot study, these findings are promising and warrant further exploration in a future large-scale clinical trial.

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CONFLICT OF INTERESTS

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A. Jult, H. Vermeulen, N. De Ruyck, L. Derycke, C. Bachert, B. Lambrecht, M. Dullaers and P. Gevaert have no conflict of interests. H. Ucar and P. Ghekiere are employed by BekaertDeslee; R. Temmerman is employed by Chrisal NV; and John Ellis is employed by Devan Chemicals NV.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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