

Dear Editor,

In a recent issue of World Nutrition Journal (2018;9(3):153-162), Dr. George Kent called into question the ability for a food product to be both new and safe. He specifically used Evivo® infant probiotic (*B. infantis* EVC001) as an example of his point, in which he inaccurately represents the scientific data, safety and biological rationale for use of the product. Here we respond to Dr. Kent's previously published article and address each inaccuracy.

Patentability and Safety are different issues

Firstly, we share Dr. Kent's passion about ensuring that products intended for use by infants and adults alike, are rigorously tested for safety and labelled appropriately for their use before they are introduced into commerce. Patents, however, are granted on the basis of novelty, inventiveness, and utility, and not safety. For example, a probiotic organism that is in common use in the food system and considered safe, may still be manufactured, presented, or packaged in a novel and patentable way to improve the product's quality or convenience of use. Patent offices around the world have literally millions of examples of such inventive improvements, and the assumption that food products cannot be patentable and safe at the same time is a misinterpretation of the concept of intellectual property that dates back to England in 1624. So, the answer to the question of can a food product be patentable and safe is, of course, yes.

Historic Prevalence of *B. infantis* in human populations

Bifidobacterium longum ssp *infantis* (*B. infantis*) is an organism that has been passed on from mother to infant during the birthing process for millennia, through a fecal oral transfer of the mother's microbiome to her infant. We know this because *B. infantis* has a unique set of genes¹. that coevolved with humans to convert undigestible human milk oligosaccharides (HMOs) comprising about 15% of the nutrients in human milk into smaller molecules (e.g., acetate and lactate) that are useable fuels for the infant². These compounds also create a gut environment that protects the newborn infant from invasion by many opportunistic enteropathogens. A probiotic organism like *B. infantis*, or any other substance used in food prior to January 1, 1958, is asserted to be safe by a historical use precedent under the Food, Drug, and Cosmetic Act. In 1913, Dr. Logan, a pediatrician at the Royal College of Physicians in Edinburgh described the stool of the breastfed baby at the time as looking like "films of almost pure culture"³. He then went on to describe the culture as gram positive bacteria which he referred to as *B. bifidus* (now renamed as *Bifidobacterium*). Although we cannot be sure that Logan's *Bifidobacterium* was indeed *B. infantis*, this would be the most likely candidate as it is consistent with Logan's written observations and we now know that *B. infantis* is the dominant consumer of HMOs².

Establishing the Safety of Evivo

Notwithstanding the above historical references, Evolve BioSystems further engaged an independent expert panel (a GRAS Panel) of four highly qualified professors from the U.S. with

expertise in Pediatrics, Toxicology, Nutrition, and Food Science to review the totality of the peer reviewed published literature and make an independent assessment of the safety of this organism for its intended use in newborn infants. Under sections 201(s) and 409 of the Food Drug and Cosmetics Act, “*general recognition of safety* [of a food substance] *through scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive.*” Through its deliberations, the panel reviewed and opined on the phenotypic and genotypic characterization of Evolve’s *B. infantis* EVC001, the manufacturing process, the intended use and dietary intake, animal safety studies, the history of the safe use of *B. infantis* in foods, and they reviewed 41 human clinical studies completed with *B. infantis* either as single strain or in multi-strain products. A key publication in their analysis was the Safety and Tolerance assessment completed using *B. infantis* EVC001⁴. The conclusion of these experts after this extensive review was: “*Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use as a FSDU [Food for Special Dietary Use] for term infants (0 to 12 months of age) consuming human milk as all or part of their diet, B. infantis EVC001 freeze-dried powder, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practices (cGMP), is GRAS based on scientific procedures.*” *B. infantis* EVC001 has also been evaluated by Health Canada for safety and efficacy in infants 0-12 months and received marketing approval in 2017.

The *B. infantis*/Human Breast Milk Symbiosis

Dr. Kent inaccurately states in his article that Evivo claims widespread deficiencies in women’s breastmilk and that Evivo is used to remedy these deficiencies. This is an unfortunate misinterpretation of the rationale for use of Evivo, as it is the absence of *B. infantis* in the general infant population that is the problem, not deficiencies in breastmilk. Evolve BioSystems is a strong supporter of breast feeding and recognizes that human breast milk contains all the nutrients required to feed a growing baby, and its gut microbiome. Indeed, research completed in the past few years has clearly indicated that when *B. infantis* is combined with the HMOs from human milk, it will dominate the infant microbiome as first demonstrated by Logan in 1913, and as can now be seen today in many populations that have minimal contact with the Western world^{5,6,7,8}. However, the absence of *B. infantis* in infants from most Western populations today is exemplified by many publications in the peer reviewed literature where the infant gut microbiome has been investigated^{9,10,11}. Although *Bifidobacterium* may be present at levels of about 30% relative abundance, it is limited to species other than *B. infantis*. In a randomized control trial where *B. infantis* EVC001 was provided to half of a cohort of breastfed babies, there was a 100% response rate in conversion of the gut microbiome to one dominated by *B. infantis*¹⁰, whereas none of the 33 infants in the control infant group had any detectable levels of *B. infantis*. Furthermore, the HMOs and the energy associated with them were being lost in the feces of the control group babies. The generational and repeated use of antibiotics which results in decreased microbiome diversity is likely the reason for the loss of *B. infantis* in Western societies¹². The good news is that when *B. infantis* ECV001 (Evivo) is given back to the baby, HMOs in breastmilk can be fully converted to important fuels like acetate and

lactate that can be utilized directly by the baby and, at the same time, lower the intestinal pH to a level that reduces the growth of many enteropathogens, as described previously^{13,14}.

Growing Data Supports a Mechanism of Action

Additional peer-reviewed data are now pointing to at least five important and immediate outcomes when the original symbiosis between breastmilk HMOs and *B. infantis* is reestablished. First, and foremost, levels of virulent pathogens such as *Escherichia coli*, *Klebsiella oxytoca*, *Clostridia difficile* and *Staphylococcus aureus* are all reduced in the gut by over 90% at a critical time (the first 100 days of life) where the infant's own immune system is being educated^{15,16,17}. Maldevelopment of the immune system in these early days has been proposed to be associated with later development of autoimmunity including Type 1 Diabetes^{18,19}. Second, breastmilk HMOs are fully utilized through conversion to acetate and lactate, which lower the gut pH and provide fuel to the gut epithelium^{2,10}. Third, the stooling frequency is decreased, and its consistency improved⁴, reflective of stooling patterns reported in the US in the 1920s¹⁴. Fourth, the antibiotic resistance gene load (primarily associated with virulent pathogens) is significantly reduced, mirroring observations of infants naturally colonized by *B. infantis*^{15,20}. Finally, elevated levels of *B. infantis* in the infant gut has been associated with significantly improved vaccine responses²¹.

In Conclusion

Due to its unique symbiosis with the HMOs found in breast milk, *B. infantis* has been shown to provide a number of important benefits to the newborn infant that cannot be provided by any other "probiotics" or bacterial species. This mechanism of pathogen colonization resistance whereby mom first provides a seed for the infant's microbiome from her own microbiome through the process of vaginal delivery, and then recruits a unique microbiome composition in her baby's gut by providing *B. infantis*-selective food for the baby (*i.e.*, breast milk) creates a protective gut microbiome to protect her baby from pathogen invasion at a time when her baby is not yet capable of mounting its own protection. Although this appears to be an ancient protective mechanism, it was only recently discovered by working backwards through the mechanistic data, showing that *B. infantis* has been disappearing in the Western world for the past 50 years or more. Indeed, one can argue that we have been the unwitting participants in a worldwide infant gut microbiome remodeling experiment over the past 100 years through a perfect storm of multigenerational antibiotic use, increased frequencies of C-Section deliveries, and the early replacement of breast milk with HMO-deficient infant formulas. However, now that we know what is missing, we can safely and easily restore *B. infantis* to the infant gut microbiome and solve for the unintended consequences of these important medical and dietary interventions.

EDITOR'S NOTE: David Kyle and Tracy Shafizadeh have declared that they are both employees of Evolve BioSystems, maker of Evivo

References

1. Sela, D. A., et al. "The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome." *Proceedings of the National Academy of Sciences* 105.48 (2008): 18964-18969. <https://doi.org/10.1073/pnas.0809584105>
2. Underwood, Mark A., et al. "Bifidobacterium longum subspecies *infantis*: champion colonizer of the infant gut." *Pediatric research* 77.1-2 (2015): 229. <https://www.nature.com/articles/pr2014156>
3. Logan, W. R. "The intestinal flora of infants and young children." *The Journal of Pathology and Bacteriology* 18.1 (1913): 527-551.
4. Smilowitz, Jennifer T., et al. "Safety and tolerability of *Bifidobacterium longum* subspecies *infantis* EVC001 supplementation in healthy term breastfed infants: a phase I clinical trial." *BMC pediatrics* 17.1 (2017): 133. <https://doi.org/10.1186/s12887-017-0886-9>
5. Huda, M. Nazmul, et al. "Stool microbiota and vaccine responses of infants." *Pediatrics* 134.2 (2014): e362-e372. [10.1542/peds.2013-3937](https://doi.org/10.1542/peds.2013-3937)
6. Davis, Jasmine CC, et al. "Growth and morbidity of Gambian infants are influenced by maternal milk oligosaccharides and infant gut microbiota." *Scientific reports* 7 (2017): 40466. <https://www.nature.com/articles/srep40466>
7. Grzeskowiak, Lukasz, et al. "Distinct gut microbiota in southeastern African and northern European infants." *Journal of pediatric gastroenterology and nutrition* 54.6 (2012): 812-816. [10.1097/MPG.0b013e318249039c](https://doi.org/10.1097/MPG.0b013e318249039c)
8. Lewis, Zachery T., et al. "Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants." *Microbiome* 3.1 (2015): 13. <https://doi.org/10.1186/s40168-015-0071-z>
9. Bäckhed, Fredrik, et al. "Dynamics and stabilization of the human gut microbiome during the first year of life." *Cell host & microbe* 17.5 (2015): 690-703. <https://doi.org/10.1016/j.chom.2015.04.004>
10. Frese, Steven A., et al. "Persistence of Supplemented *Bifidobacterium longum* subsp. *infantis* EVC001 in Breastfed Infants." *MSphere* 2.6 (2017): e00501-17. <https://doi.org/10.1128/mSphere.00501-17>
11. Vatanen, Tommi, et al. "Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans." *Cell* 165.4 (2016): 842-853. <https://doi.org/10.1016/j.cell.2016.04.007>
12. Blaser, Martin J. "Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues." (2013).
13. Henrick, Bethany M., et al. "Elevated fecal pH indicates a profound change in the breastfed infant gut microbiome due to reduction of *Bifidobacterium* over the past century." *MSphere* 3.2 (2018): e00041-18. <https://doi.org/10.1128/mSphere.00041-18>
14. TISDALL, FREDERICK F., and Alan Brown. "STUDIES ON THE ACIDITY (HYDROGEN ION CONCENTRATION) OF INFANTS' STOOLS." *American Journal of Diseases of Children* 27.4 (1924): 312-331.

15. Casaburi, Giorgio, and Steven A. Frese. "Colonization of breastfed infants by *Bifidobacterium longum* subsp. *infantis* EVC001 reduces virulence gene abundance." *Human Microbiome Journal* 9 (2018): 7-10.
<https://doi.org/10.1016/j.humic.2018.05.001>
16. Amenyogbe, Nelly, Tobias R. Kollmann, and Rym Ben-Othman. "Early-life host-microbiome interphase: the key frontier for immune development." *Frontiers in pediatrics* 5 (2017): 111. <https://doi.org/10.3389/fped.2017.00111>
17. Round, June L., and Sarkis K. Mazmanian. "The gut microbiota shapes intestinal immune responses during health and disease." *Nature reviews immunology* 9.5 (2009): 313.
18. Vatanen, Tommi, et al. "Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life." *Nature microbiology* (2018): 1.
19. Insel, Richard, and Mikael Knip. "Prospects for primary prevention of type 1 diabetes by restoring a disappearing microbe." *Pediatric diabetes* 19.8 (2018): 1400-1406.
<https://doi.org/10.1111/pedi.12756>
20. Taft, Diana H., et al. "Bifidobacterial Dominance of the Gut in Early Life and Acquisition of Antimicrobial Resistance." *mSphere* 3.5 (2018): e00441-18.
<https://doi.org/10.1128/mSphere.00441-18>
21. Huda, M. Nazmul, et al. "Bifidobacterium Abundance in Early Infancy and Vaccine Response at 2 Years of Age." *Pediatrics* 143.2 (2019): e20181489.