SPLASH!® milk science update
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This month's issue features COVID-19 and human milk, viral colonization of the infant gut, a new cattle genome, and kefir and behavior.

SARS-CoV-2 Research Highlights the Importance of Human Milk Immunobiology

- **Milk researchers all over the world are focusing on how human milk responds when mothers have COVID-19, the disease caused by SARS-CoV-2.**
- **Several research teams are investigating the use of purified milk antibodies directed at SARS-CoV-2 and possibly even whole milk collected from COVID-19-positive mothers as a therapy to treat critically ill COVID-19 patients.**
- **Although milk may contain antibodies against SARS-CoV-2, it may also be a vertical mode of transmission by passing on the virus itself.**
- **A large collaborative study is working to determine if viral RNA specific to SARS-CoV-2 is present in milk from COVID-19-positive mothers.**

Over the last six months, scientists all over the world have put their planned research programs on hold and pivoted to study SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Milk researchers are no exception. Milk from mothers that have COVID-19, the illness caused by SARS-CoV-2, could be a source of antibodies directed against the virus. Like convalescent plasma (i.e., blood from recovered COVID-19 patients), these maternally-derived antibodies offer potential as a therapeutic to help severely ill patients. But human milk could also contain RNA from SARS-CoV-2, and possibly even infectious viral material. Telling infected mothers to stop nursing "just in case" is not an option, particularly in populations without access to human milk alternatives. There is urgency in identifying both therapeutics to help those with the most severe infections and to establish informed public health policy for nursing mothers that are COVID-19 positive. The vast number of investigators tackling these questions across institutions and countries offers promise that answers will soon be available [1].

Passive (and Aggressive?) Immunity

Vaccines are an incredible scientific achievement, providing a way to develop pathogen-specific antibodies without having to suffer any of the ailments associated with the pathogen. But vaccines have nothing on passive immunity; mammalian mothers pass on their pathogen experience (aka antibodies) to their offspring in milk without the infant needing to make any energetic investment in mounting an immune response.

Human milk contains several types of antibodies (or immunoglobulins), including immunoglobulin M (IgM), immunoglobulin G (IgG), and secretory immunoglobulin A (sIgA), which is the most prevalent. The specificity of these immunoglobulins to particular viruses or pathogens allows for immature infants to mount a very mature, and aggressive, immune response that can attenuate or possibly even prevent infection and illness.

The spread of a novel coronavirus around the world means that no one has built up immunity to SARS-CoV-2. But could infants of COVID-19-positive mothers be the exception? And if so, can we take advantage of those passively obtained antibodies as a potential therapy for severe cases of COVID-19?

One researcher tackling these questions is Dr. Rebecca Powell from the Icahn School of Medicine at Mount Sinai in New York City. In early April 2020, Dr. Powell put out a request for milk samples from New York

City mothers on social media. The response from mothers was overwhelming. "I was shocked by the response I got," said Powell. "I recruit for milk studies all the time, but this was at such a high level and so unexpected."

New York City was the epicenter of the SARS-CoV-2 pandemic in the United States, so Powell expected that there might be many mothers that had either tested positive or presumed they had COVID-19, including many healthcare workers that were on the front lines. But she had not expected that so many mothers would be interested and willing to share milk samples with her for her research.

Powell and her colleagues are analyzing milk samples for the presence of SARS-CoV-2- specific antibodies, including IgM, IgG, and sIgA. "The bulk of what I expect to be the longer lasting antibody is sIgA, but I want to measure the other immunoglobulins in order to fully understand the maternal response," explained Powell. Despite decades of research on human milk immune factors, researchers are still testing hypotheses about the relationship between the maternal immune response and the types and quantities of immune factors in human milk. "We really don't know enough to focus on only one type of immunoglobulin. And we can't assume that someone who is infected and has antibodies in their blood would have them in their milk. We can't take for granted that we would just know this as a given."

In a pilot research project [1] (which has not yet undergone peer-review), Powell and colleagues report on findings from 15 milk samples from COVID-19-positive mothers compared with 10 milk samples collected prior to December 2019 (i.e., pre-COVID-19, to use as a control). All samples were analyzed for how well different antibody classes reacted to a Receptor Binding Domain on one of the virus's spike proteins. The findings, although preliminary, suggest that SARS-CoV-2-specific antibodies are passed from mothers to infants in human milk. Eighty percent of samples from COVID-19-positive mothers had reactive IgA antibodies and reactive secretory antibodies, suggesting sIgA was specific to SARS-CoV-2 [1].

The next step is to verify these findings from a larger sample to demonstrate a typical pattern in the immune response in milk. Then, Powell hopes to purify SARS-CoV-2-specific antibodies to use as a treatment for severely ill patients. "This would be for people who have a high likelihood of dying and would probably be used in combination with other treatments," said Powell. "[Purified milk antibodies] could mitigate the pathology enough to help survival." But first, she will need to get approval to use milk antibodies as a treatment. If approved, this type of research could pave the way for using isolated milk antibodies to treat other diseases. "Milk immunobiology might finally get the recognition it deserves," she explains. "We can really learn lessons that help with other diseases."

Milk as Medicine

At the same time Dr. Powell was using social media to recruit participants, the Dutch Milk Bank put out a request for milk samples on Facebook to Dutch mothers that had tested positive or suspected that they had COVID-19.

Dr. Kasper Hettinga, Dr. Hans van Goudoever, and their colleagues at the University Medical Center, Amsterdam and several other institutions throughout The Netherlands are performing similar milk antibody research to Powell's research. Hettinga and colleagues also hope to quantify the immune response in milk to SARS-CoV-2 and are asking for milk samples from a week after initial infection (or positive test result) through two months after infection. Dutch mothers have also shown strong interest in participating, with many providing daily samples that allow the researchers to see how antibody levels change over the course of the infection.

The Dutch researchers agree that these antibodies could be used as a therapeutic for the most critical COVID-19 patients. But instead of isolating antibodies, they plan to use whole milk as a therapeutic.

"The initial focus of our research is whether anti-SARS-CoV-2 antibodies in milk have a neutralizing function," explains Hettinga. "But by looking at milk as a whole, we may see additional anti-viral activity as well." These additional anti-viral milk factors include lactoferrin, lysozyme, and even free fatty acids.

"We want to look at how milk works in modulating the immune system. It has such a wide range of proteins with antiviral functions that could benefit elderly vulnerable COVID-19 patients," says Hettinga.

Giving severely ill patients human milk would allow them to take advantage of the synergistic activities of milk immune factors that evolved to enhance survival in vulnerable infants and young children. But because raw milk is not sterile, Hettinga and colleagues are also investigating various pasteurization techniques that make the milk safe to drink but also retain all of the important immune components.

Like Powell's purified antibodies, the research from Hettinga and colleagues could pave the way for using pasteurized milk as a treatment for critically ill patients suffering from illnesses other than COVID-19.

Wolf in Sheep's Clothing?

Dr. Michelle McGuire, Director and Professor in the School of Family and Consumer Sciences at the University of Idaho, is another scientist interested in the milk immune response to SARS-CoV-2—the *entire* immune response. This includes the presence of the virus itself.

McGuire is a nutritionist who started studying human milk because "it is," as she describes "the ultimate food." Her research usually focuses on identifying bacterial populations in human milk, more commonly known as the milk microbiome. But she is now part of a large collaboration across numerous institutions and one of several principal investigators looking at all of the components present in milk from mothers with COVID-19, including SARS-CoV-2.

"There are currently a lot of conflicting recommendations on breastfeeding for infected women," explains McGuire. "We really need to be able to tell mothers what to do now. But we really need to tell moms when this virus hits Africa and low-income countries where there is no safe alternative to mother's milk."

Her role in this project, funded by both The Gates Foundation and National Science Foundation, includes the establishment of a standardized milk collection procedure for studying SARS-CoV-2 and determining whether or not viral RNA from SARS-CoV-2 can be detected in milk from mothers during the first weeks after diagnosis. These are not mutually exclusive; if you are looking to see if there is viral material in milk, you need to be able to eliminate other potential contaminants, including breast tissue.

There is an important difference between looking for viral RNA and looking for infectious viral material. "Even if the virus is detectable in milk, it doesn't mean that milk is a mode of transmission," McGuire explains. "We are looking at viral RNA only, not infectious particles. You need more than genetic material to be an active and infectious virus."

McGuire and her team are not the only investigators looking for viral RNA in milk samples. To date there have been over 20 publications on the topic, with conflicting results. McGuire believes that although the quality of papers improved over time, the results should not be used to inform public policy. For starters, the methods of milk collection did not follow a standardized procedure that would eliminate other methods of contamination. Second, many of the studies used milk collected weeks after infection where viral material was less likely to be detected. And finally, only one of these papers discussed validating their method for identification of viral RNA in milk (as opposed to plasma).

McGuire and colleagues are recruiting COVID-19-positive mothers in various ways, including social media, through the American Academy of Pediatrics (AAP) website, and through UCLA and UCSF hospitals that refer mothers that test positive when they arrive in labor.

"We want to get 50 women enrolled during their first week of diagnosis," explains McGuire. In addition to collecting milk samples, they will ask mothers about their specific symptoms and the severity of their symptoms to understand the relationship between the infection and the maternal response in milk.

"Within the next three months we hope to have solid data on whether we can detect viral RNA in milk produced in the first week after diagnosis using an assay validated for human milk." If no viral RNA is present, then there is no need to look for infectious particles.

The results of McGuire's work will be of great interest to organizations that disseminate public health recommendations, including the CDC, WHO, and AAP. "We are communicating directly with policy makers and they will have the data before they are published to have time to respond and dictate public policy." As of June 2020, the WHO recommends COVID positive mothers continue to nurse as the benefits outweigh any potential risks.

McGuire has been encouraged by the collaborative nature of her research team. "Rather than a competition to be the first, the collaboration is really moving the science forward more quickly than usually happens when it is lab versus lab."

If research does indeed proceed quickly for McGuire, as well as for Hettinga and Powell, look to *SPLASH!* in the fall for an update on all of these ongoing research projects on milk and SARS-CoV-2.

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Contributed by
Dr. Lauren Milligan Newmark
Researcher, Science Writer

How Breastfeeding Influences Viral Colonization of the Infant Gut

- **Viruses are known to colonize the human gut during early infancy, but the details of this colonization are still unclear.**
- **A new study found that the viral colonization of the early infant gut occurs in a stepwise manner starting with viruses present in gut bacteria and later followed by viruses that replicate in human cells.**
- **The study found that breastfeeding influences viral colonization of the infant gut, with viruses that grow in human cells more commonly found in exclusively formula-fed babies compared with those that were fully or partially breastfed.**

The human gut microbiome is known to contain a large number of both bacteria and viruses. Viruses are absent from the infant gut at birth but colonize shortly after and can sometimes lead to gastrointestinal disorders (1–4). By one month of age, infants can have about a billion viruses per gram of stool, which is similar to the number of viruses present in older children and adults (5–7). But there is still a lot researchers don't know about how viruses colonize the early infant gut to form the virus microbiome, known as the virome.

"So far most of the studies analyzing the gut microbiome have focused on bacteria and there are not that many studies on viruses," says Dr. Guanxiang Liang of the University of Pennsylvania. "A 2015 study described the virome in infants and indicated that it is very dynamic, but there's still a lot we don't know," he says (2). "Some people think that babies are born with viruses and are colonized before they were born, while others think babies are colonized after birth," says Liang. "That's one of the questions that prompted us to analyze the infant gut virome," he says.

In a new study, Liang and his colleagues analyzed the colonization of the infant gut by viruses (8). They found that viral colonization occurs in a stepwise manner, starting with viruses present in bacteria—known

as bacteriophages—that form the predominant viral population at one month of age. By four months of age, identifiable animal viruses that can replicate in human cells become more prominent. In addition, the researchers found that breastfeeding modulates viral colonization of the infant gut, with viruses that grow in human cells more commonly found in exclusively formula-fed babies compared with those that are fully or partially breastfed.

Studying the viruses in the infant gut was no easy task. Liang says that "99.9% of them cannot be cultured, so that's the big challenge when you're doing microbiome studies." And even when researchers can sequence viruses, most of them can't be matched to known sequences in virus databases. "They could be virus or maybe they're not, and that's another big challenge," he says.

To investigate how viruses colonize the infant gut, the researchers analyzed stool samples from 20 healthy infants just after birth and at one and four months of age. "We first isolated the virus-like particles and we looked at them under the microscope," says Liang. "We were the first group to do that in newborns, and we couldn't see many viruses there," he says. "But within one month the number of viruses can reach 1 billion per gram of feces, and that's a very high number," says Liang.

Liang and his colleagues then decided to investigate the source of these viruses. "We didn't know where they were coming from," says Liang. The researchers purified DNA and RNA from the virus-like particles and from the whole gut microbial communities, and used sequencing to characterize the early virome.

The researchers found that the infant gut viral community is assembled in distinct steps. Newborns were found to have few to no viruses, and the researchers found that most of the billion virus particles identified at one month of age were not animal viruses that could replicate in human cells. Instead, most of these viruses were those present in bacteria.

"There are two kinds of phages, temperate phages and lytic phages," says Liang. "Lytic" phages get their name from their ability to "lyse" cells, i.e., to cause them to break apart by rupturing their cell membranes. "Lytic phages have ongoing replication and they infect the bacteria and lyse the bacterial cell to release themselves," he says. "Temperate phages, on the other hand, infect the bacteria and integrate themselves into the bacterial genome," says Liang. "They don't lyse the bacterial cells immediately, but when they receive certain signals they can become lytic again and lyse the cells," he says.

Liang decided to investigate which types of phages were more prevalent in the infant gut. He and his colleagues found that most of the virome community of one-month-old infants came from temperate phages rather than lytic phages. "When we checked later at four months, it seemed like more and more animal cell viruses came in, as well as some lytic phages," says Liang.

The researchers then looked into the potential influence of breastfeeding on the infant virome. "A lot of studies have looked at how breast milk inhibits the infections of some viruses," says Liang. Studies have shown that mixed feeding of formula and human milk may be protective against viruses compared with feeding only formula (9). Epidemiological studies have also shown protective effects of breastfeeding in reducing viral gastroenteritis and infant death (10,11).

Liang's initial study of 20 infants appeared to indicate that breastfeeding had an effect, as it was associated with a lower accumulation of human viruses in stool samples. To validate these results, the researchers analyzed stool samples taken at 3–4 months of age from an additional 125 infants. This analysis also showed a protective effect of breastfeeding, with 30% of formula-fed babies positive for viruses that infect human cells compared with 9% of babies who were fed human milk or human milk together with formula.

Both the initial and follow-up analyses used samples from infants from an urban area in the United States. To check whether the results were more broadly applicable, the researchers analyzed samples from a different cohort of 4-month-old infants from Botswana. They again found that viruses that grow in human

cells were more common in exclusively formula-fed babies compared with breastfed babies. "We tested the breastfeeding association again and we found similar results, which gave us confidence about these data," says Liang. The study thus describes both the stepwise colonization of the early infant gut by viruses as well as the protective effects of breastfeeding on this viral colonization.

Researchers are still investigating the mechanisms by which breastfeeding may help protect against viruses. Several factors in human milk are known to inhibit viral colonization, including maternal antibodies, milk sugars known as human milk oligosaccharides, and human milk proteins such as lactoferrin (12–14). Breastfeeding may also increase the abundance of gut bacteria that serve as a source of the early bacteriophages that populate the virome. "Breastfeeding can increase the abundance of *Bifidobacteria* and *Lactobacillus*, and the phages could come from these bacteria, so it's possible it's an indirect effect of breast milk," says Liang.

Liang is interested in performing longer-term follow-up studies of virome colonization. "One thing we haven't figured out is the long-term outcome for the infant," says Liang. "We only followed this cohort for four months, and so we could track them for one or two years or even longer to look at the dynamics of the virome and its influence on health outcomes," he says. "Another thing we could look at is preterm babies and how [premature birth] influences the virome structure," says Liang.

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Contributed by
Dr. Sandeep Ravindran
Freelance Science Writer
Sandeepr.com

Developing a Better Cattle Reference Genome

- **The original cattle genome has had many research and commercial applications since its release in 2009, but technological limitations meant that it was far from perfect.**
- **A new study describes the release of an improved cattle reference genome created using the latest technology.**
- **The new genome is 10 times more accurate than the previous one and has fewer gaps, which could help improve genetic selection of cattle and basic research on cattle.**

Cows are one of our major domestic animals, with about 1.4 billion domesticated cattle being raised for

meat and dairy all over the world [1]. Humans have long drawn from the existing genetic variation in cattle populations to select a variety of breeds with useful traits [2]. The sequencing of the cattle genome enhanced the selection of cattle by allowing the use of genomic tools to select traits [3-5].

"Cattle are a very important agricultural species both for beef production and dairy production worldwide," says Dr. Monique Rijnkels of Texas A&M University. "To understand the biology, but also to be able to select the most productive and efficient animals, having a good genome is important," she says. "There are so many applications for this kind of selection, whether it's for production traits like more milk or more fats or more protein, or being tolerant of warmer climates, being resistant to certain diseases, better quality beef, faster growth, all those kinds of things," says Rijnkels.

"Ever since the first genome was put together, people have really adopted this sort of genomic selection approach," says Rijnkels. "But if you think a certain genetic marker is somewhere connected to some gene that confers an important trait, but that turns out not to be correct because the genome assembly is not correct, then when you select for that region in the genome you very soon lose the connection with the gene because they were never really connected," she says.

A new study describes the release of an improved cattle reference genome assembly built using the latest technology [6]. "The original cattle genome project was a large, multi-institutional effort that cost tens of millions of dollars," says Dr. Benjamin Rosen of the USDA Agricultural Research Service, one of the researchers who conducted the new study. "It was updated over the years, but improvements in sequencing technology and assembly algorithms made it clear that we could start from scratch and generate a better product at a fraction of the cost and effort," he says. "The new assembly used only a couple of instrument platforms and a budget 400-fold lower, with sequence data collection performed in a single small laboratory," says Rosen. "My collaborators Juan Medrano and Tim Smith marshalled the resources and generated the data while I drove the more technical aspects of assembling the genome," he says. The new genome assembly's name, ARSUCD1.2, comes from the affiliations of Tim Smith from the Agricultural Research Service (ARS) and Juan Medrano from the University of California at Davis (UCD).

"The first bovine assembly—UMD3.1—was released in 2009, and from that time there have been significant advances in sequencing technology and technologies to assemble a genome, as well as improved bioinformatics platforms that allow the development of significantly improved, more continuous assemblies," says Medrano. "Having a continuous and reliable genome reference assembly is fundamental for all aspects of genomics research," he says. Using newer sequencing technology resulted in fewer gaps in the new genome and greater accuracy. "The new assembly was improved by more than 200-fold in continuity and 10-fold in accuracy," says Medrano.

"Assembling a genome is similar to putting together a very large puzzle," says Rosen. "The most important difference between the new assembly and the previous reference is in the continuity of the genome, i.e., how many pieces your genome is broken up into," he says. "The old assembly was made up of more than 72,000 pieces while the new assembly contains 345, making it more than 200-fold more continuous," says Rosen. "This is a direct result of improvements in sequencing and assembly methods," he says.

"Before this we had several iterations of an assembly based on sequences that were derived with older technology and there were substantial issues with that assembly," says Rijnkels, who was not involved in the new study. "There were lots of gaps and there were a lot of mis-assemblies and so they improved significantly on that by basically going back to the drawing board," she says. "They used new sequence technologies and improved alignment or assembly algorithms to put it all together in a more continuous way and with improved base accuracy," says Rijnkels. "That has all kinds of advantages over what we had before," she says.

The improvements allowed for better gene annotations, and should allow researchers to be more certain about the location of the genes and genetic markers that are used for basic research and genetic selection. "New extensive gene expression data and the availability of more and longer transcripts for gene placement and orientation of sequences across gaps had a large impact on significantly improving gene annotation," says Medrano.

The improved genome has many potential applications. "An improved assembly has benefits for identification, reconstruction and fine mapping of loci important for production and health traits, for the identification of regulatory regions of genes, and for fine mapping genes associated with economically important traits," says Medrano. "The improved annotation corrected mis-assembled regions of the genome, identified missing genes, and allowed the reconstruction of complex, highly repetitive regions of the genome," he says [7].

"That has implications for genomic selection because now we have more confidence in where genes and genetic markers are," says Rijnkels. The new genome enables researchers to create more accurate gene models and more accurately determine the genetic variants associated with different traits that researchers want to select for. "Having a more accurate genome makes it much more reliable now to select for variations and also to then try to understand mechanisms that underlie these variations," says Rijnkels. "This really will help us understand the biology and help in the genetic selection, and help in every aspect of understanding cattle biology," she says.

The new reference genome sequence is already available in the GenBank repository. "The new reference has been publicly available and in use since April 2018," says Rosen. "It is utilized by a very broad and diverse research community across academia, government and industry around the world," he says. "Millions of animals have been genotyped across various platforms and the improved accuracy of the genome will allow for better translation between the various platforms," says Rosen.

"Immediately after ARSUCD1.2 was released with an inherent increased accuracy, it was adopted in December 2018 as the cattle reference genome by the US genomic evaluation system and by the 1000 Bull Genomes Project, which is a large database of genetic variants for genomic prediction, and practically by all the cattle genomics community," says Medrano [8,9].

"It's good that now there is a paper that serves as a landmark that this genome is out there for everybody," says Rijnkels. "The community is really happy with this new reference genome, and the paper allows those maybe not following the field so much to know that we do have an improved assembly and we encourage everybody to use it because it really is better and more accurate," she says.

The new genome was built using the same animal, the Hereford cow L1 Dominette, that was used for the previous cattle reference genome [5]. "I think that makes it a lot easier to go back and reuse data that was already analyzed against the old genome, so you don't have to worry about whether differences you see are because of using data from a different animal," says Rijnkels. "That was a good call," she says.

The new study also improved on the previous one by choosing a different source of DNA. Where the original Hereford assembly used blood as the source of DNA, the new one uses genomic DNA extracted from frozen lung tissue as the source. Specific genomic regions, particularly those with important immune function loci, undergo rearrangement in blood cells that can make it hard to properly align and organize them in a genome assembly. "I think that was a good call too to use lung tissue, as the genetic structure is a little bit more stable and it makes research into these immune loci more accessible," says Rijnkels.

"Extracting DNA from lung rather than blood was done intentionally to help us better assemble immune gene clusters," says Rosen. "An important outcome of this assembly is the ability to better interrogate immune loci to identify variants affecting health traits," he says.

By providing a 200-fold improvement in sequence continuity and a 10-fold improvement in per-base accuracy over previous cattle assemblies, the new cattle reference genome promises to serve as a solid foundation for a new era of basic research and genetic selection in cattle.

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Contributed by
Dr. Sandeep Ravindran
Freelance Science Writer
Sandeepr.com

Kefir Milk Influences Behavior in Mice

- **Drinking kefir, a fermented milk beverage, can alter the composition of gut bacteria in animal models and has been linked to mood changes in human studies.**
- **A new study on mice investigated how kefir consumption influenced stress-inducing behaviors.**
- **Behavioral changes in mice were attributed to differences in gut microbiota induced by kefir consumption.**
- **Mice consuming kefir had higher levels of bacteria with the potential to produce chemical compounds linked to a decrease in anxiety and depression.**

The nearly 100 trillion bacteria that live in our gastrointestinal tract aren't just involved in food digestion; they influence the health and function of the entire body. Mounting evidence suggests gut microbes may even influence the brain, including behavior [1, 2]. This connection between the gut and the brain is called the gut-brain axis and is a complex network of signaling pathways linking the central nervous system with the enteric (or gastrointestinal) nervous system.

Fermented foods are known to influence the composition and quantity of bacteria living in our guts—does this mean that fermented foods could alter behaviors, and possibly even our moods? A new study [1] in mice demonstrated that kefir, a fermented milk drink, influenced the host's gut microbiome and in turn, the host's behavior. If these findings are confirmed in humans, it will give a whole new meaning to the phrase "emotional eating."

From Milk to Kefir

SCOBY may sound like a 1980s cartoon about a crime-fighting dog but is actually a living culture of bacteria and yeast used to make fermented foods and drinks. The addition of SCOBY (symbiotic culture of bacteria and

yeast) to foods transforms them in taste and composition; tea becomes kombucha, cabbage becomes kimchi, and milk becomes kefir. These fermented foods are usually more acidic and "tangy," and have health benefits beyond those attributed to the starter food [3].

The SCOBY used to make kefir are called kefir grains, which look more like cauliflower than a grain of wheat or rice. Types of kefir grains vary in microbial species but usually include lactic acid bacteria, like *Lactobacillus*, and acetic acid bacteria, along with yeasts. These bacteria break down much of the lactose in milk, making it easier to digest. But lactic acid bacteria also break apart milk proteins, which increases the concentration of biologically active peptides. Kefir also has significantly more microbes than does milk; as the bacteria and yeast from the SCOBY dine on milk sugars, they increase in numbers.

Higher concentrations of biologically active peptides and micro-organisms give kefir unique health benefits beyond those found in milk, including anti-inflammatory effects, reduction in hypertension, stimulation of the immune system, and anti-microbial properties [1, 4]. In animal models, kefir has altered the composition of the gut microbiome, and a small number of human studies suggest that kefir can improve mood, such as decreased social anxiety [1]. A new study [1] from a team of Irish researchers looks to bridge these two findings by testing the effects of two types of milk kefirs on behaviors related to anxiety and depression in mice.

Is Kefir a Psychobiotic?

Psychobiotics are microbes that influence mental health and mood. Fermented foods like kefir could be classified as psychobiotics if they are shown to regulate mood or behaviors associated with mood (e.g., reward-seeking behavior).

To test their hypothesis, the research team created four treatment groups of mice: group 1 were fed cow's milk as a control, group 2 were fed kefir type Fr1, group 3 were fed kefir type UK4, and group 4 were undisturbed (as oral tube feeding of milk or kefir could potentially influence behavior). The two kefir types were made with different kefir grains, meaning they differed in the types of bacteria present in the SCOBY. Using two different kefirs allowed the team to attribute the changes in behavior to the action of microbiota, as opposed to some other ingredient found in all kefirs. Behavioral assessments took place after three weeks of daily feeding and involved a series of tests that are commonly used to assign a behavioral phenotype to mice [1].

The researchers predicted that the differences in bacterial species between Fr1 and UK4 would result in behavioral differences between group 2 and group 3, and this was supported by their results. Kefir Fr1 increased reward-seeking behavior whereas UK4 decreased repetitive behavior. Moreover, they could link these behaviors to specific changes in gut microbiota associated with each kefir. For example, Fr1 increased reward-seeking behavior and also increased the gut bacteria *Parabaceroides goldsteinii.* Higher levels of *P. goldsteinii* were then linked to an increase in the synthesis of a neuroactive compound, S-adenosylmethionine, known to increase reward-seeking behavior [1]. In humans, depression is associated with a decrease in reward-seeking behavior. S-adenosylmethionine supplementation has been studied as a treatment for depression, providing additional support for a connection between *P. goldsteinii* and the observed behavioral changes.

Both kefirs were found to regulate the functional capacity of the host's gut microbiota and both also led to changes in the quantity of compounds that interact with the central nervous system, what the researchers refer to as gut-brain modules or GBMs [1]. Of particular importance was the finding that both types of kefir increased levels of *Lactobacillus reuteri*, which was linked to an increase in the ability to make the neurotransmitter gamma-aminobutyric acid (GABA) [1]. Low levels of GABA in humans and animal models are linked to anxiety and mood disorders, but further studies are needed to determine whether levels of *gut-derived* GABA can improve mood [1].

These results suggest that kefir may indeed be a psychobiotic—for mice, at least. The potential influence of kefir on the human gut-brain axis is still unknown, but the results suggest that further research is

warranted on kefir, and other fermented foods, as potential dietary interventions for mental health.

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Contributed by
Dr. Lauren Milligan Newmark
Researcher, Science Writer

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