Your probiotic may be lying to you

Validating bifidobacterial species and subspecies identity in commercial probiotic products

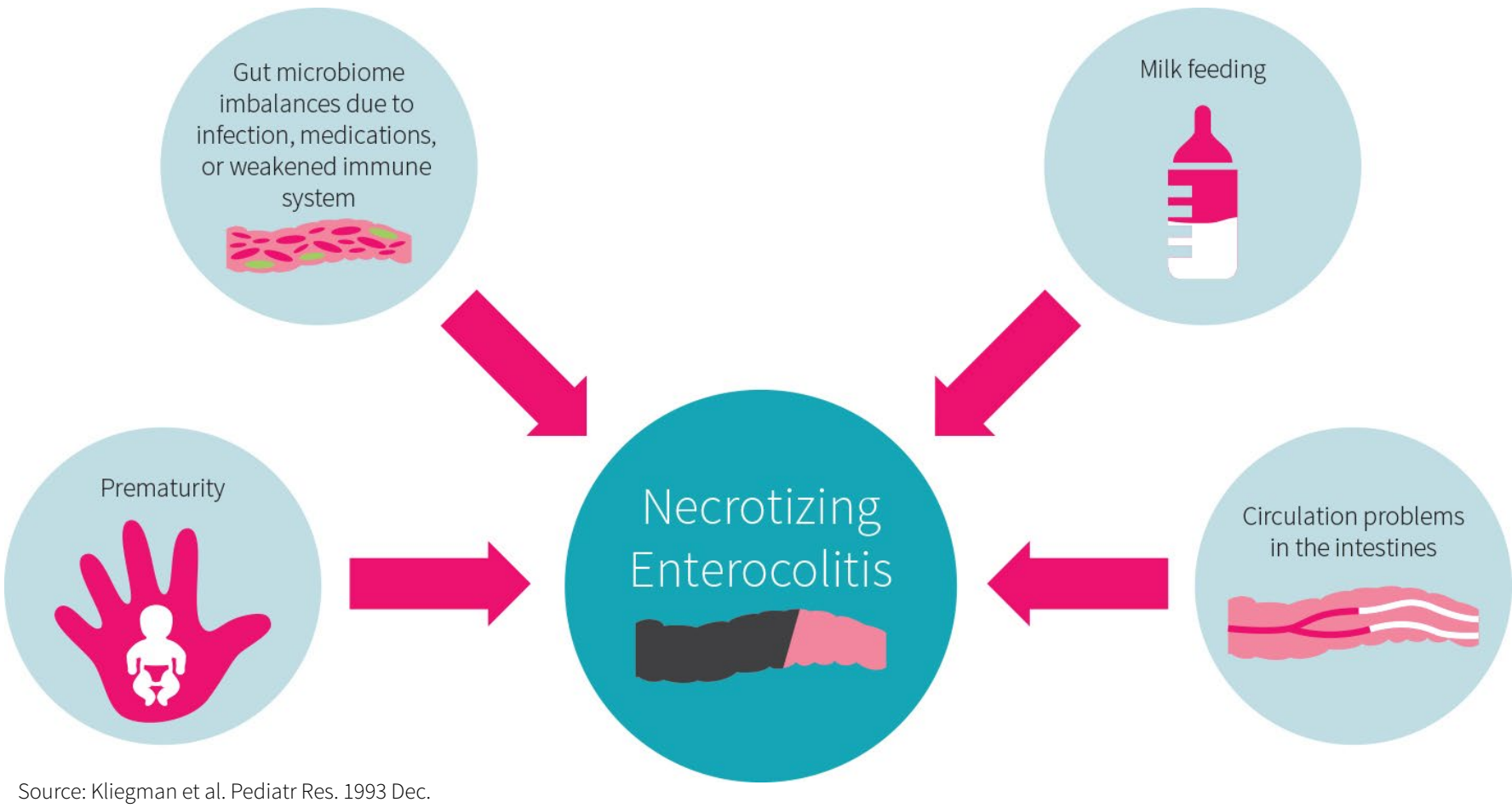
Introduction

Necrotizing enterocolitis (NEC) is a life-threatening infection that causes the tissues of the intestine to die. First described in the medical literature back in 1965, this illness can require surgical intervention, extensive hospital stays often ranging from 90 days to more than 6 months, and has been a significant cause of disease and death in premature infants. The vast majority of those who contract NEC are born premature and the annual medical costs for treating NEC has been estimated to be around $5 billion in the U.S.

While the root causes of NEC are not entirely understood, it has been theorized that the infection could be due, in part, to the invasion of the gut by harmful bacteria. Other factors that may play a part in infant NEC are shown in Figure 1. The use of probiotic therapy may be able to reduce the overgrowth of these invasive species and decrease the risk of contracting NEC. Probiotic bacteria, such as *Bifidobacterium*, produce acidic end products that can lower the pH of the intestine, creating unfavorable breeding grounds for pathogens. A recent meta-analysis of 24 trials found that probiotic use for the prevention of NEC in preterm infants was able to decrease the mortality rates and severity of NEC. However, there still remain questions about the best strain of bacteria, dosage to be used, and appropriate duration of therapy.

Bifidobacteria are of particular interest as a possible treatment to combat NEC. Research has shown this bacterial species to be ubiquitous in the gut of healthy infants, demonstrated an inclination for it to resist the colonization of certain pathogens, and has associated the bacteria with the development of the immune system. *Bifidobacterium* contains many different subspecies, some of which may be more protective against NEC than others. Probiotics containing higher amounts of these subspecies would likely be sought out in the treatment of NEC. Commercially available *Bifidobacterium*-containing probiotics usually have an array of these subspecies.

Figure 1: Risk factors for NEC in newborns



Source: Kliegman et al. Pediatr Res. 1993 Dec.

For medical practitioners to be able to effectively utilize these products, they must be tested to verify bacterial classification, viability, and purity. Researchers in the present study evaluated 17 probiotic products to determine if the detectable species of *Bifidobacterium* match the listing on the product label.

Necrotizing enterocolitis (NEC) is a life-threatening condition that disproportionately affects infants born prematurely. The use of probiotics containing *Bifidobacterium* may be useful in the treatment of NEC. However, these probiotics must be tested to ensure the contents claimed on the label match the product. This study tested 17 commercially available probiotic products.

Who and what was studied?

The researchers collected 17 probiotic products that listed *Bifidobacterium* as an ingredient. Two bottles of each product were purchased, with each coming from different product lots, or batches.

Two samples were assessed from each bottle, for a total of four samples tested per product. This was done so that variations within the same product could be measured. DNA analyses were performed on each sample to assess the strains of bacteria present in each product. Results were then compared to the label to see if the type of subspecies identified in the tests was the same as those listed on the product. In order to accurately test these samples, the researchers needed to differentiate between the DNA of various *Bifidobacterium* strains. To

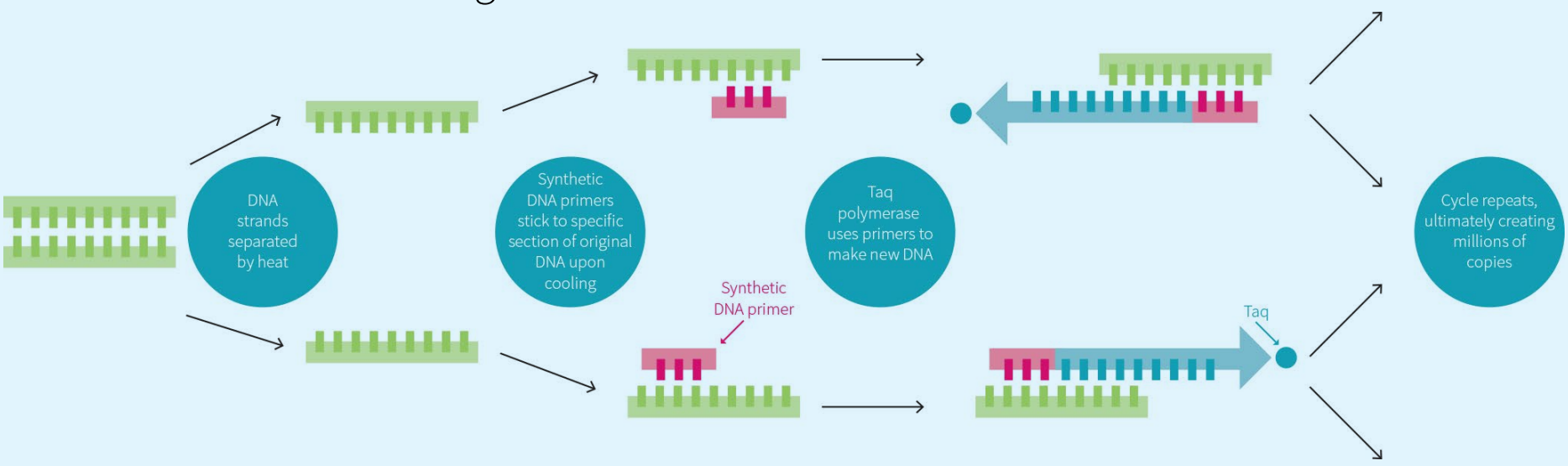
What is PCR?

PCR is used to increase, or amplify, segments of DNA. It is difficult to run genetic analyses on small quantities of DNA. PCR allows significant amounts of DNA to be copied so that proper tests can be conducted, in this case making DNA copies of Bifidobacterium subspecies.

The process, depicted in Figure 2, works by first separating the two strands of DNA that form the double helix. From there, an enzyme called Taq polymerase builds two new strands of DNA identical to the original. These new exact copies can then be used to make additional copies, and so on, until enough duplicates have accumulated for testing.

The testing that complemented the PCR technique in this study allowed the identification of Bifidobacterium subspecies and their ratio by conducting multiple reactions which selectively amplified only the DNA of one over the other. PCR actually revolutionized the field of molecular biology and led to the mapping of the entire human genome. Its creator, Dr. Kary B. Mullis, won the Nobel Prize for Chemistry in 1993.

Figure 2: The basics of how PCR works



accomplish this, they used a testing method based on Polymerase Chain Reaction (PCR).

It should be noted that while this study was funded by public grants, two of the authors, David A. Mills and Steve A. Frese, work at Evolve Biosystems, a company that is developing "proprietary and effective probiotic bacteria…[to] maintain a healthy microbiome in all infants during the first six months of life."

Researchers examined two samples from different batches of the 17 probiotic products. DNA samples of various *Bifidobacterium* strains were extracted from the probiotics and the results were compared against each product's label.

What were the findings?

The contents of the probiotics were generally consistent from sample to sample and between different lots of the same product. One product had considerable variation between samples and three showed significant differences between different lots.

Across all tested samples for each product, nine probiotics tested positive for strains of *Bifidobacterium* not listed on the label, 12 were missing strains listed on the label, and four contained non-*Bifidobacterium* species that were not on the label. There was only one product that perfectly matched its label claims on all tests. After testing certain pills, PCR was unable to amplify the DNA of the *Bifidobacteria*. Lack of amplification could mean that there were no strains or species of *Bifidobacteria* present or that inhibitors from the DNA isolation process affected the PCR. When testing for multiple strains of bacteria, those that are present at levels under 5% of the total DNA content may not be picked up in the final data output. This is a known limitation of the testing method employed in this study.

An intriguing finding of the study was that certain subspecies of *Bifidobacterium* are commonly mislabeled on commercial probiotics. One popular product that had previously listed two separate subspecies, *B. longum* subsp. *longum* and *B. longum* subsp. *infantis*, has since reclassified them as *B. animalis* subsp. *lactis* in their labeling. This can cause confusion among practitioners who may be seeking out a specific subspecies. In fact, there is evidence that the use of *B. longum* subsp. *infantis* may be favorable for infants. To help alleviate this issue, the researchers developed and validated a new testing method to help distinguish between the longum and infantis subspecies, a differentiation that was not previously possible with traditional testing alone.

Only one out of 17 probiotic products was a perfect match to label claims. Nine samples contained *Bifidobacterium* not listed on the label, 12 were missing strains claimed on the label, and four had unidentified non-*Bifidobacterium* species. New valid testing methods to differentiate *Bifidobacterium* subspecies developed by the authors can be used in future studies to check probiotic composition.

What does the study really tell us?

"These results suggest that quality control of probiotics is lacking. In order for clinical trials to provide meaningful data about the benefits of specific probiotic strains and enable clinicians to make informed decisions about prescribing or recommending probiotics, increased standards of strain identification are needed, particularly given the lack of regulatory oversight for certification of probiotics in the United States."

ERD #13, Volume 2 "Not-so-safe supplements" discussed how the current state of regulatory affairs in the U.S. does not provide the FDA adequate oversight of supplements to ensure their quality. The study under

review illustrates why this continues to be an issue. Without a reliable supplement screening infrastructure, clinicians can't be certain of the contents of a probiotic if they choose to provide it to a patient.

Further challenges arise when attempting to conduct clinical trials with commercially available products. Strain viability, dosing, misidentification of the bacterium, and contaminants are all potentially confounding variables. To avoid these issues, individual researchers conducting trials must independently verify the composition and purity of the probiotics they use.

Probiotics supplements are generally recognized as safe, and the misidentification of probiotics is not likely to pose substantial harm. However, problems can arise when people are seeking out the specific benefits of a particular subspecies of bacteria. The use of the tools and testing methods developed in this study will help discriminate between subspecies with differing metabolic capabilities that can influence the health of the user.

Quality control of probiotics is lacking. The tests developed in this study will help scientists conduct clinical trials in which specific bacterial strains in probiotics can be identified and isolated. Proper identification of probiotic species will ensure that positive or negative results are accurately ascribed to the correct strain, improving the quality of scientific literature on probiotics.

The big picture

The debate over the role *Bifidobacterium* probiotics can play in treating NEC in premature infants continues. Some have argued that probiotics should be used as a routine preventative measure. However, questions remain regarding what strain or combination of strains would be appropriate. Further clinical trials are needed to find the answers. These studies would not only have to assess strain, but dosing, timing, and therapy duration as well.

It is challenging for practitioners to identify high-quality commercially available probiotics that could be used in their practice. Quality control plays a significant role in the viability of probiotics as a treatment. Lack of such control was made evident in 2014, when a premature infant who had received the probiotic supplement Solgar ABC Dophilus Powder as a preventative treatment for NEC died of mucormycosis, a fungal infection that affects the sinuses or lungs. Investigations by the Centers for Disease Control and Prevention (CDC) and U.S. Food and Drug Administration (FDA) determined that the Solgar probiotic had been contaminated with the fungus Rhizopus oryzae.

In order for probiotics like *Bifidobacterium* to be widely integrated into routine medical care, rigorous quality control measures must be put in place by the producer to ensure good manufacturing practices. Laboratories would then need to confirm the bacterial composition, viability, and purity.

Before probiotics like *Bifidobacterium* can be utilized as a widespread NEC treatment, two things must happen. First, further clinical trials must narrow down which strains or combination of strains are the most efficacious. Second, high manufacturing standards must be set to ensure a consistent and quality probiotic is being delivered to patients.

Frequently asked questions

I'm not a premature infant. What are the other applications of Bifidobacterium?

This species of bacteria is one of the most well studied and has been tested in a number of potential treatments, with some shown in Figure 3. The *Bifidobacterium infantis* strain has been shown to have

certain antimicrobial properties that may inhibit the growth of pathogens in the intestines. Bifidobacteria-supplemented formula has also demonstrated protective properties against diarrhea in infants and rotavirus infection. It may also have a favorable effect in treating human inflammatory bowel diseases. Lastly, in the treatment of irritable bowel syndrome, *Bifidobacterium* supplementation may be a more effective therapy over supplementation with other probiotic strains like *Lactobacillus*.

How common is it for supplements to be mislabeled?
It is hard to say just exactly how prevalent the issue of supplement mislabeling is, but there are a number of studies that have raised concern over this issue. Most recently, a study from a group of scientists in Australia found that many traditional Chinese herbal remedies contained herbs and pharmaceutical agents not declared on the label. Potentially toxic heavy metals were also detected in levels above the recommended daily intake. Another study testing 44 herbal products found that 59% contained plant species not listed in the ingredients.

From the consumer's standpoint, one way to help ensure the quality of the supplement being purchased is to seek out those that have been independently tested by third parties such as NSF, USP, Informed Choice, and Consumer Lab.

What should I know?

Probiotics such as *Bifidobacterium* may play a role in the treatment and prevention of NEC. Further clinical trials are needed to assess commercially available probiotics for composition and purity in order to help ascertain clinical efficacy, especially since these treatments are intended for use on high-risk patients. Issues to be considered are the accuracy of the probiotic label, bacterial strain, consistency of product formulation from batch to batch, dosing, timing, bioavailability, and duration of therapy. The testing methods employed in this study will help answer these questions in future trials. ◆

Have you thanked your Bifidobacteria lately? Express your gratitude then head to the Facebook ERD forum to discuss this topic.

Figure 3: Some other effects of Bifidobacterium