



GIVING GOOD BACTERIA TO CHICKENS TO KEEP HUMANS FROM GETTING SICK

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YOUNG REVIEWERS:



ANSHUL
AGE: 10



LUVENA
AGE: 12



MARIANA
AGE: 15



PRANATEE
AGE: 13



ZARA
AGE: 15

The bacteria *Salmonella* is a major cause of food poisoning. Poultry products are one of the leading foods that cause *Salmonella* outbreaks. While farmers, food processors, and the public health community already do a lot to prevent these illnesses, people are still getting sick. Our group is studying how we can use the “good” bacteria in the intestines of chickens to drive *Salmonella* out of chickens. To test this idea, we used various diets to change the bacterial populations in chicken intestines. We found that changes in the numbers of good bacteria can lead to lower levels of *Salmonella*. We are currently working to identify which bacteria are responsible for the changes in the amount of *Salmonella* in the chicken intestines, with the goal of making a diet that will eliminate *Salmonella* from

chickens. Hopefully, this will reduce the number of people who get sick from eating poultry products.

SALMONELLA BACTERIA CAUSE FOOD-BORNE ILLNESS

Have you ever eaten something and a few days later gotten really sick? You likely had what is commonly called food poisoning, also known as a **food-borne illness**. These illnesses are caused by bacteria or viruses that can infect you if the food you eat is not cooked properly. Different types of food can carry different types of bacteria or viruses, but chicken, eggs, and other poultry products are commonly associated with food-borne illness (Figure 1A) [1].

Many kinds of bacteria and viruses can be associated with poultry products but, in the U.S., the bacteria *Salmonella* accounts for the largest number of food-borne illnesses from eating poultry products (Figure 1B) [2]. If you eat a chicken sandwich that is contaminated with *Salmonella*, 48–72 h later you could develop an infection, with symptoms including nausea, abdominal cramps, fever, chills, headache, vomiting, and diarrhea. This infection can last up to 7 days.

FOOD-BORNE ILLNESS

Sickness caused by eating or drinking something containing harmful bacteria, parasites, viruses, or chemicals. Symptoms include vomiting, diarrhea, abdominal pain, fever, and chills.

Figure 1

What types of food cause the most food-borne illness? (A) In the U.S., there were 5,760 outbreaks of food-borne illness from 2009 to 2015. Of those, 1,281 could be associated with a single food source. Poultry products (meat and eggs) caused the most illnesses, followed by vegetables, beef and dairy products, pork products, fruit, fish and seafood, sprouts and herbs, and finally nuts and beans [1]. (B) In 149 food-borne disease outbreaks in which people got sick from eating poultry products, almost half of them (43%) were caused by *Salmonella* [2].

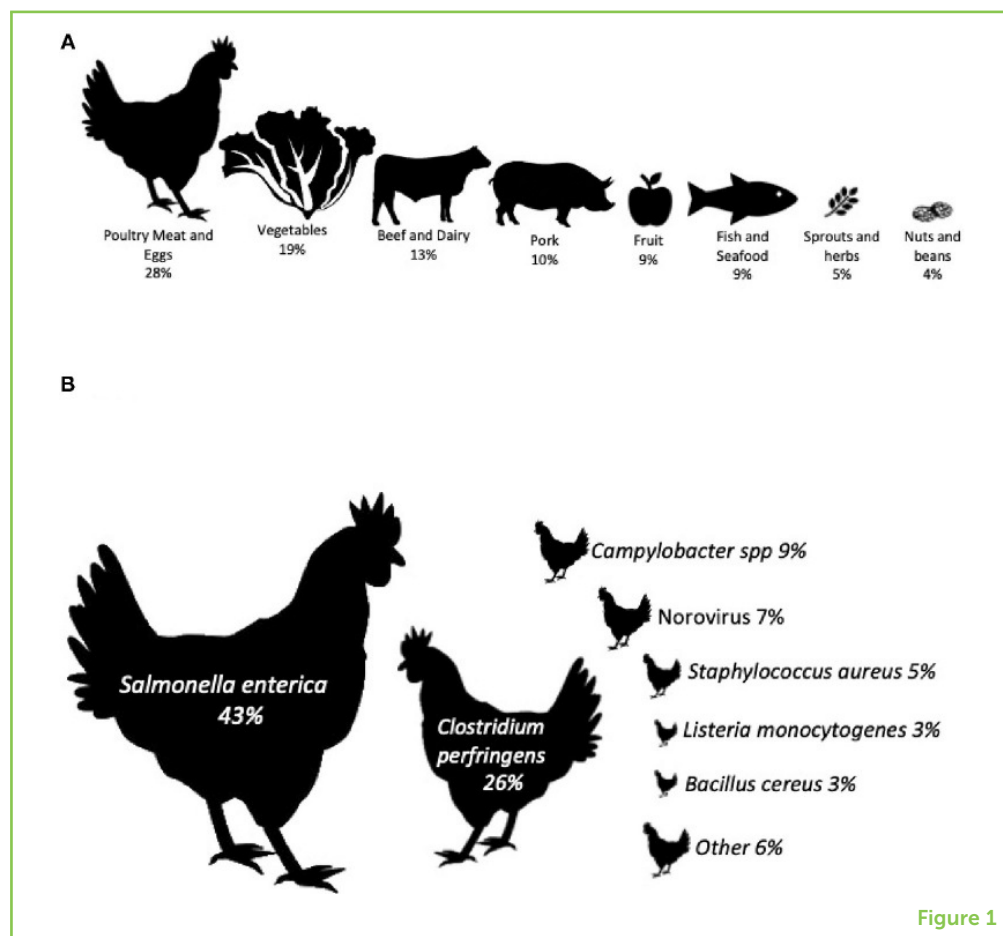


Figure 1

Sometimes the infection can be so severe that you may have to go to the hospital¹.

But how did *Salmonella* get into your chicken sandwich in the first place? *Salmonella* bacteria live in the environment. Chickens can pick up *Salmonella* when they peck at the food on the ground. All chickens, including those sold as organic, free-range, or natural, can pick up the bacteria. While *Salmonella* can make us sick, these bacteria do not make chickens sick. That means *Salmonella* can live inside the intestines (gut) of a chicken without us knowing. From there, *Salmonella* can find its way into the chicken's eggs. Bacteria can also spread from the gut to the meat when a chicken is cut up. If the eggs and meat are stored and cooked properly, any *Salmonella* that may be present will be killed, so it will not make us sick. However, sometimes people do not cook their chicken well enough, or they accidentally spread *Salmonella* from raw chicken to other food².

CAN WE FIGHT SALMONELLA IN CHICKENS?

Farmers and food producers spend a lot of money trying to keep *Salmonella* out of food [3]. On the farm, chickens are often given vaccines to prevent *Salmonella*. However, there are over 2,600 different types of *Salmonella*. That is too many to make vaccines against all of them. At food processing plants, the eggs and meat go through many steps to wash away *Salmonella* before the food is packaged and sent to grocery stores and restaurants. Even with all of these efforts, poultry products still cause thousands of *Salmonella* illnesses each year [2]. To successfully keep *Salmonella* out of us, we need to figure out new ways of keeping *Salmonella* out of chickens.

One approach is to try to get other bacteria to help. Just like humans, chickens have trillions of "good" bacteria that live inside their guts. These good bacteria help chickens digest their food and they also produce nutrients that the chickens cannot make for themselves. These bacterial populations change in response to what the chickens eat. The various types of bacteria in the gut fight over food and resources to survive. We asked whether we could make chicken safer to eat by helping the good bacteria in chickens' guts fight off *Salmonella* [4].

To test this hypothesis, we used three groups of 100 chickens each. We gave normal chicken feed to one group. This was our **control** group, and it was used to show us what the population of bacteria in the chicken gut normally looks like. We fed the second group chicken feed mixed with a type of fiber that only certain good bacteria can use as food. This bacteria food is known as a **prebiotic**. The types of bacteria that can eat the prebiotic make chemicals that should drive out *Salmonella*. We thought that increasing the numbers of these

¹ Food poisoning - Causes, symptoms & treatment | Dr. Claudia" YouTube, Babylon Health, Jan 16, 2020, <https://www.youtube.com/watch?v=JR6yHykfYJE>.

² "How salmonella spreads from farm to table" YouTube, The Oregonian, Mar 26, 2015, <https://www.youtube.com/watch?v=dusDTYFNTi8>.

CONTROL

A group of animals that does not receive any experimental treatment and is used to represent what normally happens and is compared to the other groups of animals to help the researchers determine how the effect the treatment.

PREBIOTIC

Nutrients used as food by gut bacteria. Prebiotics are used to encourage the growth of "good" bacteria in the gut.

good bacteria might make the gut a place where *Salmonella* do not want to live. Our third group received normal chicken feed, but at the start of the experiment we gave them a *Salmonella* type that had been modified so it cannot make people sick. This was to test the possibility that adding a “safe” *Salmonella* to the chickens’ guts might prevent other *Salmonella* types from being able to live there. This is the bacterial equivalent of what you might do when you do not want someone to sit next to you at school: you get other people to sit around you first.

We let each group of chickens eat their assigned foods from the day they hatched until they were 35 days old. This gave the good bacteria plenty of time to grow. By day 35, there could be thousands of different types of bacteria in the chickens’ guts.

DIET CHANGES BACTERIA IN CHICKEN INTESTINES

Our hypothesis was that the prebiotic treatment and the safe-*Salmonella* treatment would change the populations of good bacteria in the chicken gut. We needed a way to measure how the gut bacteria in these chickens might be different from the bacteria in the guts of our control group. So how could we measure all the bacteria in the guts of these chickens? It would take a very long time and cost a lot of money to grow all these bacteria in the lab and count them by hand; not to mention that most of the bacteria that live in the gut cannot be grown in the lab. So, we performed a bacterial version of a crime scene investigation—we determined which bacteria were present based on the bacterial **DNA** we found in the gut. To do this, we collected samples of the partially digested food from inside the chickens’ guts, and we isolated the bacterial DNA from those gut samples.

To identify which bacteria were present in each gut sample, we tested for a specific bacterial **gene** known as **16S ribosomal RNA**. Testing for this gene could tell us which types of bacteria were in each gut sample *and* how many of each type were there [4]. For each chicken, we then made a list of all the bacteria we found and the percentages of the bacterial population each type made up. We used a computer program that looked at *all* the bacteria we found in *all* chickens, and we grouped the chickens together based on the similarity of the bacterial populations in their guts. This analysis showed us whether there were differences among our groups of chickens.

GOOD BACTERIA CAN REDUCE SALMONELLA IN CHICKENS

If our prebiotic treatment or our safe-*Salmonella* treatment affects which bacteria can live in the gut, then we would expect the numbers and types of bacteria to be different between our experimental groups.

DNA (DEOXYRIBONUCLEIC ACID)

Material found in cells that contain all the instructions a living thing needs to work and function.

GENE

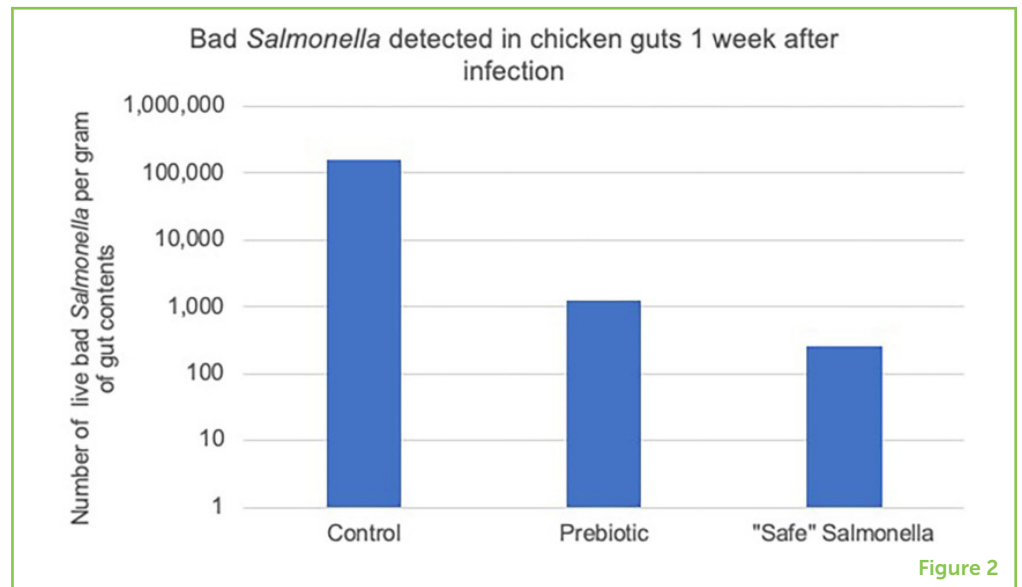
Specific regions of DNA that contain the instructions for individual components of a living thing.

16S RIBOSOMAL RNA

A gene (segment of DNA) found in bacteria that can be used to identify the types of bacteria in a population.

Figure 2

Changing the “good” bacteria in the chicken gut helps get rid of disease-causing *Salmonella*. After 4 weeks on either the control diet, the prebiotic diet, or the safe-*Salmonella* diet, we gave the chickens a dose of disease-causing *Salmonella*. One week later, we collected gut samples and measured how much disease-causing *Salmonella* was in the guts of seven chickens from each of our three groups. The safe-*Salmonella* group and the prebiotic group had less disease-causing *Salmonella* than the control group.



This is essentially what we found! At the start of the experiment, there were no differences between the groups. The gut bacteria found in the chickens from all three groups were the same. However, 4 weeks after the start of the experiment, there were three distinct groups of bacterial populations: one for the control group, one for the prebiotic group, and one for the safe-*Salmonella* group.

Once we knew that our diet treatments could change the bacterial populations in the gut, we wanted to see if the altered bacterial populations could prevent disease-causing *Salmonella* from living in the gut. Four-week-old chickens from each group were given disease-causing *Salmonella*. One week later, we collected gut samples from the chickens, like before. This time, we grew the bacteria in the lab, so we could count how many disease-causing *Salmonella* were in each chicken and compare those numbers among the three groups. In the control group, we found that chickens had an average of 100,000 disease-causing *Salmonella* bacteria per gram of gut material. However, chickens that had either the prebiotic treatment or the safe-*Salmonella* treatment had much lower levels of disease-causing *Salmonella* in their guts (Figure 2).

KEEPING HUMANS HEALTHY

From these experiments, we learned that feeding chickens a prebiotic or giving them a safe *Salmonella* changes the kinds and amounts of bacteria living in chickens' guts. We also learned that these changes can lead to lower levels of disease-causing *Salmonella*. Now we are trying to find out exactly which bacteria in the chicken guts are responsible for decreasing the growth of disease-causing *Salmonella*. We hope that by identifying the specific bacteria that prevent disease-causing *Salmonella* from living in chickens, and understanding

exactly how these good bacteria manage to stop the *Salmonella*, we will be able to develop diets for chickens that will completely eliminate *Salmonella* from their guts. Since *Salmonella*-contaminated eggs and poultry products are a major source of *Salmonella* outbreaks in humans, eliminating *Salmonella* in the guts of chickens could greatly reduce the number of people who get sick from food-borne illnesses in the future.

ORIGINAL SOURCE ARTICLE

Azcarate-Peril, M. A., Butz, N., Cadenas, M., Koci, M., Ballou, A., Mendoza, M., et al. 2018. A *Salmonella*-attenuated strain and galacto-oligosaccharides accelerate clearance of *Salmonella* infections in poultry through modifications to the gut microbiome. *Appl. Environ. Microbiol.* 84:e02526-17. doi: 10.1128/AEM.02526-17

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CONFLICT OF INTEREST: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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YOUNG REVIEWERS



ANSHUL, AGE: 10

Hello! My name is Anshul and I am a fifth grader in North Wales, Pennsylvania, which is close to Philadelphia. I am very interested in Biology and Entomology. I am an active member for the Johns Hopkins CTY program, and my favorite hobby is to read.



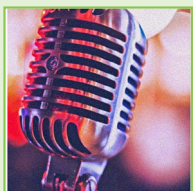
LUVENA, AGE: 12

Hi, my name is Luvena! I love music, sports, and food. My favorite subjects in school are math and language arts. In my spare time, I enjoy playing piano and reading books with my sister. When I grow up, I would like to be a neurosurgeon.



MARIANA, AGE: 15

Hi! My name is Mariana and I am 15 years old. I like reading books, spending time with animals, and swimming. I consider every day brings an opportunity for me to learn something new and for being happy.



PRANATEE, AGE: 13

Hello! I love to bake, especially tarts and pies. In school, my favorite subjects are science, lunch, and recess. I like spending time outdoors and going hiking. I also love going to the beach and have an interest in photography. Watching my favorite TV shows, painting, listening to music, singing, and hanging out with friends are my favorite things to do in my free time. In the future, I would like to either like to be a scientist, or a singer/songwriter and actress.



ZARA, AGE: 15

My name is Zara and I am 15 years old. In my spare time, I enjoy reading, helping my community, and playing volleyball. I think it is important to work hard for achieving our dreams and for being a better person every day.

AUTHORS

MARY MENDOZA

Mary Mendoza is a research specialist at North Carolina State University. After graduating from the University of Maryland with a B.S. in Biology, Mary worked at the National Institutes of Health studying the signaling pathway of mast cells.



Upon relocating to North Carolina in 2008, Mary joined Hosni Hassan's lab at North Carolina State University as a research specialist where current research has focused on *Salmonella* and its interaction with the microbiome of the chicken.



RIZWANA ALI

Rizwana Ali is a, recently retired, research specialist at North Carolina State University. She has a B.S. in Pharmacy from the University of Karachi and a M.S. degree in Physiology from N. C. State University.



NATALIE ROBERTS

Natalie Roberts is a Research Specialist in Dr. Matt Koci's lab at North Carolina State University. She is excited about exploring how microbes like bacteria affect health and immunity. In her free time, she enjoys reading, gardening and spending time with her three daughters.



LAUREN BOOP

Lauren Boop taught for 9 years in Wake County in North Carolina where she became really interested in intersecting STEM education and the humanities. Through an inquiry-based classroom, she teaches students to explore all aspects of the world around them from tiny microbes to large landscapes. Her own love of exploring the world has brought her to her current role as a middle school teacher at an international school in Portugal.



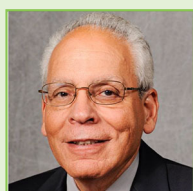
KRISTIN BEDELL

Kristin Bedell has taught kindergarten through college. She currently writes STEM curriculum for North Carolina 4-H and is working on her Ph.D. in Education at the University of North Carolina at Chapel Hill. Her favorite microbes turn milk into cheese and flour into sourdough bread.



BRAD RHEW

Brad Rhew is the STEM Coach in Winston-Salem/Forsyth County Schools, North Carolina and serves on the North Carolina Science Teachers Association as the District 5 Leader and the Grants Committee Chair. He works closely with his school district and state science leaders to increase hands-on inquiry based learning in the science classroom. He is currently working on his doctorate in Curriculum and Instruction through Gardner-Webb University.



HOSNI M. HASSAN

Dr. Hassan is Professor of Microbiology, Biochemistry, Food Science and Toxicology at North Carolina State University. Dr. Hassan's research focuses on applying microbial physiology, genetics, and molecular biology to understand host/microbe interactions to promote the growth of good bacteria and inhibit the growth of the bad ones (i.e., Pathogenic Bacteria). His research interests include—Oxygen free radical biology, oxidative stress, microbiology and food safety, poultry microbiome and metabolome, developing patented *Salmonella* vaccines, isolation/application of species-specific probiotics, and developing best practices to eliminate human salmonellosis.

**M. ANDREA AZCARATE-PERIL**

Dr. M. Andrea Azcarate-Peril, is an Associate Professor of Medicine at UNC Chapel Hill. She uses molecular biology, genomics, and next-generation sequencing tools to address questions relevant to the role of the intestinal microbiota in human health and disease.

**MATTHEW D. KOCI**

Dr. Matthew D. Koci is a professor of immunology and infectious diseases at North Carolina State University. Our bodies encounter millions of bacteria and viruses every day. The vast majority of them do not pose any threat to us and our immune system generally leaves them alone. His research is focused on trying to find how our immune system can tell the difference between the helpful microbes and the bad ones, and why some of the microbes and viruses that make humans sick do not make animals sick. *mdkoci@ncsu.edu