

## *Lactobacillus rhamnosus* probioticum vermindert postpartum depressie en angst

*Effect of Lactobacillus rhamnosus HN001 in pregnancy on postpartum symptoms of depression and anxiety: A randomized double-blind placebo-controlled trial.*  
*Slykerman et al. (EBioMedicine, 2017)*

Ernstige depressies tijdens de zwangerschap en na de bevalling komen in de Westerse wereld voor **bij 10 tot 15% van de vrouwen**. Een **postnatale depressie** kan niet alleen de zorg en de band van de moeder met het kind beïnvloeden, maar kan ook langdurige gevolgen hebben op de cognitieve en sociaal-emotionele ontwikkeling van het kind.

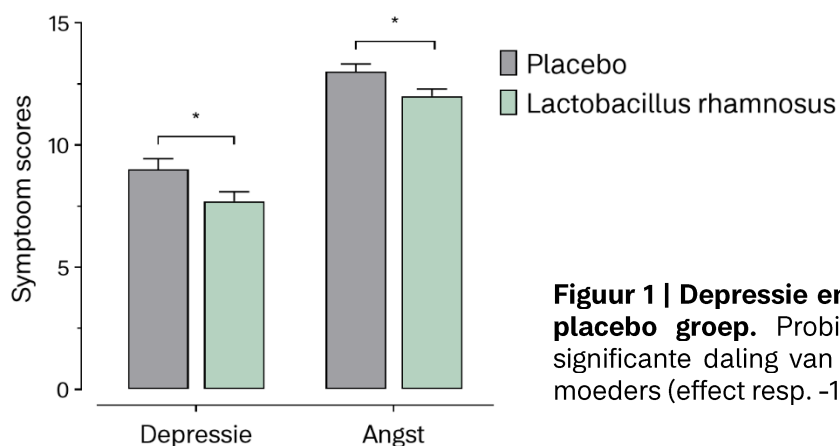
Uit voorgaand onderzoek is reeds geweten dat een gezond dieet geassocieerd is met een verlaagde kans op depressie en **dieet verbeterende interventies effectief zijn in patiënten met matige tot ernstige depressie**. De toename in literaire evidentie die het darm microbiom linkt aan de hersenchemie en het gedrag zorgt voor **meer inzicht in hoe veranderingen in de compositie van ons microbiom de gemoedstoestand kan beïnvloeden**.

Zo toonde een recent systematisch review artikel aan dat **het gebruik van probiotica de gemoedstoestand verbetert en stress en angst verlaagt** in respectievelijk 60 en 70% van de onderzochte studies. Aangezien geen enkel klinisch onderzoek reeds het effect van probiotica bij postnatale depressies heeft bestudeerd, **onderzochten de auteurs van dit artikel of het gebruik van *Lactobacillus rhamnosus* de symptomen van depressie of angst kon beïnvloeden bij zwangere en bevallen vrouwen**.

### Resultaten en Conclusie:

Voor deze studie werden in totaal 423 vrouwen gerecruteerd waarvan de helft het probioticum innamen tot 6 maanden na de geboorte. De resultaten toonden aan dat bij **het gebruik van *Lactobacillus rhamnosus* significant lagere depressie en angst scores** werden gerapporteerd dan in de placebo groep (Figuur 1). Dit verschil was onafhankelijk van de aanwezigheid van huilbaby's.

Uit deze studie werd besloten dat het **gebruik van *Lactobacillus rhamnosus* nuttig kan zijn** tijdens de zwangerschap en tot 6 maanden na de bevalling, **zowel in de preventie als in de behandeling van postpartum depressie of angst**.



**Figuur 1 | Depressie en angst scores in de probioticum en placebo groep.** Probioticum behandeling leidde tot een significante daling van de depressie en angst scores bij de moeders (effect resp. -1.2 en -1.1 vs. placebo; \*p<0.05).





## Research Paper

# Effect of *Lactobacillus rhamnosus* HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial



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## ABSTRACT

**Background:** Probiotics may help to prevent symptoms of anxiety and depression through several putative mechanisms.

**Objective:** The aim of this study was to evaluate the effect of *Lactobacillus rhamnosus* HN001 (HN001) given in pregnancy and postpartum on symptoms of maternal depression and anxiety in the postpartum period. This was a secondary outcome, the primary outcome being eczema in the offspring at 12 months of age.

**Design, Setting, Participants:** A randomised, double-blind, placebo-controlled trial of the effect of HN001 on postnatal mood was conducted in 423 women in Auckland and Wellington, New Zealand. Women were recruited at 14–16 weeks gestation.

**Intervention:** Women were randomised to receive either placebo or HN001 daily from enrolment until 6 months postpartum if breastfeeding.

**Outcome Measures:** Modified versions of the Edinburgh Postnatal Depression Scale and State Trait Anxiety Inventory were used to assess symptoms of depression and anxiety postpartum.

**Trial Registration:** Australia NZ Clinical Trials Registry: ACTRN12612000196842.

**Findings:** 423 women were recruited between December 2012 and November 2014. 212 women were randomised to HN001 and 211 to placebo. 380 women (89.8%) completed the questionnaire on psychological outcomes, 193 (91.0%) in the treatment group and 187 (88.6%) in the placebo group. Mothers in the probiotic treatment group reported significantly lower depression scores (HN001 mean = 7.7 (SD = 5.4), placebo 9.0 (6.0); effect size -1.2, (95% CI -2.3, -0.1),  $p = 0.037$ ) and anxiety scores (HN001 12.0 (4.0), placebo 13.0 (4.0); effect size -1.0 (-1.9, -0.2),  $p = 0.014$ ) than those in the placebo group. Rates of clinically relevant anxiety on screening (score > 15) were significantly lower in the HN001 treated mothers (OR = 0.44 (0.26, 0.73),  $p = 0.002$ ).

**Interpretation:** Women who received HN001 had significantly lower depression and anxiety scores in the postpartum period. This probiotic may be useful for the prevention or treatment of symptoms of depression and anxiety postpartum.

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## 1. Introduction

Major depression in pregnancy and after birth occurs in 10–15% of women in New Zealand, a rate comparable to other western countries

(Abbott and Williams, 2006). Postnatal depression (PND) is associated with persistent depression, and even, in a few cases each year, death from suicide (PMMRC, 2014). This disorder may affect a mother's ability to care for and bond with her new infant, as well as her quality of life and daily functioning (Da Costa et al., 2006). In addition, maternal depression can produce long-lasting effects on children's cognitive, social-emotional and health outcomes (Tronick and Reck, 2009; Grace

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et al., 2003). In addition to depressed mood, PND is associated with hopelessness, excessive fatigue, psychomotor agitation, appetite and sleep disturbances, guilt, or feelings of inadequacy, particularly regarding one's ability to care for the newborn and co-morbid anxiety. Anxiety often coexists with depression and like PND, the prevalence varies widely depending on the timing and the type of disorder (generalised anxiety disorder (GAD) vs obsessive compulsive disorder (OCD)) and the way it is measured (self-report vs structured interview). Despite this, most women with PND are either not recognised as being depressed, are unable to access psychological therapy or are reluctant to take antidepressant medication in pregnancy or while breastfeeding. Furthermore it takes several weeks for the therapeutic effect of antidepressants to appear and there is a 15–30% discontinuation rate (Gartlehner et al., 2005). Safe and effective therapies to prevent and treat PND are needed (Battle et al., 2003).

A “healthy” diet comprising higher intakes of fruit and vegetables, whole grains and lean meat and fish is associated with a reduced likelihood of depression (Lai et al., 2013). These observational studies are supported by a recent randomised controlled trial (RCT) showing that a dietary improvement intervention was effective as an adjuvant therapy in patients with moderate to severe depression who were being treated with psychotherapy or antidepressants (Jacka et al., 2017). Furthermore, it has been suggested that fermented foods alter dietary items before they are ingested, resulting in phytochemical transformation into bioactive chemicals which reduce oxidative stress and inflammation (Selhub et al., 2014).

There is a growing literature linking the gut microbiota to brain chemistry and behaviour via multiple bi-directional pathways (the microbiome-gut-brain axis), including the immune system, neuroendocrine, hypothalamic pituitary adrenal axis (HPA axis), short chain fatty acids or tryptophan and sympathetic and parasympathetic arms of the autonomic nervous system including the enteric nervous system, the vagus nerve, and the gut microbiota (Wang and Kasper, 2014; Dinan and Cryan, 2015). Microbial dysbiosis is associated with many health problems including neuropsychiatric disorders, such as autism spectrum disorder, depression and anxiety, and are associated with elevated levels of pro-inflammatory cytokines, increased oxidative stress, altered gastrointestinal (GI) function, and lowered micronutrient and omega-3 fatty acid status (Dawson et al., 2016). Intriguingly, alterations in the pattern of gut microbial composition in healthy adults influences mood (Li et al., 2016).

Probiotics are live microorganisms that when consumed in adequate amounts provide health benefits to the host (Hill et al., 2014). In 2005 it was first suggested that probiotics might be an adjuvant therapy for major depression (Logan and Katzman, 2005) and others have also suggested that probiotic enhancement of gut microbiota may improve mood outcomes (Dinan and Cryan, 2016). Pre-clinical studies have demonstrated that the anxiety phenotype of mice can be changed with fecal transplantation and that the changes in microbiota are accompanied by changes in brain chemistry (Collins et al., 2013). Furthermore, probiotic treatment has also been shown to have a positive effect on anxiety-like and depressive-like behaviour in animal studies (Bravo et al., 2011; Desbonnet et al., 2010), with mediating mechanisms including GABA receptor expression in specific locations of the central nervous system (Bravo et al., 2011), the HPA axis (Desbonnet et al., 2010) and the vagus nerve which transmits information from the gut luminal environment to the CNS (Carabotti et al., 2015). Interestingly, probiotic supplementation in adults has not been found to substantially alter their gut microbiota as sampled by fecal samples (Kristensen et al., 2016), although this does not exclude their potential effect higher up in the small intestine or on the adherent mucosal gut microbiome.

Clinical trials of probiotic treatment have yielded mixed results, and systematic reviews of human trials concluded that the evidence for beneficial effects of probiotics on mood may not be as strong as some recent narrative studies purport (Romijn and Rucklidge, 2015). A recent systematic review identified 10 clinical trials of the effect of probiotics on

symptoms of depression (Wallace and Milev, 2017). Seven studies were in healthy subjects, 2 in chronic fatigue syndrome and one in depression. Three of 5 studies reported improved mood with probiotics, and 5 of 7 studies reported improvements in stress and anxiety. A recent study that was published after these reviews reported that obese women treated with a weight-reduction programme and probiotic had reduced symptoms of depression compared with the comparison group, but this effect was not seen in men (Sanchez et al., 2017). There was no effect on anxiety. Both reviews suggested further RCTs were needed. To date probiotic effects on postnatal depression have not been studied in a clinical trial.

Our aim was to evaluate whether probiotic supplementation with *Lactobacillus rhamnosus* HN001 (HN001) had a beneficial effect on postnatal symptoms of depression and anxiety in a group of healthy women. This was a secondary outcome, the primary outcome being eczema in the offspring at 12 months of age.

## 2. Methods and Materials

### 2.1. Study Design

The Probiotics in Pregnancy Study (PIP Study) is a two-centre (Wellington and Auckland, New Zealand) randomised double-blind placebo-controlled trial testing the effect of the probiotic HN001 on the development of eczema and atopic sensitization in offspring (the primary outcome) and pregnancy outcomes (secondary outcomes) in women. The full protocol is published (Barthow et al., 2016).

### 2.2. Study Population

The selection of participants, randomisation process and quality control measures have been described in detail previously (Barthow et al., 2016). In brief, 423 women were recruited at 14–16 weeks gestation between December 2012 and November 2014. 212 women were randomised to HN001 and 211 to placebo. Women were considered eligible if they were English-speaking, planning to breastfeed, and if either they or the unborn child's biological father had a history of asthma, hayfever or eczema requiring medication. Women were excluded from the study if aged < 16 years, planning to move outside the study centres during study duration, planning on taking probiotics, or if they had serious medical or health problems related to the pregnancy.

### 2.3. The Intervention

Women were randomised to receive either HN001 at a dose of  $6 \times 10^9$  colony-forming units (cfu) or placebo (corn-derived maltodextrin), to be taken daily from enrolment until birth and, from birth up till six months post-birth whilst breastfeeding. The capsules were indistinguishable. Both researchers and participants were blinded to treatment assignment of participants. To assess adherence, capsule bottles were collected at regular intervals and counts of remaining capsules were completed by an independent person.

### 2.4. Randomisation

Randomisation was managed by Fonterra Co-operative Group Ltd. and concealed from all study staff and participants. Randomisation was stratified by study centre and performed in blocks of random lengths according to a computer-generated randomisation list. Research staff screened and enrolled eligible participants, and provided enrolled participants with the next available sequentially-numbered capsule container.

### 2.5. Data Collection

Mothers were interviewed at baseline (14–16 weeks gestation) to collect information about maternal characteristics and demographics. When children were aged 6 months and approximately 12 months old, mothers visited the research centres or were visited at home and were invited to complete a questionnaire about their psychological wellbeing, thinking back to when their child was 1–2 months of age. If children were older than 12 months when the questionnaire was being used, mothers were posted the questionnaire or invited to complete it online via a secure link. Mothers and researchers remained blind to treatment assignment of participants at all follow-up stages of the study.

### 2.6. Primary Outcomes

**Edinburgh Postnatal Depression Scale (EPDS):** The EPDS is a 10 item screening questionnaire widely used to assess maternal mood (Cox et al., 1987). For the purposes of analysis, the standard cut-off of >12 was used to identify mothers at higher risk of postnatal depression.

**State Trait Anxiety Inventory 6 item version (STAI6):** The STAI6 is a short 6 item scale validated as an anxiety screening questionnaire based on the longer State Trait Anxiety Inventory (Marteau and Bekker, 1992). A cut-off score > 15 was used as an indicator of clinically significant levels of anxiety.

For both the EPDS and the STAI6 the questions were altered to use the past tense as mothers were asked to remember back to when their child was 1–2 months old and complete the questions based on how they were feeling at that time. It should be noted that the modified questionnaires have not been validated.

### 2.7. Explanatory Variable

**Infant Colic:** Infant colic was assessed at the 6 month interview when mothers were asked if they had contacted a health professional because their child had colic at any time between birth and six months of age.

### 2.8. Sample Size and Statistical Analysis

With a sample size of 200 in each group and 13% drop-out rate the study had a 79% power to detect a 26% reduction in EPDS at the 5% level of significance.

Data were analysed as intent-to-treat. Adherence was calculated as the number of capsules taken as a proportion of the expected number taken. Statistical analysis was conducted in SAS 9.4 using a generalised linear model for the continuous outcomes and logistic regression for categorical outcomes. Multivariable analysis of the relationship between probiotic supplementation and postnatal depression and anxiety scores, adjusted for the time since birth at which the questionnaires were completed and infant colic.

The trial was registered at the Australia New Zealand Clinical Trials Registry: ACTRN12612000196842.

### 2.9. Ethics

The study received ethical approval from the New Zealand Multiregional Ethics Committee (MEC/11/09/77). Participants gave written informed consent. The study conforms to the standards indicated by the Declaration of Helsinki.

### 2.10. Role of Funding Sources

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The

corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

## 3. Results

Fig. 1 shows the numbers assessed for eligibility, the number excluded, and the number eligible, recruited and allocated in the PIP Study. Of the 423 randomised women, 380 (89.8%) completed the psychological outcome measures, 193 (91.0%) were in the treatment group and 187 (88.6%) in the placebo group. Of the 380 participants in this study, 11 completed the questionnaires at the 6 month infant visit, 112 completed them at the 12 month infant visit and the remaining 257 completed the measures online at a median child age of 2.1 years (IQR 1.8–2.4). One participant did not complete all questions in the anxiety questionnaire, and the STAI6 score was recorded as missing.

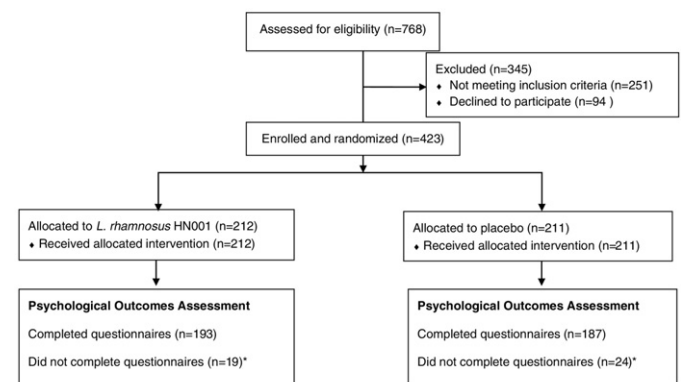
Table 1 shows the maternal characteristics of the study participants. Of particular note there was little difference in the use of medications for psychological problems prior to the index pregnancy.

The median adherence to allocated treatment did not differ (probiotic 92.3% (IQR = 83.9–97.2%), placebo 91.1% (IQR = 81.8–96.9%;  $p = 0.30$ )).

Depression and anxiety scores tended to increase with increasing interval between delivery and when the questionnaire was completed (depression score increased by 0.85 per year,  $p = 0.065$ ; anxiety score increased by 0.66 per year,  $p = 0.060$ ). Infant colic was associated with higher depression (multivariable  $p < 0.0001$ ) and anxiety (multivariable  $p < 0.0001$ ) scores, but was not significantly associated with probiotic supplementation group ( $p = 0.456$ ).

Table 2 shows the mean and standard deviation of depression and anxiety scores in the probiotic treatment and placebo groups. Mothers in the probiotic treatment group reported significantly lower depression scores (HN001 mean = 7.7 (SD = 5.4), placebo 9.0 (6.0), effect size -1.2, (95% CI -2.4, -0.1),  $p = 0.035$ ) and anxiety scores (HN001 12.0 (4.0) placebo 13.0 (4.3), effect size -1.1 (-1.9, -0.2),  $p = 0.014$ ) than those in the placebo group. After controlling for infant colic and time since birth that questionnaires were completed, probiotic supplementation remained significantly associated with reduced depression ( $p = 0.037$ ) and anxiety ( $p = 0.014$ ) scores.

Table 3 shows the number of women in the probiotic treatment and placebo groups who reported clinically significant depression or anxiety scores (that is above the cut-off points). The number of women reporting depression scores above the cut-off point did not differ significantly between the probiotic treatment and placebo groups (OR = 0.64 (0.38, 1.07),  $p = 0.086$ ). However, women in the probiotic treatment group were significantly less likely to have anxiety scores above the cut-off point than the placebo group (OR = 0.44 (0.27, 0.73)  $p = 0.001$ ), this association remained statistically significant after



**Fig. 1.** Participant flow showing the numbers of participants who were randomly assigned, received intended treatment and were analysed for the psychological outcomes.



**Table 1**  
Characteristics of study population at enrolment.

	HN001 (N = 193)	Placebo (N = 187)
Previous pregnancy		
0	66 (34.2%)	52 (27.8%)
1	46 (23.8%)	68 (36.4%)
2	43 (22.3%)	40 (21.4%)
3	24 (12.4%)	11 (5.9%)
>3	14 (7.3%)	16 (8.6%)
Age (years), mean (SD)	33.5 (4.24)	33.7 (4.44)
Weight (kg), median (interquartile range)	69.2 (63.1–79.1)	71 (63.5–81.7)
BMI, median (interquartile range)	25.1 (23.0–28.6)	25.9 (23.0–29.5)
Parental allergies		
Mother only	70 (36.3%)	64 (34.2%)
Father only	26 (13.5%)	28 (15.0%)
Both	97 (50.3%)	95 (50.8%)
Ethnicity		
Maori	20 (10.4%)	29 (15.5%)
Pacific	5 (2.6%)	3 (1.6%)
Asian	13 (6.7%)	14 (7.5%)
European	155 (80.3%)	140 (74.9%)
Other	0 (0.0%)	1 (0.5%)
Household income (\$NZ)		
0–49k	15 (7.8%)	10 (5.4%)
50–99k	59 (30.6%)	63 (33.7%)
100–149k	69 (35.8%)	66 (35.3%)
150 + k	50 (25.9%)	48 (25.7%)
Maternal smoking	42 (21.8%)	36 (19.3%)
Maternal education		
School education	23 (11.9%)	25 (13.4%)
Post school education	26 (13.5%)	17 (9.1%)
University education	144 (74.6%)	145 (77.5%)
Medication for psychological problem ever taken prior to index pregnancy		
Yes	41 (21.2%)	36 (19.3%)
No	152 (78.8%)	151 (80.7%)
Maternal antibiotics from enrolment (14–16 weeks gestation) to birth		
Yes	57 (30.3%)	58 (31.2%)
No	131 (69.7%)	128 (68.8%)
Maternal antibiotics from birth to 3 months postpartum		
Yes	64 (33.2%)	72 (38.5%)
No	129 (66.8%)	115 (61.5%)

controlling for infant colic and time since birth at questionnaire completion ( $p = 0.002$ ).

Use of medications for psychological problems during the index pregnancy was low and did not differ between treatment groups (HN001 3.6% vs. placebo 3.2%).

#### 4. Discussion

This study demonstrated a significantly lower prevalence of symptoms of depression and anxiety postpartum in women supplemented with the probiotic HN001 during and after pregnancy than in those given a placebo. Furthermore, the number of women reporting clinically significant levels

of anxiety on screening was significantly lower in the probiotic group. To our knowledge this is the first double-blind RCT of probiotics that has evaluated symptoms of depression and anxiety in the postpartum period. In addition, our sample size was substantially larger than many previously reported RCTs of probiotics on mood and behaviour.

The finding that women supplemented with probiotics had fewer symptoms of postnatal anxiety and depression is consistent with two previous clinical studies of the effect of probiotics on mood in different populations. A RCT of 40 people with major depressive disorder treated with a combination of three probiotics (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*) or placebo also found a significant reduction in symptoms of depression on the Beck Depression Inventory (BDI) in the treatment group (Akkasheh et al., 2016). A reduction in anxiety symptoms in a sample of 39 chronic fatigue patients randomised to receive *Lactobacillus casei* or placebo has also been reported, but the same study did not find a reduction in symptoms of depression on the BDI in the treatment group (Rao et al., 2009). However, not all studies have demonstrated a significant positive effect of probiotic treatment on mood outcomes (Romijn and Rucklidge, 2015; Romijn et al., 2017). A recent study involving 69 subjects who successfully completed the study found no evidence that a probiotic preparation (containing *Lactobacillus helveticus* and *Bifidobacterium longum*) for 8 weeks was effective in treating adults with moderate scores on self-reported symptoms of depression, stress and anxiety (Rao et al., 2009). The diversity of study populations, including those with schizophrenia (Dickerson et al., 2014) smokers (Reale et al., 2012), and irritable bowel syndrome patients (Dapoigny et al., 2012), the range of probiotic strains used, varying length of treatment, small sample sizes and varying measures of mood make it difficult, if not impossible, to undertake any meta-analysis of these studies. It is clear that more large studies of probiotic treatment are needed including those that measure psychological outcomes.

In our study infant colic was associated with higher depression and anxiety scores. There has been a suggestion in the literature that probiotic supplementation may benefit maternal mood by reducing infant colic. One study reported that direct probiotic supplementation of infants reduced infant colic and this in turn was associated with lower rates of maternal depression (Mi et al., 2015). While infants in our study are likely to have been exposed to a small amount of probiotic indirectly, either in utero or via breastmilk, they were not administered the probiotic directly; furthermore, we found that prevalence of infant colic did not differ between the probiotic and placebo groups and hence there was little difference in the effect size when adjusted for infant colic. Multivariable analysis showed that probiotic supplementation and absence of infant colic were independently associated with lower postnatal depression and anxiety scores.

The prevalence of scores for depression and anxiety above the cut-off at 1–2 months post-partum in this study was higher than the 10% to 15% usually reported. In part, this may be due to the mothers and or fathers having a history of asthma, hayfever or eczema requiring medication, as it is known that those with allergies are at higher risk of mental health problems (Scott et al., 2007). It may also be due to mothers in our study completing the questionnaire retrospectively. Possibly when mothers reflect back to how they felt 1 to 2 months after

**Table 2**  
Depression and anxiety scores in the probiotic treatment (HN001) and placebo groups.

	Mean	Standard deviation	Univariable effect size (95%CI), p-value	Multivariable <sup>†</sup> effect size (95%CI), p-value
Depression scores <sup>a</sup>				
HN001 N = 194	7.7	5.4	-1.2 (-2.4, -0.1)	-1.2 (-2.3, -0.1)
Placebo N = 187	9.0	6.0	p = 0.035	p = 0.037
Anxiety Scores <sup>a</sup>				
HN001 N = 192	12.0	4.0	-1.1 (-1.9, -0.2)	-1.0 (-1.9, -0.2)
Placebo N = 187	13.0	4.3	p = 0.014	p = 0.014

<sup>a</sup> Three participants had incomplete anxiety data on the STAI6 and one had incomplete depression data on the EPDS, therefore scores could not be calculated.

<sup>†</sup> Adjusted for infant colic and time since birth that questionnaires were completed.

**Table 3**

Number and percentage of participants scoring at or above the cut-off point for depression and anxiety in the treatment (HN001) and placebo groups.

			Univariable odds ratio (95% CI), p-value	Multivariable <sup>†</sup> odds ratio (95% CI), p-value
Depression score <sup>a</sup>	Depressed N (%)	Not depressed N (%)		
HN001	32 (16.5)	162 (83.5)	0.64 (0.39, 1.07) <i>p</i> = 0.086	0.65 (0.38, 1.10) <i>p</i> = 0.11
Placebo	44 (23.5)	143 (76.5)	Reference	Reference
Anxiety score <sup>a</sup>	Anxious N (%)	Not anxious N (%)		
HN001	30 (15.6)	162 (84.4)	0.44 (0.27, 0.73) <i>p</i> = 0.001	0.44 (0.26, 0.73) <i>p</i> = 0.002
Placebo	55 (29.4)	132 (70.6)	Reference	Reference

<sup>a</sup> Three participants had incomplete anxiety data on the STAI6 and one had incomplete depression data on the EPDS, therefore scores could not be calculated.<sup>†</sup> Adjusted for infant colic and time since birth that questionnaires were completed.

delivery, they realise how tiring caring for a newborn infant can be. In studies that survey prevalence of PND at an early time point, women may be less likely to rate themselves as depressed or anxious because they are expecting to feel exhausted.

Despite the prevalence of high symptom scores, the number of women who had medication for psychological problems in pregnancy was low (3.4% of total study population). Our study did not explore the reasons for this; however, it does support the contention that depression and anxiety during pregnancy is unrecognised and not treated.

Mechanisms by which probiotic supplementation influence the physiology of the brain, and thereby anxiety and depression, have been proposed using animal models. Changes in the expression of the neurotransmitter GABA receptors in regions of the mouse brain have been demonstrated along with changes in anxiety-related behaviour with *L. rhamnosus* treatment (Bravo et al., 2011). However, this relationship was not evident in mice that had had their vagus nerve removed, indicating that the vagus nerve might be the link between events in the gut and altered GABA receptor expression. However, in another study *L. rhamnosus* was not superior to placebo in modifying stress-related measures in healthy adult human males highlighting the challenge of extrapolating from animal to human studies (Kelly et al., 2017). Biochemical changes have been demonstrated in the HPA axis in rats when exposed to maternal separation, which in turn were associated with anxiety-related behaviours in the Forced Swim Test; these behaviours were reversed when the animals were treated with *Bifidobacterium infantis* (Collins et al., 2013).

Postnatal depression occurs more frequently in socio-economically disadvantaged populations. In a previous study we found that PND was more likely to occur in women who were single, <20 years of age, Maori, were unhappy with their relationship with their partner, or had a history of previous psychiatric hospitalisation (Webster et al., 1994). National Health and Nutrition Examination Survey was an observational study and found that probiotic foods or supplements were associated with a reduced risk of depression, but this was attenuated and non-significant when adjusted for factors that were associated with depression and probiotic exposure (Cepeda et al., 2017). This may be a consequence of misclassification, as probiotic food and supplement use over the 24 h period preceding data collection was used to define exposure and may not reflect usual intake. Our study was a RCT and therefore the socioeconomic and other factors are randomly distributed between the intervention and placebo group.

There are many different species and strains of probiotics. *L. rhamnosus* HN001 was chosen for the beneficial effect on the primary outcome, namely eczema (Wickens et al., 2008; Wickens et al., 2012; Wickens et al., 2013). Many *Lactobacillus* and *Bifidobacterium* strains have been studied with respect to mental health and these genera seem to show the most beneficial effects (Mayer et al., 2014).

Limitations of the study need to be considered. Firstly, the EPDS and STAI6 are screening tools for PND and anxiety, but are not diagnostic. In this report we have studied symptoms of PND and anxiety, as clinical

assessment and diagnosis of depression and anxiety were not undertaken. However, when the scores were categorised into higher risk of PND and indicators of clinically significant levels of anxiety, similar findings were seen. Secondly, the information regarding symptoms of PND and anxiety was collected retrospectively, and neither the EPDS nor the STAI6 has been validated using the questions phrased in the past tense. While this may have resulted in an increase in measurement error, it would not be expected to have introduced a differential bias in responses. Furthermore, measurement error, if introduced, would be more likely to move the results towards the null hypothesis of there being no effect of probiotic on mood outcomes. The strength of the study is the design (double-blind placebo-controlled study) with substantial group size. It is a simple, cost effective and easily implemented treatment. Furthermore, we have shown that this probiotic is safe and well tolerated in pregnancy and infants (Dekker et al., 2009).

This study provides evidence that probiotic supplementation with *L. rhamnosus* HN001 in pregnancy and postpartum reduces the prevalence of symptoms of PND and anxiety postpartum. Not all probiotic strains have the same effect on health and it is possible that the results found using HN001 are not generalisable to other probiotic strains, or at lower doses than those used in this study. Furthermore, in a recent study higher levels of anxiety-like behaviour and stress, as measured by plasma corticosterone levels, were seen in young rats treated with *Lactobacillus casei* or inulin (a prebiotic) compared with controls (Barrera-Bugueño et al., 2017). Those treated with a *L. casei* and inulin combination (synbiotic) had no anxiety-like behaviours. There are many unanswered questions, including the choice of probiotic, the dose and the duration of treatment. Can probiotics prevent the onset of symptoms? Could probiotics be used as the primary treatment for maternal mental health problems or should it be used as an adjuvant treatment to standard therapy? Such studies might incorporate inflammatory markers, cortisol, or other objective markers.

If replicated by other studies, this probiotic may be useful for the prevention or treatment of symptoms of depression and anxiety postpartum.

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## Conflict of Interests

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## Authors' Contributions

TS first suggested examining the psychological effects of probiotics. RFS independently suggested probiotics might influence mood, designed the psychological outcome measures, advised on data analysis and wrote the first draft of the manuscript. JMDT analysed the data. FH designed the data collection tool and was responsible for collecting the data in Wellington. RM critically reviewed the manuscript. CB contributed to the development of the main study and data collection in Wellington. JK assisted with data collection in Wellington. JR was project manager of the study in Auckland and collected the data. KW conceived the main study, raised the funds for both the main study and this component and managed the main project. JC had overall responsibility for the main study. EAM conceived, designed and led this component of the study, led the Auckland arm of the main study, advised on data analysis and drafted the manuscript. EAM takes responsibility for the content of the manuscript. In addition PS, RM, RMM and PA contributed to the design of the main study. All authors reviewed and approved the submitted manuscript.

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