Feasibility, safety and impact of the probiotics Lactiplantibacillus plantarum and Bifidobacterium in Papua New Guinean infants: methods and feasibility from a randomised controlled trial.

Rebecca L Ford, Andrew R Greenhill, Joycelyn Sapura, Birunu Nivio, Amelia Koale, Mary Dreyam, Tilda Orami, Joe Jude, Madeline Ong, Wendy Kirarock, Dorcas Joseph, Geraldine Masiria, Celestine Aho, William S Pomat, Anita HJ van den Biggelaar, Peter C Richmond

Submitted to: JMIR Research Protocols
on: March 17, 2025

Table of Contents

Feasibility, safety and impact of the probiotics Lactiplantibacillus plantarum and Bifidobacterium in Papua New Guinean infants: methods and feasibility from a randomised controlled trial.

Rebecca L Ford[1]; Andrew R Greenhill[2]; Joycelyn Sapura[1]; Birunu Nivio[1]; Amelia Koale[1]; Mary Dreyam[1]; Tilda Orami[1]; Joe Jude[1]; Madeline Ong[3]; Wendy Kirarock[1]; Dorcas Joseph[1]; Geraldine Masiria[1]; Celestine Aho[1]; William S Pomat[1]; Anita HJ van den Biggelaar[3]; Peter C Richmond[3, 4]

[1] Papua New Guinea Institute of Medical Research Goroka PG
[2]Microbiology Research Group Institute of Innovation, Science and Sustainability Federation University Churchill AU
[3] Wesfarmers Centre for Vaccines and Infectious Diseases The Kids Research Institute Australia Perth AU
[4]Discipline of Paediatrics Medical School University of Western Australia Perth AU

Corresponding Author:
Rebecca L Ford

Papua New Guinea Institute of Medical Research
PO Box 60
Cnr of Homate & Leigh Vial Streets
Goroka
PG

Abstract

Background: Childhood mortality in low- and middle-income countries (LMICs) remains a major public health concern, with infections being a leading cause of infant death. Probiotics have shown promise in reducing infection-related morbidity and mortality in preterm infants, but their use in full-term newborns in LMICs requires further investigation.

Objective: This study aims to assess the feasibility, safety, and initial outcomes of administering one of two synbiotic formulations or a placebo to newborns in Papua New Guinea's Eastern Highlands Province.

Methods: Between November 2020 and June 2023, healthy neonates (< 72 hours old, n=244) were randomly assigned in a double-blinded manner (1:1:1) to receive an oral preparation containing Lactiplantibacillus plantarum, Bifidobacterium longum subspecies infantis, or placebo for 7 consecutive days. Follow-up continued for 6 months, with rectal swabs, stool, blood, saliva, and/or nasopharyngeal swabs collected pre-intervention, on day 7, at 2 weeks, and at 1, 3, 4 and 6 months of age. Ongoing analyses will assess probiotic gut colonisation, bacterial nasopharyngeal carriage, antibody responses to Hib, Hepatitis B, PCV-13, and dTP, as well as hospitalisation and infection rates among intervention groups.

Results: Funding and ethical approvals were obtained in 2018 and 2019. Initial synbiotic preparations were received in August 2020, with participant recruitment beginning in November 2020. All follow-up visits concluded in December 2023. Of the enroled infants, 218/244 (89%) completed the full 7-day synbiotic/placebo course, and 169/244 (69%) completed the study. Disruptions due to the COVID-19 pandemic led to family relocations outside the study area, preventing some infants from completing the study. High rates of sample collection were achieved, with >90% of rectal swabs, nasopharyngeal swabs, saliva, and blood samples successfully obtained at multiple time points. Sample analysis is ongoing.

Conclusions: This study demonstrates that probiotic supplementation is feasible in full-term infants in an LMIC setting. The findings from this study will inform the need for larger trials of probiotics and synbiotics to complement existing efforts to reduce infection-related infant mortality in LMICs while also providing insights into their clinical, immunological, and microbiological impacts in infants. Clinical Trial: ANZCTR.org.au Identifier: CTRN12620001369910

(JMIR Preprints 17/03/2025:73926)
DOI: https://doi.org/10.2196/preprints.73926

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✔ **Please make my preprint PDF available to anyone at any time (recommended).**
Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.
No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✔ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**
Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v
Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http
No. Please do not make my accepted manuscript PDF available to anyone.

Original Manuscript

Original paper

Rebecca L Ford[1], Andrew R Greenhill[2], Joycelyn Sapura[1], Birunu Nivio[1], Amelia Koale[1], Mary Dreyam[1], Tilda Orami[1], Joe Jude[1], Madeline Ong[3], Wendy Kirarock[1], Dorcas Joseph[1], Geraldine Masiria[1], Celestine Aho[1], William S Pomat[1], Anita HJ van den Biggelaar[3], Peter C Richmond[3,4]

[1] Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea
[2] Microbiology Research Group, Institute of Innovation, Science and Sustainability, Federation University Australia, Victoria, Australia

[3] Wesfarmers Centre for Vaccines and Infectious Diseases, The Kids Research Institute Australia, Perth, Australia

[4] Discipline of Paediatrics, Medical School, University of Western Australia, Perth, Australia

Corresponding Author:
Rebecca L Ford, PhD
Papua New Guinea Institute of Medical Research
PO Box 60/Cnr of Homate & Leigh Vial Streets
Goroka, EHP 441
Papua New Guinea
Phone: +675 532-2800
Facsimile: +675 532-1998
Email: rebecca.ford.imr@gmail.com

Feasibility, safety and impact of the probiotics *Lactiplantibacillus plantarum* and *Bifidobacterium* in Papua New Guinean infants: methods and feasibility from a randomised controlled trial.

ABSTRACT

Background: Childhood mortality in low- and middle-income countries (LMICs) remains a major public health concern, with infections being a leading cause of infant death. Probiotics have shown promise in reducing infection-related morbidity and mortality in preterm infants, but their use in full-term newborns in LMICs requires further investigation.

Objectives: This study aims to assess the feasibility, safety, and initial outcomes of administering one of two synbiotic formulations or a placebo to newborns in Papua New Guinea's Eastern Highlands Province.

Methods: Between November 2020 and June 2023, healthy neonates (< 72 hours old, n=244) were randomly assigned in a double-blinded manner (1:1:1) to receive an oral preparation containing *Lactiplantibacillus plantarum*, *Bifidobacterium longum* subspecies *infantis*, or placebo for 7 consecutive days. Follow-up continued for 6 months, with rectal swabs, stool, blood, saliva, and/or nasopharyngeal swabs collected pre-intervention, on day 7, at 2 weeks, and at 1, 3, 4 and 6 months of age. Ongoing analyses will assess probiotic gut colonisation, bacterial nasopharyngeal carriage, antibody responses to Hib, Hepatitis B, PCV-13, and dTP, as well as hospitalisation and infection rates among intervention groups.

Results: Funding and ethical approvals were obtained in 2018 and 2019. Initial synbiotic preparations were received in August 2020, with participant recruitment beginning in November 2020. All follow-up visits concluded in December 2023. Of the enroled infants, 218/244 (89%) completed the full 7-day synbiotic/placebo course, and 169/244 (69%) completed the study. Disruptions due to the COVID-19 pandemic led to family relocations outside the study area, preventing some infants from completing the study. High rates of sample collection were achieved, with >90% of rectal swabs, nasopharyngeal swabs, saliva, and blood samples successfully obtained at multiple time points. Sample analysis is ongoing.
Conclusion: This study demonstrates that probiotic supplementation is feasible in full-term infants in an LMIC setting. The findings from this study will inform the need for larger trials of probiotics and synbiotics to complement existing efforts to reduce infection-related infant mortality in LMICs while also providing insights into their clinical, immunological, and microbiological impacts in infants.
Trial Registration: ANZCTR.org.au Identifier: CTRN12620001369910
Keywords: probiotics, synbiotics; Bifidobacterium; *Lactiplantibacillus plantarum*; infant health; neonatal infections; gut microbiota; colonisation

INTRODUCTION

Childhood mortality and interventions

Childhood mortality remains one of the greatest public health challenges of our time. The under 5 year mortality rate was estimated at 38 per 1,000 live births in 2021, which equates to an estimated 5 million deaths per year in this age group. The mortality rate has decreased over the past 30 years, with the greatest improvements in the 1 month to 5 year age bracket. Deaths within the first 28 days of life (neonates) account for almost half of all deaths within the first 5 years of life[1].
The majority of childhood deaths occur in low-middle income countries (LMICs). Poor sanitation, limited access to improved water, poor access to health care, limited childhood vaccination coverage, inadequate nutrition and cost of healthcare relative to income contribute to high infectious disease burden and poor health outcomes. Many of these long-recognised contributors are difficult to rectify in the short-term; to expediate improvements in child health in LMICs novel approaches are required to complement longer-term initiatives.

Probiotics and synbiotics

Probiotics have long been ingested by humans through the consumption of fermented foods. The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”[2] with a panel of experts in 2013 supporting the ongoing use of this definition[3,4]. In addition to fermented foods, probiotics have been isolated from various sources and commercialised as health supplements, and more recently as therapeutic agents. A promising strategy for the use of probiotics is in combination with prebiotics – non-digestible food ingredients which selectively promote microbial growth. Termed “synbiotics”, these combinations are thought to be more efficacious in enhancing the survival and

activity of the probiotic bacteria once ingested, and have the potential to lower the risk of developing many illnesses and diseases[5,6]. However, the quality of synbiotic products available on the market varies significantly, with differences in probiotic strains, prebiotic types, and dosage levels, which may affect their effectiveness[7]. Furthermore, while initial studies show promise[8–10], more robust clinical trials are needed to confirm their therapeutic benefits and safety, as much of the current evidence is based on *in vitro* or animal models[11–13] rather than large-scale human studies. Standardisation in product formulation and more rigorous clinical proof are essential to fully understand their potential health impacts.

Study rationale and objectives

One novel approach that has garnered interest in recent years is the use of probiotics early in life. In particular, probiotics have been shown to improve health outcomes in preterm infants. Meta-analyses of health outcomes of probiotics administered to preterm infants, demonstrate that probiotics can decrease the risk of necrotising enterocolitis (NEC)[14–16], and death[17]. The administration of probiotics to pre-term infants is now standard practise in neonatal intensive care units in many high-income settings. Its applicability has been investigated in some LMIC settings for at least 20 years[18]; with a systematic review on randomised control trials in India suggesting probiotics reduce the risk of NEC, late onset sepsis and death[19]. Similarly, another review of trials conducted in 10 LMICs demonstrated that probiotics decreased the risk of NEC, late onset sepsis and all-cause mortality[20].

With evidence suggesting that probiotics are beneficial to pre-term babies, there has been interest over the past decade in the potential role of probiotics in improving health in full-term babies in LMICs. A large community-based study in India demonstrated the potential health benefits of probiotics in infants[21]. In a randomised control trial over 2000 children in the intervention arm were administered a strain of *Lactobacillus plantarum* (now *Lactiplantibacillus plantarum*) supplemented with fructooligosaccharide (FOS) (together forming a synbiotic) once-daily over 7 days, starting on day 2-4 of life. Compared to the placebo group, children in the synbiotic intervention arm had significantly lower rates of sepsis causing death (the study primary outcome) and sepsis combined with lower respiratory tract infections. Rates of diarrhoea, omphalitis and local infections were also significantly lower in the intervention group.

Papua New Guinea (PNG) is a LMIC, in which approximately 40% of the population live in poverty[22]. In PNG approximately 4.1% of children die before they reach 5 years old, and the under-one-year mortality rate is estimated to be around 33 deaths per 1,000 live births: higher than the global averages[23]. Leading causes of death in children in PNG include neonatal complications (such as pre-term birth, birth asphyxia and birth trauma, and sepsis), lower respiratory tract infections (LRTIs), tuberculosis, malaria and diarrhoea. The neonatal mortality rate alone is estimated to currently be around 9.25%[24]. LRTIs, often driven by pneumococcal infections, play a particularly critical role in child morbidity and mortality in PNG. The high diversity and prevalence of nasopharyngeal pneumococcal carriage in children, compounded by limited vaccine coverage, underscore the importance of understanding local serotype distribution and the effectiveness of PCV13 (pneumococcal conjugate vaccine)

antibodies in this setting[25,26]. Given the high burden of serious infections in children in PNG, there is a need to explore novel approaches to reducing this burden.

Here we report on the implementation and participant recruitment of a randomised double blinded placebo controlled trial conducted in the Highlands of PNG in which newborns were administered one of two synbiotic formulations, *Lactiplantibacillus plantarum* or *Bifidobacterium longum* subspecies *infantis*, or a placebo. This was the first study of probiotics supplementation in PNG infants and the primary aim of this pilot study was therefore to demonstrate the feasibility and tolerability of giving infants in PNG probiotic supplementation for 7 consecutive days starting within the first 3 days of life before embarking on a larger-sized clinical impact trial. Secondary and exploratory aims include assessing gut colonisation by probiotic species at multiple time points, hospitalisation and infection rates, nasopharyngeal carriage of respiratory bacteria, vaccine-specific immune responses, microbiome development, and maternal vaginal colonisation at delivery.

METHODS

Study location

PNG is one of the largest countries in the Oceania region by both landmass and population. There has been rapid population growth over the past 30 years from less than 4 million in 1990 to an estimated 11.8 million inhabitants in 2021[27,28].

This study was conducted in Eastern Highlands Province (EHP) of PNG, where an estimated 785,000 people live. The capital of EHP is Goroka, with the town (~33,000) and surrounding peri-urban areas having a total population of ~212,000[28]. Goroka is located ~1600 metres above sea level and is situated on the Highlands Highway (the main highway in PNG). The climate is typically highland subtropical: rainfall occurs year-round with lower amounts falling from June to September. The terrain is mountainous and rugged, making access to many rural villages and communities outside of town challenging and requiring off-road 4WD vehicles.

The study was conducted by the Papua New Guinea Institute of Medical Research (PNGIMR) and international collaborators. The PNGIMR is headquartered in Goroka. Participants were recruited at the Goroka Provincial Hospital, the major referral hospital for EHP.

Study design and objectives

In this pilot study, we aimed to demonstrate the feasibility and tolerability of administration of two synbiotic (probiotic plus prebiotic) formulations in PNG newborn in the first week of life. The study design was a double-blinded randomised placebo controlled trial in which newborns received one of two different synbiotic formulations or placebo, over 7 consecutive days, with the first dose given before the newborn was 72 hours old and administration

completed within the first 10 days of life.
The primary study objectives were: a) safety in terms of tolerability during the 7-day supplementation period; and b) feasibility of daily probiotic administration as measured by the proportion of children who successfully completed 7 days of the full daily supplementation (7 out of 7). The secondary objective was to demonstrate colonisation of the gut by probiotic strains at 1 week, 1, 3, 4 and 6 months of age. Exploratory objectives include: hospitalisation rates until 6 months of age; nasopharyngeal carriage of *Streptococcus pneumoniae* and other respiratory tract bacteria at 2 weeks, 1 and 4 months of age; IgG antibody responses to childhood vaccination with 13-valent pneumococcal conjugate vaccine (PCV13), diphtheria, tetanus and whole cell pertussis (DTwP), and *Haemophilus influenzae* type b (Hib); kinetics of colonisation of probiotics at various time points over first 6 months of life; and maternal carriage/colonisation with probiotic species and pathogens at time of delivery.

Ethics

Ethical approval was obtained from the University of Western Australia (2018/RA/1/2301/4) and PNGIMR Institutional Review Board (# 1809) in 2018, and the PNG Medical Research Advisory Committee (No. 18.20) in 2019. The study is registered as a clinical trial in PNG under the National Department of Health Pharmaceutical Standards Branch (CT04 MRAC18.20) and is registered with the Australian and New Zealand Trials Registry (ACTRN12620001369910).

Assent, consent and recruitment

Prior to and throughout the duration of the study, clinic and field staff conducted awareness and information sessions within communities in and near Goroka, the Goroka Provincial Hospital antenatal clinic, and rural health centers. Formal letters and information about the study were also sent to the Eastern Highlands Provincial Health Authority and senior management of Goroka Provincial Hospital, from whom approval was obtained prior to commencement.
Healthy, pregnant women late in the third trimester were approached in the antenatal clinic at Goroka Provincial Hospital or in their local communities by a study health extension officer (HEO) or nurse. The women were given information about the study and given the opportunity to discuss participation with their families. Women who assented to be part of the study were approached within 3 days after giving birth to confirm consent and enrol the infant when meeting all the inclusion criteria. Written parental consent was obtained prior to enrolment.
Inclusion criteria for the infants to be enroled in the study were:

- Birth weight ≥ 1.5 kg
- < 72 hours old
- Feeding orally
- Residing within 60-minute driving distance from IMR, Goroka
- Intending to stay in the study area for at least 6 months (duration of the study)
- Written informed consent.

Exclusion criteria were:

- Infant had received antibiotics at the time of screening (in first 72 hours of life)
- Infant had signs/symptoms of (suspected) infection at the time of screening
- Infant had major congenital abnormality
- Mother was HIV positive/status unknown
- Apgar score <8
- Mother had fever (>38°C) within 2 days before or after delivery
- Mother had foul smelling amniotic fluid within 2 days after delivery
- Mother had abdominal tenderness within 2 days after delivery
- Meconium staining of amniotic fluid
- Unable or did not provide consent.

Infants were determined to be eligible to participate and to not have any congenital abnormalities based on clinical assessments conducted routinely by hospital paediatricians on newborns delivered at Goroka Provincial Hospital, and by a study HEO.

Study interventions

The synbiotic products and placebo were manufactured by DuPont Nutrition and Health (Madison, WI, USA), now International Flavors & Fragrances (IFF), and supplied in September 2020 prior to the commencement of the study for the full cohort target number. An extra 10% of products were provided to allow for any repeat doses that may be required in the case of vomiting after administration and any additional recruitments to cater for anticipated loss to follow-ups. A full replacement batch of intervention products was provided by IFF in November 2022 due to expiry of the initial products before recruitment had been completed.

Two probiotic supplements: single-strain *Lactiplantibacillus plantarum* and *Bifidobacterium longum subsp. infantis* compared to placebo were used in this study. These two probiotic supplements were prepared as synbiotics and supplied, as with the placebo, lyophilized in glass vials. Each vial contained a single dose of one of the following: (i) *L. plantarum* ATCC strain 202195 (1.0×10^9 CFU) + 150 mg of fructo-oligosaccharide, with 100 mg potato maltodextrin as excipient (corresponding with the preparation used in the Indian trial [8]); (ii) *B. infantis* Bi-26 (at least $1x10^9$ CFU) + 1.2g human milk oligosaccharide 2′-fucosyllactose, with 100 mg potato maltodextrin as excipient; or (iii) placebo (100 mg potato maltodextrin).

Randomisation

A randomisation list was computer-generated and kept by Dupont/IFF such that the research investigators, research and laboratory staff, and study participants were blinded to the intervention arms. A single randomisation code (sequential number 1 to 215) was assigned to a carton of intervention product containing 7 vials of product, and allocated to each enroled child consecutively. Each vial of product was labelled with the specific trial randomisation code and the day of administration (D1 – D7).

Intervention administration, vaccination and follow-up

The intervention, vaccine and specimen collection schedules are shown in Figure 1. Just prior to administration, a single vial of intervention product corresponding to the appropriate day of administration (D1, D2, D3, D4, D5, D6 or D7), was diluted by adding 3 mL of either breast milk or sterile water directly to the vial, and inverting five times to mix and resuspend. The suspension was then delivered in oral drops to the infant using a Pasteur pipet (comparable to giving oral polio vaccine). Before administering, infants were breastfed and burped. The intervention was administered by IMR study staff (HEO or study nurse). The baby was monitored for 30 min after receiving the intervention. In the case of vomiting, the dose was repeated an hour later. Unwell babies during the observation period were taken to the IMR clinic for examination and treatment (if intervention had taken place elsewhere) and referred to Goroka Provincial Hospital when needed. Obstetrics information and baseline characteristics were obtained at the day of enrolment. A review of the babies' health was conducted at each follow-up visit prior to any intervention administration, vaccination or specimen collection. Weight and length measurements were recorded and information on feeding practices were also collected.

Routine childhood vaccines (pentavalent: whole-cell pertussis, diphtheria, tetanus, Hepatitis B and *Haemophilus influenzae* type B (Hib) vaccine, PCV13 and oral polio vaccine; see Figure 1) were obtained through the Eastern Highlands Provincial Health supply chain and administered by the study HEOs/nurses at 1, 2, and 3 months of age as per PNG's Expanded Programme on Immunisation. Inactivated polio vaccine was also administered at 3 months of age, and the first dose of the measles vaccine was administered at the completion of the study (6 months). Birth doses of Bacillus Calmette-Guérin (BCG) vaccine and Hepatitis B vaccines were generally administered by hospital staff. In instances where this did not occur, study HEOs/nurses administered them at the time of enrolment. All vaccinations administered were recorded in the child's health book held by the parent or guardian.

Follow-up visits were conducted at either the IMR clinic or at the participant's home. At time points where blood was scheduled to be collected (day of enrolment, day of last intervention administration, 1 month and 4 months of age), participants were brought to the IMR clinic for review and sample collection. All other follow-up visits were carried out by the clinical and field staff at the participants' place of residence. Up to three home visit attempts were made for each participant follow-up before a child was withdrawn from the study after being deemed unlocatable. Children were also withdrawn from the study if information had been received that the family had moved out of the study area permanently, if parents or guardians withdrew their consent for continuation in the study, by the investigators following a protocol violation, or if deemed appropriate for the well-being of the child.

Morbidity surveillance

All serious adverse events (SAEs) that occurred during the study period were recorded. The variability in diagnostic terms and lack of gold-standard case definitions for neonatal sepsis make it difficult to compare incidence, severity, aetiology, or outcomes of disease across studies and settings. Instead of sepsis,

we therefore studied ‘Severe Infection’ as a clinical outcome to recognize that many infants will not manifest overt signs of sepsis, and with the qualifier ‘severe’ indicating that localised infections without systemic features (e.g., skin pustules) will be excluded. Severe clinical infection was defined as the presence of at least one of the following signs or symptoms to align the a similar study of synbiotics in Bangladesh[29]:

i) Poor feeding (not sucking effectively or not sucking at all, on direct observation)
ii) Lethargy (movement only when stimulated or not moving at all, on direct observation)
iii) Convulsions (observed, or strongly suspected by clinician based on caregiver or community health worker report)
iv) Severe chest in-drawing (observed)
v) Fever (axillary temperature greater than or equal to 38°C)
vi) Hypothermia (axillary temperature less than 35.5°C);

and any subsequent positive sterile site cultures, and/or hospitalisations with intention to treat with antibiotics for at least 5 days.

Throughout the study the paediatric ward at Goroka Provincial Hospital was monitored daily for possible study infants admitted. For any participant admitted, clinical information on health status, reason for admission, treatment and discharge outcomes were recorded. Additionally, parents or guardians were encouraged to bring their infants to the IMR clinic for any illness experienced to enable SAEs to be recorded in this period. If clinically indicated, specimens were collected to aid in health assessment and diagnosis. Clinical samples included small volumes of blood (~2 ml), faeces and/or an NPS. Samples were processed at the PNGIMR laboratory, and results returned to the study clinicians for dissemination to the parent or guardian of the participant.

If a child was unwell during a scheduled study visit, routine procedures such as administration of the intervention and collection of samples were still conducted unless there were contra-indications to do so.

In addition, as with previous clinical trial studies led by PNGIMR, unwell family members of study children were also seen at the PNGIMR clinic as required throughout the 6-month duration of participation.

Specimen collection

For secondary and exploratory objectives of the study, a number of biological samples were collected at several timepoints throughout the study (Figure 1). To assess intestinal colonisation of probiotic species, rectal swabs and stool were collected. Rectal swabs were collected at the day of enrolment, at the day of the last intervention (7-10 days old), and at 2 weeks, 1 month, 3 months, 4 months and 6 months of age, and stored in eNAT® preservation and transport media (Copan Diagnostics, USA). Stool samples were collected in addition to rectal swabs if infants were passing stool at the time of the study visit to allow for sensitivity comparisons of detecting probiotics strains in rectal swabs versus stool. Stool samples were collected in stool specimen containers and transferred to cryovials by laboratory staff. Rectal swabs and stool samples were then stored at −80 °C.

Nasopharyngeal swabs to assess bacterial carriage and load were collected at

ages 2 weeks, 1 and 4 months using a flocked swab (Copan Diagnostics, USA). Two swabs per time point were collected: one swab was stored in skim-milk tryptone-glucose-glycerol broth (STGGB) (priority) for culturing of bacterial pathogens and the second was stored in PrimeStore® MTM (ThermoFisher, Australia) for deep sequencing. Both samples were stored at −80 °C.

Venous blood samples (max 2 mL at visit 1 & 2; max 4 mL at visits 9 & 12) and saliva samples (visits 1, 7, 9 & 12) were obtained to assess systemic and mucosal vaccine responses and identify intervention-associated pathways or markers of immune maturation using systems biology approaches. Blood samples were obtained using small butterfly gauge 20 needles and syringe techniques to lower the chances of veins collapsing. A maximum of two attempts were made to collect a blood sample, after which no further attempts were made. For systems biology studies up to 2 mL of blood were collected in heparin tubes. These samples were processed at the PNGIMR laboratory within 15 min of collection for RNA (transcriptomic analysis), plasma (proteomic and metabolomic analysis), flow (single cell immunophenotyping) and WBC (proteomic, metabolomic and epigenetic analysis) as per an established protocol by the EPIC-HIPC clinical core Boston Children's Hospital group[30]. For serology, up to 2 mL of blood were collected in standard serum tubes, and the serum separated and stored in cryovials by laboratory staff. Blood for serology was prioritized at 1 month and 4 months over blood for systems biology. Saliva samples were collected from infants, ideally at least 30 min after being breastfed, using 4-6 surgical eye spears (Defries Industries, Australia) placed in floor of mouth to soak up saliva from the gums until saturated. The eye spears were then transported in 15 mL falcon tubes to the PNGIMR laboratory where they were centrifuged at 10,000 *g* at 5 °C for 10 min. Saliva recovered after centrifugation was transferred to cryovials for storage. If no liquid (saliva) was obtained after centrifugation, the sample was discarded. All blood products and saliva samples were initially stored at -80 °C at PNGIMR, before being shipped on dry ice to The Kids Research Institute Australia (formerly Telethon Kids Institute) in Perth, Australia.

Mothers were asked to self-collect vaginal swabs shortly after delivery using eNAT® flock swabs. This was to collect information on the background prevalence of maternal vaginal carriage of probiotic species, in addition to pathogens associated with neonatal sepsis, such as Group B *Streptococcus* and *Escherichia coli*, which may be vertically transmitted to the infant at the time of delivery. The swabs were stored at -80 °C.

Excess expressed breast milk was also collected at each intervention visit (visits 1-7) and stored at -80 °C at PNGIMR for later measurement of proteins (including antibodies) and carbohydrate contents.

Study data and management

All data collection forms were checked manually before entry into a password-protected electronic database management system (REDCap) housed at the The Kids Research Institute Australia. Access to the de-identified database was accessible to authorised researchers and study personnel only at PNGIMR and collaborating institutions. To ensure any problems or queries were addressed as soon as possible, investigators in PNG and Australia attended fortnightly teleconferences.

Data safety monitoring board

A data safety monitoring board (DSMB) of independent clinicians from PNG and Australia was established. All serious adverse events (SAEs) were reported within 24 hours to an appointed PNG Safety Monitor and the Chair of the DSMB for review, followed by a final report with outcome. Quarterly reports including a summary of all SAEs, all morbidities, protocol violations and status of enrolment and follow-up were prepared by the investigators and sent to the Chair of the DSMB and distributed to other DSMB members. A teleconference with DSMB members and the study investigators was held every quarter.

Sample size calculation

It was determined that sample sizes of n = 50 per group would achieve 90% power to detect the colonisation rate of 30% in the probiotic versus 5% in the placebo groups when using a two-sided test of proportions with continuity correction at 5% significance level. A final sample size of 65 per group was decided upon, which takes into account a presumed 10% loss to follow-up and difficulties in collecting timely bio-samples. Based on this sample size, it was estimated that the study would have 80% power to detect a 50% reduction in pneumococcal colonisation rate in the probiotic versus control group at a 5% significance level.

Statistical analyses

The data will be cleaned prior to analysis. All data will be downloaded from REDCap and a back-up copy archived before data cleaning. Missing data, outliers, and inconsistencies will be queried and verified against source documentation. Where data requires changing, the database will be updated accordingly. All queries and corresponding amendments will be documented. Per-protocol and intention-to-treat analyses will be performed, analysing those with available outcome data. The distribution of missing data across the treatment groups will be described, and reasons for missing data will be explored where possible to check if they occurred randomly.

The primary objective is to determine the feasibility and safety of treatment. The number of solicited and unsolicited adverse events, as well as the number of discontinued, missed, and completed doses will be summarized descriptively by treatment groups. Pearson $\chi 2$ and Fisher's exact tests will be used as appropriate to determine the significance of differences between groups.

The secondary objective is to measure gut colonisation of probiotic strains on the last day of treatment (Day 7 up to Day 10), 1 month, 3 months, and 4 months. Prevalence of colonisation at each timepoint will be summarised descriptively by treatment group. Gut colonisation, categorised as positive or negative, in each treatment group will be compared using Pearson $\chi 2$ test, while continuous descriptors of colonisation (i.e. Ct or RFU) will be analysed using one-way ANOVA.

Exploratory objectives include measuring the hospitalisation rates throughout the study, nasopharyngeal carriage of *S. pneumoniae* and other respiratory bacteria, IgG antibody titers to PCV13, DTwP, and Hib, kinetics of probiotic gut colonisation, and maternal carriage of probiotic species at the time of delivery.

Hospitalisation rates will be reported descriptively per treatment group and

compared using Pearson χ2 or Fisher's exact tests as appropriate.
Nasopharyngeal carriage of *S. pneumoniae* and other respiratory bacteria, as well as gut colonisation, will be summarised descriptively at each corresponding time point. A Cox regression analysis will be done, adjusting for birth weight, sex, previous antibiotic use, and breastfeeding status. Hazard ratios, their 95% confidence intervals, and p-values will be reported.
IgG antibody levels to PCV13, DTwP, and Hib will be reported descriptively at baseline (prior to vaccination at one month) and at 4 months (one month after the third dose). The differences between treatment groups will be estimated using linear regression, adjusting for birth weight, sex, breastfeeding status, and previous antibiotic use at each time point. The model will also adjust for maternal antibody titers to diptheria, tetanus, and pertussis when comparing IgG titers to DTwP, using pre-vaccination infant titers as proxy measures. All IgG levels will be log-transformed prior to analysis. The corresponding coefficients, 95% confidence interval, and *p*-values will be reported.
Maternal vaginal carriage of probiotic species will be reported descriptively and compared using Pearson χ2 test.
Ad hoc analyses will include comparison of IgG antibody levels, nasopharyngeal carriage of respiratory bacteria, adverse events, and other growth outcomes among those colonised and not colonised by *B. infantis* or *L. plantarum*. Pearson χ2, Fisher's exact test, and regression modelling will be used as appropriate. The models will adjust for similar variables as above.
The results of the analysis will be reported according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

RESULTS

Assent and enrolment

A total of 244 children were enroled between 29 October 2020 and 30 June 2023 and randomised to receive one of either *L. plantarum* 202195, *B. infantis* (Bi-26), or placebo (maltodextrin). A flow chart (Figure 2) shows the number of women assented, number of infants randomised, number of children seen at subsequent visits and number of early terminations. In the first year of the study, only 32% of the target 195 infants were enroled. This was largely due to the onset of the COVID-19 pandemic, which brought multiple government-imposed lockdowns and curfews that restricted field activity and hindered recruitment efforts. However, recruitment increased during the second year and by the end of December 2022 the full 195 of the initial cohort had been recruited with 48% (94/195) of participants being enroled during March through to the end of June 2022 (Figure 3). Due to a higher loss to follow-up than anticipated as a result of families leaving the study area, largely due to the COVID pandemic, ethical approval was obtained at the end of March 2023 for the recruitment of an additional 45 participants (an increase from 195 to 240 (n=80/treatment group)), who were enroled between April to June 2023 (Figure 3).

Follow-ups and early study terminations

169/244 (69%) infants successfully completed the study by December 2023. Of

the 75 early study terminations, 37 were lost to follow-up (15% of study cohort); 28 moved out of the study area before study completion (11% of study cohort); 9 had consent withdrawn by the parents (4% of study cohort); and 1 participant was withdrawn by physician for medical reasons not related to the study intervention. The majority of these early terminations (76%) occurred after the 1 month visit (Figure 2).

Protocol violations

There were no protocol violations; however, there was one incident of an accidental delivery of study intervention to a non-study participant. This child was monitored over several days by study staff to ensure their well-being, and the parents were informed to contact one of the study team in the event of any illness. No adverse events were subsequently reported for this child.

In addition, during July and August of 2023 there was a shortage of routine vaccines available in Goroka which delayed some of the follow-up visits and specimen collections for 45 study participants. Of the birth vaccines, 11 participants did not receive Hepatitis B and 15 did not receive BCG; 5 participants received neither Hepatitis B nor BCG, with 4 of these subsequently lost to follow-up before 1 month. Twenty-four participants were delayed in receiving BCG, with 14 receiving it 2-3 months after birth.

Among the routine monthly vaccination visits, delays were as follows: 7 participants had their 1 month vaccines delayed, 11 had their 2 month vaccines delayed, and 4 had their 3 month vaccines delayed. For participants with a delayed monthly vaccination visit, the timing of all subsequent scheduled follow-up visits for sample collections and immunisation were readjusted accordingly. For instance, if a participant's 1 month vaccines were delayed by 24 days, their 2 and 3 month vaccines were also delayed by 24 days. Of the 22 participants with delayed monthly vaccinations and sample collections, 3 were subsequently lost to follow-up: 1 prior to receiving their 1 month immunisations and 2 prior to receiving their 3 month immunisations. No participant remaining in the study experienced more than one additional delayed monthly immunisation.

Feasibility

218/244 (89.3%) of the study infants successfully completed the daily intervention from day 1-7. Of the 26 newborns who did not complete daily treatment, 10 (4 *L. plantarum* group; 5 *B. infantis* group; 1 placebo group) were lost to follow-up before completion of the 7 doses, and 16 newborns (5 *L. plantarum* group; 6 *B. infantis* group; 5 placebo group) missed one or more doses (15 missed a single dose and 1 missed 2 doses) but continued in the study.

Specimen collection

Rectal swabs, nasopharyngeal swabs, saliva and blood were successfully collected from more than 91% of infants who were still enroled in the study at the time of their follow-up visits (Table 2). Sufficient blood volumes were collected from 80-81% of participants at the two timepoints where more than one analysis was proposed for the sample. Rectal swabs were collected from over 99% of participants at each of the seven timepoints, except at the 3 month visit, where the collection rate was 91.3%. Collection of stool was low with the

majority (40.4%) being obtained within the first week of the study. Two NPS were collected from more than 98% of participants at each of the three timepoints.
Of the saliva sponge specimens collected, 21% (n=184) failed to recover a viable sample for subsequent analyses after centrifugation and were discarded. Excess breastmilk was successfully obtained from mothers for up to 7 days during the first week of the study in 77% of cases. However, only 18% of vaginal swabs were obtained.

Table 2: Participant specimens collected at designated time points during the 6 month follow-up.

Sample Type		Age						
		<72 hrs n=244	**8-10 Days n=234**	**2 weeks n=227**	**1 month n=226**	**3 months n=196**	**4 months n=178**	**6 months n=169**
Maternal:								
Vaginal swabs, n (%)	43/244 (18)							
Breast milk, n (%)	1,281/1,708 (75)							
Infant:								
Rectal swabs, n (%)		244 (100)	233 (99.6)	225 (99.1)	226 (100)	179 (91.3)	177 (99.4)	168 (99.4)
Stool, n (%)		89 (36)	104 (44.4)	27 (11.9)	28 (12.4)	22 (11.2)	10 (5.6)	8 (4.7)
Nasopharyngeal swabs (x2), n (%)				448 (98.7)	451 (99.8)		356 (100)	
Saliva, n (%)		244 (100)	232 (99.1)		226 (100)		178 (100)	
Blood								
OMICs, n (%)		244 (100)	232 (99.1)		182 (80.5)		144 (80.9)	
Serology, n (%)					225 (99.6)		175 (98.3)	

DISCUSSION

Feasibility of probiotic administration in PNG

Evidence of the benefits of probiotics in the health of full-term infants is scarce, but warrants further exploration given the health benefits of administering probiotics to pre-term neonates. The study conducted in India demonstrated reduced risks of NEC and mortality in children receiving probiotics early in life, highlighting their potential as an effective health intervention in neonates in LMICs[31]. Given there are numerous variables in probiotics studies, additional studies will be required before such an intervention is widely adopted. This pilot study aimed to assess the feasibility and tolerability of probiotics (or placebo) supplementation administered to neonates in PNG for 7 consecutive days—marking the first such study in this population.
Our study is one of very few examining the effects of probiotics in full-term

babies in a community setting within an LMIC, and particularly in exploring immunological outcomes. As highlighted by a systematic review conducted by Imdad et al (2021)[32], research on probiotics in community settings in LMICs is lacking. By addressing this gap, we have demonstrated both successes and challenges in participant recruitment and field work, with laboratory outcomes to follow (once analyses are completed).

We successfully completed participant recruitment, probiotic administration and specimen collection in a challenging and labour-intensive research environment. Crucial to this success was maintaining a strong rapport with hospital staff, particularly those in the labour ward, and was a testament to the study team. Daily home visits were conducted by study staff for synbiotic administration, resulting in over 90% of study infants receiving all doses, and more than 95% receiving at least six of seven doses. Just over two-thirds of participants recruited were followed through to completion, despite the impact of the COVID-19 pandemic on families. These results demonstrate the feasibility of implementing such an intervention in the PNG community.

Specimen collection: successes and challenges

This study ambitiously sought to collect up to 27 specimens per infant across 13 visits. For rectal swabs, nasopharyngeal swabs, saliva and blood for serology the rate of collection was >98% at all timepoint-specimen combinations except for one (at 3 months 91% of participants provided a rectal swab). Blood for serology (vaccine immunogenicity) was prioritised over blood for potential omics studies at 1 and 4 month collection points, thus the collection rate was lower for omics research at those time points (~80%). Stool samples (collected in addition to rectal swabs) were sought “when feasible” and had a low collection rate, particularly at 4 and 6 months where only ~55 of participants provided a stool sample. Likely reasons for this include timing (the baby did not pass faecal matter during the visit) and perhaps a cultural reluctance to provide stool samples in this setting[33].

Viable saliva was unable to be recovered from 21% of samples collected. Over half were from samples collected at the first visit, within 72 hours of birth, and therefore likely a reflection of age and the ability to produce sufficient quantities of saliva. The high overall collection rates demonstrated the feasibility of intensive specimen collection in this setting. Future studies will streamline specimen collection based on the findings from this study.

In addition to specimens from infants, we also sought breast milk during the first 7 days of participation and a vaginal swab self-collected from mothers of infant participants prior to giving birth. Collection of breast milk was successful, with 77% of potential samples being collected. However, <20% of mothers provided a vaginal swab. This is likely due to the allowable time for recruitment and enrolment in this study of up to 72 hours post-delivery. While not critical to this study’s outcomes, vaginal microbiota could be relevant in future research on infant gut colonisation.

Logistical and operational barriers

Implementing this study in PNG required overcoming significant logistical challenges, including impassable roads, frequent road tolls and maintenance of study vehicles. Due to the intensive nature of the follow-up visits, study staff

had to remain flexible, and it was often the case that a different member of the team attended different follow-up visits for a particular participant. This was the reason for the accidental probiotic delivery to a non-study infant on one occasion, but also reflects the trust of PNGIMR in the community. On this particular visit the study participant was not at their place of residence, but another infant was. The staff member administering the intervention had not attended this participant before and the parent/guardian of the infant that was present gave no indication that the child was not part of the study and allowed for the intervention to be administered. It is likely there was a misunderstanding on the part of the parent/guardian and the probiotic was seen as a medicine and of benefit to the infant.

Additional logistical hurdles included a vaccine supply shortage in the EHP in 2023. Study participants received their routine vaccinations from study nurses, with the vaccines acquired through the government medical supply. This supply was temporarily disrupted through July and August and delayed vaccine administration for 45 participants. Furthermore, we also experienced difficulties in importing a second batch of synbiotics into PNG due to delays in obtaining approval to do so from the relevant government organisations. Subsequent delays in obtaining ethical approval to expand the study size by more than 10% contributed to a lapse in recruitment from January to April in 2023 (Figure 3).

Adding to the complexity of this study and further enhancing the challenges, recruitment occurred during the COVID pandemic, commencing in PNG in late October 2020. COVID cases remained in low numbers until March 2021; however, for the following ~12 months there was high COVID transmission, including the rise of the Delta variant around September 2021 and the first Omicron case reported in December 2021. This impacted study recruitment in numerous ways. First and foremost, there were government sanctioned lockdowns and enforced curfews. This resulted in reduced field activities, including participant recruitment, particularly during the periods of high transmission. Secondly, the high burden of COVID meant periods of sick leave for study staff themselves, as well as family-care obligations, both in terms of trying to keep family members safe from COVID and assisting in care and treatment of COVID patients. Another impact of COVID worthy of mention is that some families moved during COVID times, meaning some children were lost to follow-up or withdrew from the study.

In PNG the concept of probiotics and ‘good germs’ is not well understood, and considerable investment of time and resources was required by the study team to promote awareness in the community prior to and throughout the study. This is likely one reason why recruitment was slow to gather momentum. Compounding this lack of knowledge of probiotics was the rollout of the COVID vaccine. There was considerable hesitancy around the COVID vaccine in PNG, resulting in a very low vaccine uptake: <5% of the population received one or more doses[34]. At the time of the vaccine rollout, commencing in May 2021 in EHP, there was confusion within some study communities that the probiotics were COVID vaccines, and that we were trialling COVID vaccines on the babies.

In PNG, like many countries, there are times of political and civil unrest. Elections can be highly contested, thus conducting field work in the lead up, during and shortly after elections can be dangerous. During the National election in mid-2022 there was considerably less field work conducted. Not long

after the election in 2022 (but not related to it) in September was the death of a senior bureaucrat in Goroka, leading to violence and arson attacks, with an estimated 3000 people fleeing their home to avoid it. This also led to a pause in recruitment and follow-up. At around the same time an earthquake hit the highlands of PNG which caused damage to infrastructure including roads: this caused some further minor delays in recruitment and follow up.

Implications for future research

While intervention studies can be challenging to implement in resource constrained settings, it is in these settings where health interventions are most needed to improve health outcomes. Through this pilot study, we have demonstrated the feasibility of administering probiotics to PNG infants in a clinical trial environment, supporting the implementation of a larger RCT to study clinical impact.

The aforementioned challenges no doubt contributed to the 75 early study terminations, accounting for 31% of all infant participants. However, almost 90% of participants completed their 7-day course of probiotic (or placebo), and a further 15 participants missed only one of seven doses (>95% of participants had at least six of seven doses). The comparatively high number of participants completing six or more doses provides some evidence that such an intervention could be feasible and be met with public acceptance provided sufficient education and dissemination of information accompanied the intervention.

ACKNOWLEDGEMENTS

We extend our heartfelt gratitude to the families who participated in this study for their invaluable contributions. Special thanks go to the PNGIMR study field team for their dedication and perseverance in challenging conditions. We also wish to acknowledge the staff of the Eastern Highlands Provincial Hospital, particularly paediatrician Dr Casparia Mond and the maternity ward nurses, for their unwavering support and assistance throughout the study.

This work was generously funded by the Bill & Melinda Gates Foundation and the maternal and neonatal immunisation network, IMPRINT. We also sincerely thank DuPont/IFF for their generous development and provision of probiotics used in this study. We confirm that the funders and DuPont/IFF had no involvement in the study’s design, data collection, analysis, interpretation, manuscript preparation, or the decision to submit the manuscript for publication.

AUTHOR CONTRIBUTIONS

AHJB and PCR conceived the study and led its design. AHJB, PCR, WSP, and RLF contributed to the development of country-specific protocols. Study implementation and data collection were led by RLF, WK, JS, and WSP, with assistance from BN, AK, MD, GM, and DJ. Laboratory specimen processing was conducted by JJ and TO. AG, RLF, and TO developed the probiotic gut colonisation laboratory protocol. CA developed the nasopharyngeal microbiological culturing protocol. MO managed the data and helped design the

analysis plan with PCR and AHJB. RLF and AG prepared the initial draft of the manuscript, while AHJB, PCR, and MO contributed to subsequent revisions. All authors reviewed and approved the final version.

CONFLICTS OF INTEREST

No conflicts of interest declared.

ABBREVIATIONS

BCG	Bacillus Calmette-Guérin Vaccine
B. *infantis*	*Bifidobacterium longum* subspecies *infantis*
DSMB	Data Safety Monitoring Board
DTwP	Diphtheria, Tetanus, and Whole Cell Pertussis Vaccine
EHP	Eastern Highlands Province
Hib	*Haemophilus influenzae* type b Vaccine
HEO	Health Extension Officer
LMICs	Low- and Middle-Income Countries
L. *plantarum*	*Lactiplantibacillus plantarum*
LRTIs	Lower Respiratory Tract Infections
NEC	Necrotizing Enterocolitis
NPS	Nasopharyngeal Swab
PCV13	13-valent Pneumococcal Conjugate Vaccine
PNG	Papua New Guinea
PNGIMR	Papua New Guinea Institute of Medical Research
RCT	Randomised Controlled Trial
REDCap	Research Electronic Data Capture
SAEs	Serious Adverse Events
STGGB	Skim-Milk Tryptone-Glucose-Glycerol Broth
WBC	White Blood Cells

DATA AVAILABILITY

The datasets generated and analysed during this study will be available from the corresponding author upon reasonable request, once analysis has been completed and results published.

REFERENCES

1. Newborn mortality. Available from: https://www.who.int/news-room/fact-sheets/detail/newborn-mortality [accessed Oct 22, 2024]

2. Araya M, Morelli L, Reid G, Sanders M, Stanton C, Pineiro M, Ben Embarek P. Joint FAO/WHO Working Group report on drafting guidelines for the evaluation of probiotics in food. Lond Can World Health Organ Food Agric Organ U N 2002;

3. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani

RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014 Aug;11(8):506–514. PMID:24912386

4. Guarner F, Sanders ME, Szajewska H, Cohen H, Eliakim R, Herrera-deGuise C, Karakan T, Merenstein D, Piscoya A, Ramakrishna B, Salminen S, Melberg J. World Gastroenterology Organisation Global Guidelines: Probiotics and Prebiotics. J Clin Gastroenterol 2024 Jul;58(6):533–553. doi: 10.1097/MCG.0000000000002002

5. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol 2015 Jul 22;52(12):7577. PMID:26604335

6. Yadav MK, Kumari I, Singh B, Sharma KK, Tiwari SK. Probiotics, prebiotics and synbiotics: Safe options for next-generation therapeutics. Appl Microbiol Biotechnol 2022 Jan 11;106(2):505. PMID:35015145

7. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients 2017 Sep 15;9(9):1021. PMID:28914794

8. Mahboobi S, Rahimi F, Jafarnejad S. Effects of Prebiotic and Synbiotic Supplementation on Glycaemia and Lipid Profile in Type 2 Diabetes: A Meta-Analysis of Randomized Controlled Trials. Adv Pharm Bull 2018 Nov;8(4):565–574. PMID:30607329

9. Ford AC, Harris LA, Lacy BE, Quigley EMM, Moayyedi P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. Aliment Pharmacol Ther 2018 Nov;48(10):1044–1060. PMID:30294792

10. Chan CKY, Tao J, Chan OS, Li H-B, Pang H. Preventing Respiratory Tract Infections by Synbiotic Interventions: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr Bethesda Md 2020 Jul 1;11(4):979–988. PMID:31996911

11. Pham VT, Mohajeri MH. The application of in vitro human intestinal models on the screening and development of pre- and probiotics. Benef Microbes 2018 Sep 18;9(5):725–742. PMID:29695182

12. Aabed K, Shafi Bhat R, Moubayed N, Al-Mutiri M, Al-Marshoud M, Al-Qahtani A, Ansary A. Ameliorative effect of probiotics (Lactobacillus paracaseii and Protexin®) and prebiotics (propolis and bee pollen) on clindamycin and propionic acid-induced oxidative stress and altered gut microbiota in a rodent model of autism. Cell Mol Biol Noisy--Gd Fr 2019 Jan 31;65(1):1–7. PMID:30782287

13. Shelby RD, Janzow GE, Mashburn-Warren L, Galley J, Tengberg N, Navarro J, Conces M, Bailey MT, Goodman SD, Besner GE. A novel probiotic

therapeutic in a murine model of Clostridioides difficile colitis. Gut Microbes Taylor & Francis; 2020 Nov 9;12(1):1814119. PMID:32954922

14. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2014 Apr 10;(4):CD005496. PMID:24723255

15. Aceti A, Gori D, Barone G, Callegari ML, Di Mauro A, Fantini MP, Indrio F, Maggio L, Meneghin F, Morelli L, Zuccotti G, Corvaglia L, Italian Society of Neonatology. Probiotics for prevention of necrotizing enterocolitis in preterm infants: systematic review and meta-analysis. Ital J Pediatr 2015 Nov 14;41:89. PMID:26567539

16. Chi C, Li C, Buys N, Wang W, Yin C, Sun J. Effects of Probiotics in Preterm Infants: A Network Meta-analysis. Pediatrics 2021 Jan 1;147(1):e20200706. doi: 10.1542/peds.2020-0706

17. Chi C, Buys N, Li C, Sun J, Yin C. Effects of prebiotics on sepsis, necrotizing enterocolitis, mortality, feeding intolerance, time to full enteral feeding, length of hospital stay, and stool frequency in preterm infants: a meta-analysis. Eur J Clin Nutr Nature Publishing Group; 2019 May;73(5):657–670. doi: 10.1038/s41430-018-0377-6

18. Agarwal R, Sharma N, Chaudhry R, Deorari A, Paul VK, Gewolb IH, Panigrahi P. Effects of oral Lactobacillus GG on enteric microflora in low-birth-weight neonates. J Pediatr Gastroenterol Nutr 2003 Mar;36(3):397–402. PMID:12604982

19. Balasubramanian H, Ananthan A, Rao S, Patole S. Probiotics for Preterm Infants in India - Systematic Review and Meta-Analysis of Randomized Controlled Trials. Indian J Pediatr 2020 Oct;87(10):817–825. PMID:32185693

20. Deshpande G, Jape G, Rao S, Patole S. Benefits of probiotics in preterm neonates in low-income and medium-income countries: a systematic review of randomised controlled trials. BMJ Open British Medical Journal Publishing Group; 2017 Dec 1;7(12):e017638. PMID:29222137

21. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, Baccaglini L, Mohapatra A, Mohapatra SS, Misra PR, Chaudhry R, Chen HH, Johnson JA, Morris JG, Paneth N, Gewolb IH. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017 Aug;548(7668):407–412. doi: 10.1038/nature23480

22. UNICEF Papua New Guinea Country Office Annual Report 2022 | UNICEF. Available from: https://www.unicef.org/reports/country-regional-divisional-annual-reports-2022/Papua-New-Guinea [accessed Oct 31, 2024]

23. End preventable deaths of newborns and children under 5 years of age. Available from: https://www.who.int/data/gho/data/themes/topics/sdg-target-3_2-newborn-and-child-mortality [accessed Oct 30, 2024]

24. Annual Child Morbidity and Mortality Reports – The Paediatric Society of Papua New Guinea. Available from: http://pngpaediatricsociety.org/reports/annual-child-morbidity-and-mortality-reports/ [accessed Oct 30, 2024]

25. Aho C, Michael A, Yoannes M, Greenhill A, Jacoby P, Reeder J, Pomat W, Saleu G, Namuigi P, Phuanukoonnon S, Smith-Vaughan H, Leach AJ, Richmond P, Lehmann D, Team for the NPCVTS. Limited impact of neonatal or early infant schedules of 7-valent pneumococcal conjugate vaccination on nasopharyngeal carriage of Streptococcus pneumoniae in Papua New Guinean children: A randomized controlled trial. Vaccine Rep 2016 Dec;6:36. PMID:28580433

26. Britton KJ, Pickering JL, Pomat WS, de Gier C, Nation ML, Pell CL, Granland CM, Solomon V, Ford RL, Greenhill A, Hinds J, Moore HC, Richmond PC, Blyth CC, Lehmann D, Satzke C, Kirkham L-AS, 10v13vPCV trial team. Lack of effectiveness of 13-valent pneumococcal conjugate vaccination against pneumococcal carriage density in Papua New Guinean infants. Vaccine 2021 Sep 7;39(38):5401–5409. PMID:34384633

22. World Bank Open Data. [Available at https://data.worldbank.org]

23. Population | National Statistical Office | Papua New Guinea. [Available at https://www.nso.gov.pg/statistics/population/]

29. Pell LG, Qamar H, Bassani DG, Heasley C, Funk C, Chen C-Y, Shawon J, O'Callaghan KM, Pullenayegum E, Hamer DH, Haque R, Kabir M, Ahmed T, O'Kelly C, Hossain MI, Khan AZ, Loutet MG, Islam MS, Morris SK, Shah PS, Sherman PM, Sultana S, Mahmud AA, Saha SK, Sarker SA, Roth DE. Neonatal administration of Lactiplantibacillus plantarum ATCC 202195 with or without fructooligosaccharide in Bangladesh: a placebo-controlled randomized trial. mSphere American Society for Microbiology; 2025 Feb 24;0(0):e01032-24. doi: 10.1128/msphere.01032-24

30. Lee AH, Shannon CP, Amenyogbe N, Bennike TB, Diray-Arce J, Idoko OT, Gill EE, Ben-Othman R, Pomat WS, van Haren SD, Cao K-AL, Cox M, Darboe A, Falsafi R, Ferrari D, Harbeson DJ, He D, Bing C, Hinshaw SJ, Ndure J, Njie-Jobe J, Pettengill MA, Richmond PC, Ford R, Saleu G, Masiria G, Matlam JP, Kirarock W, Roberts E, Malek M, Sanchez-Schmitz G, Singh A, Angelidou A, Smolen KK, Brinkman RR, Ozonoff A, Hancock REW, van den Biggelaar AHJ, Steen H, Tebbutt SJ, Kampmann B, Levy O, Kollmann TR. Dynamic molecular changes during the first week of human life follow a robust developmental trajectory. Nat Commun Nature Publishing Group; 2019 Mar 12;10(1):1092. doi: 10.1038/s41467-019-08794-x

31. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, Chandel DS, Baccaglini L, Mohapatra A, Mohapatra SS, Misra PR, Chaudhry R, Chen HH, Johnson JA, Morris JG, Paneth N, Gewolb IH. A randomized synbiotic trial to prevent sepsis among infants in rural India. Nature 2017 Aug 24;548(7668):407–412. PMID:28813414

32. Imdad A, Rehman F, Davis E, Ranjit D, Surin GSS, Attia SL, Lawler S, Smith AA, Bhutta ZA. Effects of neonatal nutrition interventions on neonatal mortality and child health and development outcomes: A systematic review. Campbell Syst Rev John Wiley & Sons, Ltd; 2021 Mar 1;17(1):e1141. doi: 10.1002/cl2.1141

33. Abdad MY, Soli KW, Pham B, Bande G, Maure T, Jonduo M, Kisa D, Rai G, Phuanukoonnon S, Siba PM, Horwood PF, Greenhill AR. Diarrhoeal disease surveillance in Papua New Guinea: findings and challenges. West Pac Surveill Response J WPSAR 2020 Mar 30;11(1):7–12. PMID:32963886

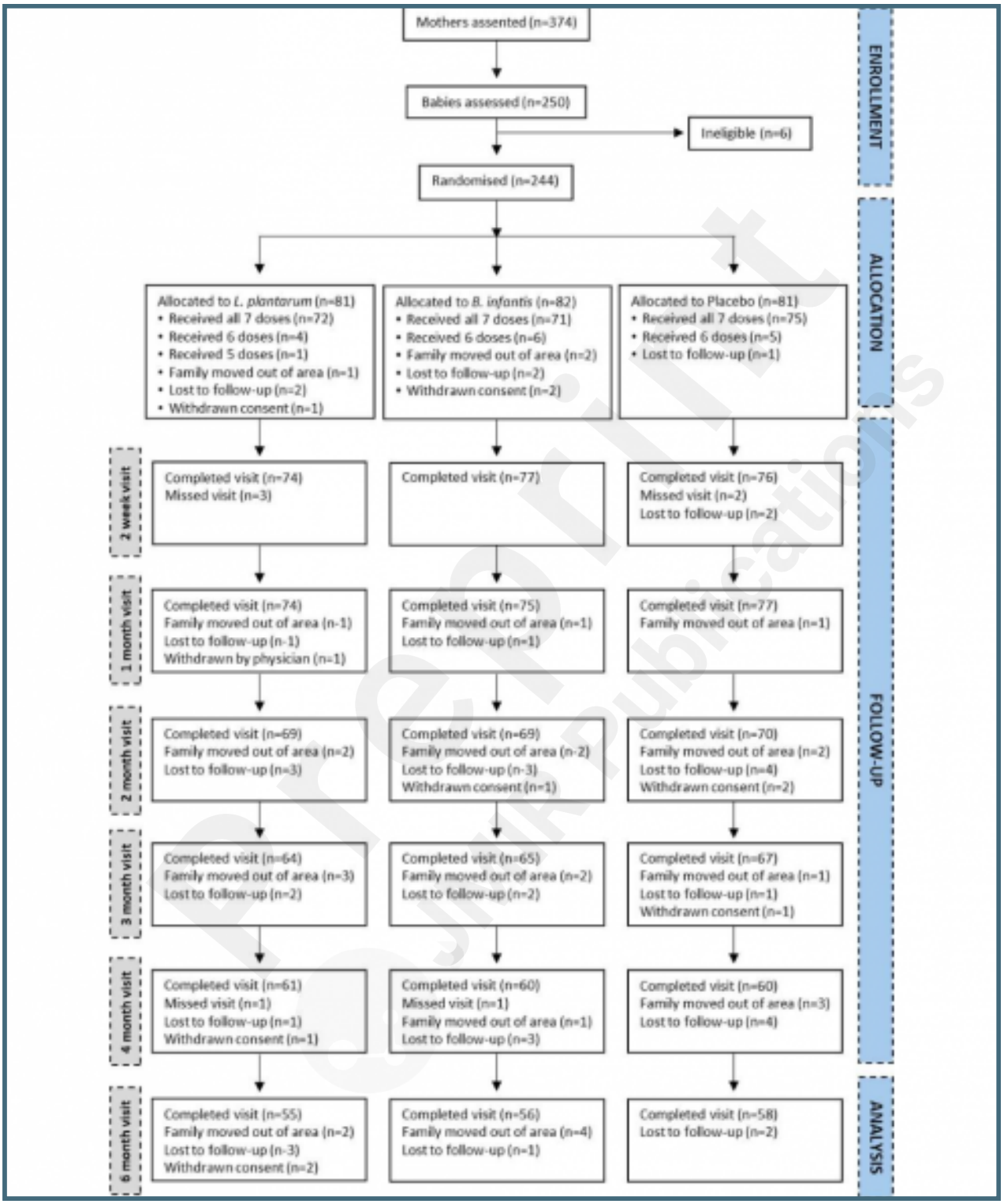
34. COVID-19 vaccines | WHO COVID-19 dashboard. Available from: https://data.who.int/dashboards/covid19/vaccines [accessed Oct 31, 2024]

Supplementary Files

Figures

Intervention, routine vaccination and specimen collection schedule. The timeline for synbiotic administration, routine infant vaccinations, and designated specimen collection points throughout the study for each participant are illustrated.

Flow chart of participants enroled and visits completed. Over two-thirds of participants enroled completed the study with no significant differences observed between any of the treatment groups.



Participant recruitment trends from October 2020 to June 2023. Overall, recruitment increased as the study progressed. Periods of disruption due to COVID-19 enforced lockdowns, local elections, civil unrest, probiotic importation delays and ethics approval delays for study expansion are reflected as lulls in recruitment.