

## Short Report: A Phase One Safety Study of *Lactobacillus reuteri* Conducted in the Peruvian Amazon: Observations from the Field

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**Abstract.** Clinical research on probiotics presents challenging issues for researchers, regulators, and funding agencies, and these issues become more complex when United States federally funded research is conducted outside the United States. Here, we describe the design and results of a Phase I safety study of *Lactobacillus reuteri* DSM 17938 conducted as a community-based trial under the Food and Drug Administration Investigative New Drug (FDA IND) program in a small town in the Peruvian Amazon. Forty-five healthy adults 18 to 65 years of age were randomized in a 2:1 ratio to receive either *Lactobacillus reuteri* 10<sup>8</sup> organisms once daily for 5 days or an identical appearing placebo. Results showed no evidence of invasive infection resulting from probiotic administration and no differences between groups. Although we encountered several challenges in conducting an FDA-approved safety trial in this setting, the rigorously collected contextually relevant data will be very valuable to support later Phase II/III studies of *L. reuteri* for use in similar settings.

Probiotic products, including nutritional supplements and preparations designed to provide health benefits, have generated increased attention from both the biomedical community and the lay press during the last 10 years, as consumers in many countries are eager for alternative, natural, and cost-effective ways to improve health and prevent or treat diseases.<sup>1–4</sup> “Probiotics” are intended to contain non-pathogenic microorganisms generally administered orally to colonize the gastrointestinal tract, where they have the potential to improve health through modulating the microbiome. Human microbiome research has rekindled interest in probiotics,<sup>5–8</sup> as the structure of the microbiome is linked with metabolic phenotypes in the host, and thus the use of select probiotic organisms to orchestrate beneficial changes is gaining scientific support.<sup>9</sup>

Clinical research on probiotics for prevention or treatment of human diseases, such as infectious diarrhea, presents new and challenging questions and issues for researchers, regulatory agencies, and funding agencies<sup>10,11</sup>; most probiotic products are produced by food manufacturers and marketed as nutritional supplements, without claiming any specific health benefits. The U.S. Food and Drug Administration also categorized some of these probiotic preparations as “Generally Regarded as Safe or GRAS” products. The use of probiotics is broad in the extreme, and they are present in yogurt, beverages, dietary supplements, and animal feed in an increasing number of countries. However, when investigators in the United States evaluate the therapeutic effect of probiotics or any other products to treat, mitigate, prevent, or cure disease states, United States regulations determine that they are being used as pharmaceuticals for a medical indication, and regulates them accordingly. Pharmaceutical products to be used for medical indications are produced and controlled in a far more stringent way than food products and dietary supplements intended for the general population. Appropriately documented and controlled manufacturing is a requirement, which is particularly important in vulnerable populations such as, for example, very low-birth-weight premature infants.

There are reasons for stricter regulations that are in fact beneficial to the research community as well as stakeholders.<sup>12</sup> It is well documented that many probiotics sold in pharmacies often do not contain the correct strains or the quantity of viable organisms indicated on the label,<sup>10</sup> or are contaminated with other organisms such as fungi or possibly harmful bacteria such as *Enterococcus faecium*, or simply contain contaminants or adulterants that are not indicated on the label.<sup>13</sup> Stricter control of the manufacturing process and environment reduces risk factors for human exposure, again particularly important in vulnerable populations associated with medical indications. Clinical trials have shown highly divergent findings regarding the induction of carriage, and clinical outcomes,<sup>4,8</sup> indicating the need for more stringency with independently scrutinized clinical documentation, with adequate protocol design, study execution, and reporting, which is a demand for use in pharmaceutical indications.

Although the data supporting the use of probiotics for clinical indications is mixed, the demand from the public is high. The global market in 2011 was recently estimated at 27.9 billion US\$ and is projected to grow at a rate exceeding 6% for the next 5 years.<sup>14</sup> Although most investigators would agree that viability is important, few trials document the identity, viability, and potency of the probiotic throughout the study period. Because many studies are performed using commercial products that may have inadequate production standards, there is reason to doubt the integrity of probiotic research conducted without strict documentation of the chemistry, manufacturing, and controls data (CMC) and Certificate of Authenticity (CoA), provided by the manufacturer in the drug manufacturing file (DMF) held by the U.S. Food and Drug Administration/Center for Biologics Evaluation and Research (US FDA/CBER).

International research on probiotics adds another level of complexity to this situation.<sup>15,16</sup> Regulations regarding medical research on probiotics are non-existent in many countries, and where they do exist, the guidelines and standards vary greatly. The majority of clinical research on probiotics centers on two general thematic areas: infectious diarrhea,<sup>17,18</sup> which poses a much larger clinical burden in resource-poor areas of the world; and inflammatory bowel disease,<sup>19–21</sup> which occurs across a broad spectrum of income levels. Our research group

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has been interested in probiotics as a simple, cost-effective intervention for pediatric diarrhea in Peru for the past 15 years, and we receive support from the National Institutes of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM) for these studies. Although clinical trials carried out in Peru are not mandated to be done under U.S. FDA oversight, NCCAM requires that their funded clinical research on probiotics for medical indications be conducted with regulatory approvals through the FDA in the United States, and following regulatory agency policies in the country where the research is being conducted.

As part of this research program we recently completed a Phase I safety study of *Lactobacillus reuteri* DSM 17938, a widely used probiotic that has been shown to be effective for treatment of pediatric diarrhea, through a community-based clinical trial for adults completed under an approved investigational new drug (IND) protocol in Santa Clara, a small town in the Peruvian Amazon. Because our original intent was to investigate the impact of *L. reuteri* on pediatric diarrhea in this Amazonian community, the corresponding Phase I adult study (required by FDA before doing Phase II efficacy studies under IND) was also completed in the same location. This report summarizes our experience conducting a clinical trial under IND in an isolated, resource poor area outside the United States.

Lactic acid-producing bacteria, including several *Lactobacillus* species, have been the most frequently studied probiotic agents. *Lactobacillus reuteri* is considered one of the few true autochthonous or naturally occurring species of *Lactobacillus* in the human gastrointestinal tract, and *Lactobacillus reuteri* ATCC 55730 is a widely used probiotic strain first isolated from breast milk in Peru and introduced commercially in Sweden in 1991. The parent strain was cured of plasmids carrying antibiotic resistance<sup>22</sup> and the daughter strain *L. reuteri* DSM 17938 is now commercially produced by BioGaia, and more than 3 billion doses have been distributed to date in more than 50 countries. *L. reuteri* strains including DSM 17938 have been shown to possess several characteristics that may contribute to their probiotic efficacy, including excretion of the antimicrobial reuterin, lactic acid, and competitive exclusion by steric hindrance.<sup>23</sup> Further research integrating metabolomics and microbiome research may elucidate which of these microbial characteristics are most important for interfering with pathogenesis of other organisms, to inform development of more effective probiotics in the future.

The primary objective of this study was to assess the safety and tolerability of *L. reuteri* (Lr) strain DSM 17938 in healthy adult volunteers. Upon enrollment subjects were randomized using a computer generated block schedule to one of two treatment groups in a ratio of 2:1 (treatment to placebo). Subjects received either *L. reuteri* (Lr):  $10^8$  (100 million) organisms per dose, given once daily for a 5-day treatment period, or placebo oil preparation (PL), administered as 5 drops of the oil vehicle used for the Lr suspension, given once daily for 5 days (BioGaia AB). The chosen dose level of  $1 \times 10^8$  colony-forming units (CFU)/day for *L. reuteri* has been shown to be safe and efficacious for all ages in numerous studies, including some cited here.<sup>24–26</sup> The preparation in sunflower oil and medium chain triglyceride oil was selected as it is the same preparation that is used for dosing in infants and children, populations that will be studied in

subsequent trials under the same IND. Study products were numerically coded and both subjects and investigators were blinded to the study group assigned. Quality assurance measures adopted included installation of a back-up generator to assure cold chain, directly observed study product administration, and documentation of product viability during the study.

In this phased study design recommended by FDA/CBER, safety was initially evaluated in adults before IND approved use in children. Subjects enrolled were healthy adults 18 to 65 years of age residing in the Santa Clara de Nanay community (population 2,765 in 2010), located 15 km southeast of the provincial capital of Iquitos (population 422,055 in 2012). The site is located in the Department of Loreto, the largest department in Peru covering about one-third of the total area of the country but with less than a million people (population density 2.4/square kilometer). The Amazon River begins about 100 km south of Iquitos with the confluence of several rivers, and the terrain is flat tropical rain forest. Exclusion criteria included pregnancy or current breastfeeding, recent (within 30 days) antibiotic use, recent use of other probiotic products (within 90 days), recent diarrhea, presence of a monitored adverse event, or positive results on tests for antibodies to HIV, Hepatitis B core antigen, and Hepatitis C. On Days 1–5, participants had daily clinic visits where a trained health worker administered the assigned study product (Lr or PL) once daily for 5 days and recorded vital signs. Blood tests for leukocyte count, serum hemoglobin, C-reactive protein, aspartate aminotransferase, alanine aminotransferase, bilirubin, serum urea nitrogen, and creatinine were performed at baseline (Day 0), end of study product administration (Day 5), and on Day 28. Subjects were given a diary card to record any symptoms they experienced during the 5-day treatment period and the following 4 weeks, up to Day 36 following onset of study product administration. During home visits (conducted every 3–5 days from the end of the study product administration through Day 36) field workers verified information recorded and assisted subjects with completing the card as needed. Subjects were referred to the study physician if they developed fever or illness, and symptoms reported were categorized and graded in adverse event reports. After Day 36, subjects were instructed to go to the study clinic for evaluation of all episodes of febrile illness occurring up to 6 months after beginning study product administration.

Data collected in the community was transferred to central databases at the Asociación Benéfica Prisma Research Center (Unidad de Investigaciones Biomédicas, Iquitos, Peru) and entered into MS Access (Microsoft Corp., Redmond, WA), using double data entry verification and special screens with filters for invalid entries and identification of missing values. The study was monitored by a Clinical Research Organization certified to conduct FDA type regulatory monitoring activities and contracted by NCCAM, and an additional independent data monitor manually verified primary outcome data. Data analysis was conducted at Tulane University using Stata (StataCorp, College Station, TX) and SPSS (SPSS Inc., Chicago, IL). The protocol was approved by the Institutional Review Boards (IRBs) at Tulane University, Johns Hopkins University, and Asociación Benéfica PRISMA (Peru). Approval to move forward with the study was also provided by the US FDA/CBER under IND 13710, the Peruvian Instituto Nacional de Salud (INS), and NCCAM.

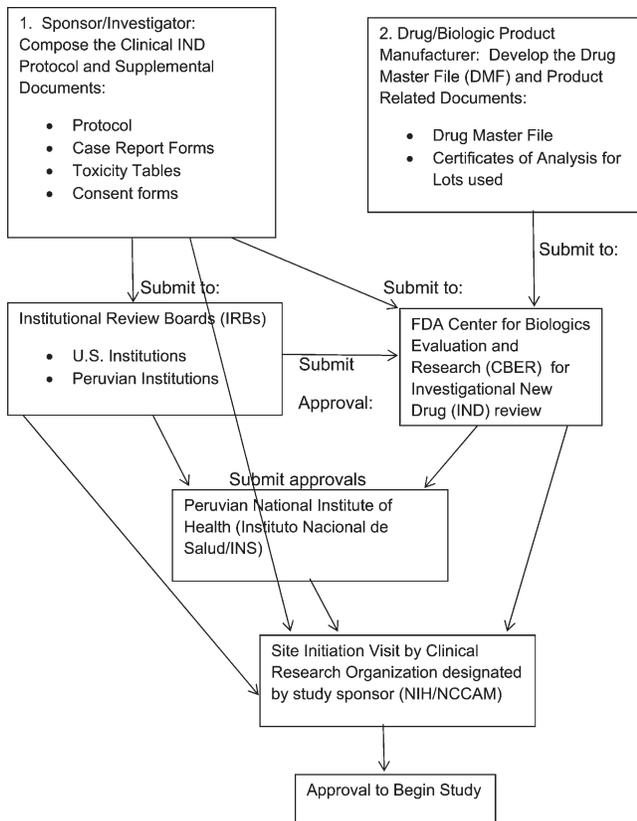


FIGURE 1. Summary of *Lactobacillus reuteri* Phase 1 Project Approval Process.

A diagram of the study approval process before initiation of human subjects' research is shown in Figure 1.

A total of 58 potential subjects were screened for participation, and 45 subjects were enrolled. Reasons for non-enrollment of 13 potential subjects were Hepatitis B seropositivity (8 of 13), concurrent antibiotic treatment (2 of 13), decided not to participate (2 of 13), and presence of another participant in the same household (1 of 13). Thirty subjects were randomized to receive Lr and 15 to receive PL. In all, 32 women and 13 men were enrolled. The Lr group included 23 women and 7 men (76.6% female) and the PL group included 9 women and 6 men (60% female; no difference in sex distribution by study group [Fisher's exact  $P$  value 0.304]). Mean age for the entire study group was 36.8 years (Lr group mean age 39.1 years [range 18.2–63.1 years] and PL group mean age 32.3 years [range 18.7–50 years]; 2-tailed  $t$ -test  $P$  value 0.043). Screened subjects who were excluded or decided not to enroll were older than participants (mean age 51.7 years, range 32–64 years) and were predominantly female (11 of 13).

Overall, results showed no evidence of invasive infection resulting from *L. reuteri* administration and no differences between groups for laboratory parameters, vital signs, clinical tolerance, or symptoms reported. None of the subjects met the criteria for drawing blood cultures (therefore no episodes of bacteremia were recorded), and there are no differences between study groups with regard to daily temperatures, leukocyte counts, or C-reactive protein detection rates. The frequency of subject-reported symptoms on the daily log sheets was similar between study groups. The only symptoms reported more frequently (with  $P < 0.05$ ) in one group were

more prevalent in the placebo group (pruritis and malaise). There was a trend toward more reporting of subjective fever in the treatment group ( $P = 0.07$ ), but this is mostly a result of events reported in Week 5 that are very unlikely to be associated with study product administration. No differences between study groups were found for any of the laboratory tests performed when controlling for study day corresponding to sample tested. The frequency of non-serious adverse events as documented by the study physician was similar between study groups, and no serious adverse events were reported. Product identification and evaluation for contaminants was done by microbiologic culture on MRS agar upon product receipt, twice during the trial and 6 weeks after the last administration of product and showed the stated amount of product on all occasions without harmful contaminants.

Conducting this Phase One study to meet requirements of the FDA IND in a community-based study completed in a semi-rural developing country setting posed many challenges. Some of the factors that extended the time required for this relatively simple study to more than 2 years included regulatory approvals in both the United States and Peru that were required to be done sequentially rather than concurrently (Figure 1), the involvement of multiple IRBs, and manual primary outcome data verification. Other challenges included the need to oversee study staff who viewed regulatory errors such as helping a subject with limited literacy to date a consent form as being a facilitator, requiring retraining of some staff members. Despite these challenges, the inherent value of conducting clinical trials under IND in resource poor settings such as our site in Peru is that they provide very valuable support for later Phase II/III studies of biologics and drugs intended for use in these settings with contextually relevant Phase I clinical data. Hopefully, the lessons learned in the process will ultimately lead to better economical and more effective therapies for diseases affecting people in developing areas of the world.

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