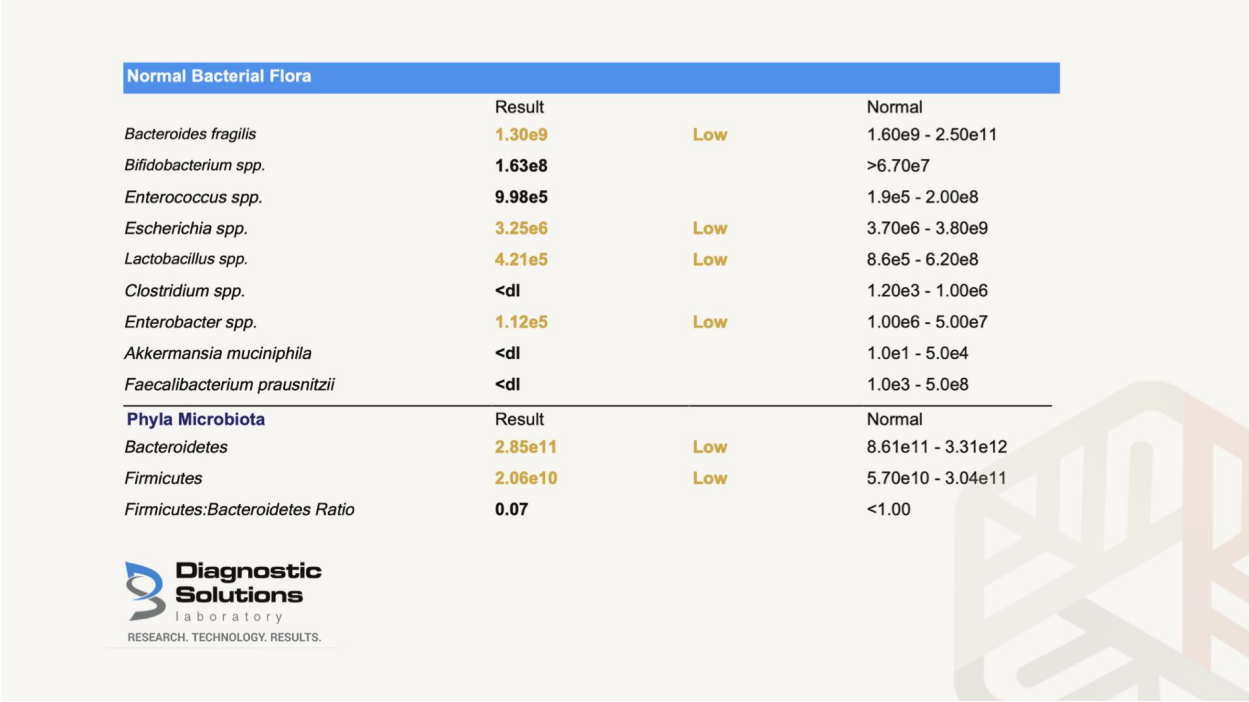
Gut: Stool Testing - Part 3

Tracey O'Shea: We'll start by talking about the beneficial bacteria section of these different labs. It's important to note that many specialists in the field think that this section of [the] lab lacks reliability and is a little difficult to correlate clinically with treatment outcomes. I'm going to talk a little bit about the beneficial bacteria section and how we use it in practice.



Normal Bacterial Flora			
	Result		Normal
Bacteroides fragilis	1.30e9	Low	1.60e9 - 2.50e11
Bifidobacterium spp.	1.63e8		>6.70e7
Enterococcus spp.	9.98e5		1.9e5 - 2.00e8
Escherichia spp.	3.25e6	Low	3.70e6 - 3.80e9
Lactobacillus spp.	4.21e5	Low	8.6e5 - 6.20e8
Clostridium spp.	<dl		1.20e3 - 1.00e6
Enterobacter spp.	1.12e5	Low	1.00e6 - 5.00e7
Akkermansia muciniphila	<dl		1.0e1 - 5.0e4
Faecalibacterium prausnitzii	<dl		1.0e3 - 5.0e8
Phyla Microbiota	Result		Normal
Bacteroidetes	2.85e11	Low	8.61e11 - 3.31e12
Firmicutes	2.06e10	Low	5.70e10 - 3.04e11
Firmicutes:Bacteroidetes Ratio	0.07		<1.00

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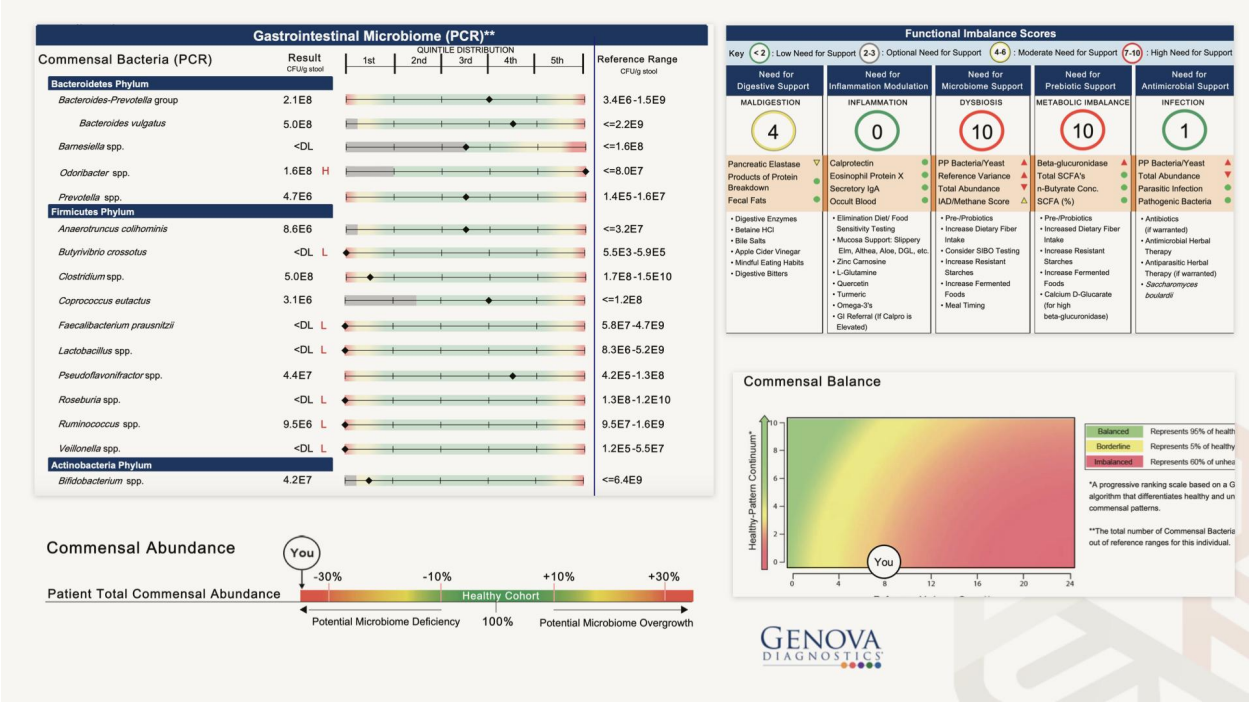
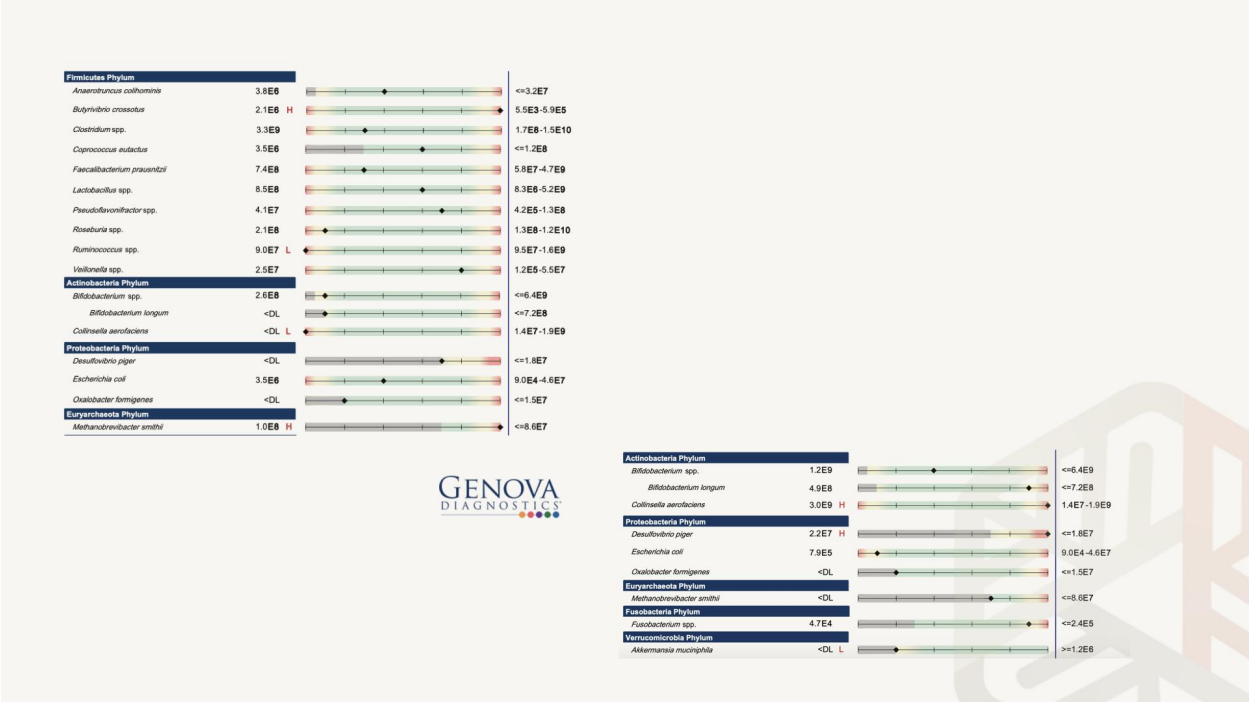
At this stage of testing, we're mostly focusing on patterns that we see in the commensal bacteria section rather than single values. So, for instance, this is a 28-year-old female with complaints of constipation, fatigue, insomnia, and weakness. We use[d] the [Diagnostic Solutions Laboratory] (DSL) GI-MAP [polymerase chain reaction] (PCR) testing for this particular patient. And as you can see, there [are] low levels of [*Lactobacillus*], [*Enterobacter*], *Bacteroides*, and so on. And with the majority of our commensals being low, this would really stick out to me as a pattern of insufficiency dysbiosis. The GI-MAP alone doesn't test for short-chain fatty acid, but I think it would have probably been a really nice secondary marker when evaluating metabolic activity of these commensals. So again, [with] one single, normal bacterial flora that's really low, while the rest are all normal, I think it's probably not something you need to go after targeting each individual species. But I do think when we're seeing [an] overall pattern, this coupled with potential opportunistic pathogens, along with other markers of poor gut health, I think it really does help move you in that direction that we need to support the beneficial species.



This is the Genova Diagnostics report. I'm showing you this as an example of the many different ways that Genova reports commensal balance, commensal abundance, giving you scores, [and] looking at the PCR of the commensal bacteria. It's really cool to see the different ways that they are showing representation of the commensal bacteria. So you can see this person, who is a 52-year-old female with hypothyroidism, [with] complaints of fatigue, high cholesterol, exercise intolerance, and insomnia. This high dysbiotic score with high potential pathogens, low total abundance, and a high metabolic imbalance store, the beta-glucuronidase is high on this person. So they've got quite a few different things going on. I would say that this person is closer to the "imbalanced category for commensal balance." And then you can also see low levels of bacteria in the Firmicutes phylum. So in this case, we have both dysbiosis that is in part insufficiency of beneficial bacteria, but also pretty significant growth of a couple [of] pathogenic species.



The groups worth mentioning in the beneficial bacteria section are the methanogens and the sulfate-reducing organisms. The most dominant methanogen and probably the most represented on stool testing is the *Methanobrevibacter*. Higher levels tend to be seen in [patients with irritable bowel syndrome] (IBS) [with] constipation. I think it's important to note that as of now, there's no real research or support literature for using methanogens in stool to diagnose methane-dominant [small intestinal bacterial overgrowth] (SIBO). Some practitioners and some people believe that and can use that to help support treatment decisions, like if someone is unable to afford a SIBO test. I haven't seen strong enough literature at this point. So I'm really just using it as helping me understand the ecosystem a little bit better.

The other group that I mentioned was the sulfate-reducing organisms, including *Desulfovibrio* species, *Fusobacterium*, and sometimes [*Escherichia coli*]. The most prevalent and dominant of those is the *Desulfovibrio* species. It is suspected that higher quantities seen in the stool represent more potential for hydrogen sulfide production and a higher capacity for sulfate metabolism. So these higher-density numbers tend to be seen more often with [IBS with diarrhea]. [This is] not a hard and fast rule. But again, a trend. A reminder that I'm referring to PCR testing here and not culture-based methods since as we mentioned earlier, there are concerns with abundance representation in culture-based testing. So we'll dive into this a little bit more in the SIBO section, but I just wanted to mention it here in the beneficial bacteria section.

Now, let's talk about *Klebsiella*, which is the pathogenic bacteria that's often associated with joint pain, more specifically, autoimmune conditions like ankylosing spondylitis, reactive arthritis, [and] rheumatoid arthritis. It's been reported in IBS and other gut issues, and when you see a positive result for *Klebsiella*, especially in someone with joint pain, you should consider running a [human leukocyte antigen B27] (HLA-B27) test through Labcorp or Quest. HLA-B27 is a protein found on the surface of white blood cells that's encoded by the B locus and the major histocompatibility complex on chromosome 6, and presents antigenic peptides both derived from self- and non-self antigens to the T cells. So 95 percent of people with ankylosing spondylitis have HLA-B27. But only a small percentage of people with HLA-B27 will actually go on to develop ankylosing spondylitis.

The prevalence [varies] significantly. About 8 percent of Caucasians, 4 percent of North Africans, 2 to 9 percent of Chinese, and 0.1 to 0.5 percent of persons of Japanese descent possess this gene.

Potential Autoimmune Triggers	Result		Normal
Citrobacter spp.	<dl		<5.00e6
Citrobacter freundii	<dl		<5.00e5
Klebsiella spp.	2.34e4	High	<5.00e3
Klebsiella pneumoniae	5.35e2		<5.00e4
M. avium subsp. paratuberculosis	<dl		<5.00e3
Prevotella spp.	3.75e7		<1.00e8
Proteus spp.	<dl		<5.00e4
Proteus mirabilis	<dl		<1.00e3
Fusobacterium spp.	1.90e7		<1.00e8
Fungi/Yeast			
	Result		Normal
Candida spp.	<dl		<5.00e3
Candida albicans	<dl		<5.00e2
Geotrichum spp.	<dl		<3.00e2
Microsporidium spp.	<dl		<5.00e3
Rodotorula spp.	<dl		<1.00e3
Viruses			
	Result		Normal
Cytomegalovirus	<dl		<1.00e5
Epstein Barr Virus	<dl		<1.00e7

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Intestinal Health			
Digestion	Result		Normal
Steatocrit	<dl		<15 %
Elastase-1	247		>200 ug/g
GI Markers	Result		Normal
b-Glucuronidase	139		<2486 U/mL
Occult Blood - FIT	13	High	<10 ug/g
Immune Response	Result		Normal
Secretory IgA	752		510 - 2010 ug/g
Anti-gliadin IgA	54		0 - 157 U/L
Inflammation	Result		Normal
Calprotectin	19		<173 ug/g

HLA B 27 Disease Association

HLA-B27 Positive 04

HLA-B*27 Positive
This patient is positive for HLA-B*27. This procedure rules out the B*27:06 and 27:09 alleles, which the literature suggests are not associated with spondyloarthropathies.
B27 allele interpretation for all loci based on IMGT/HLA

LabCorp

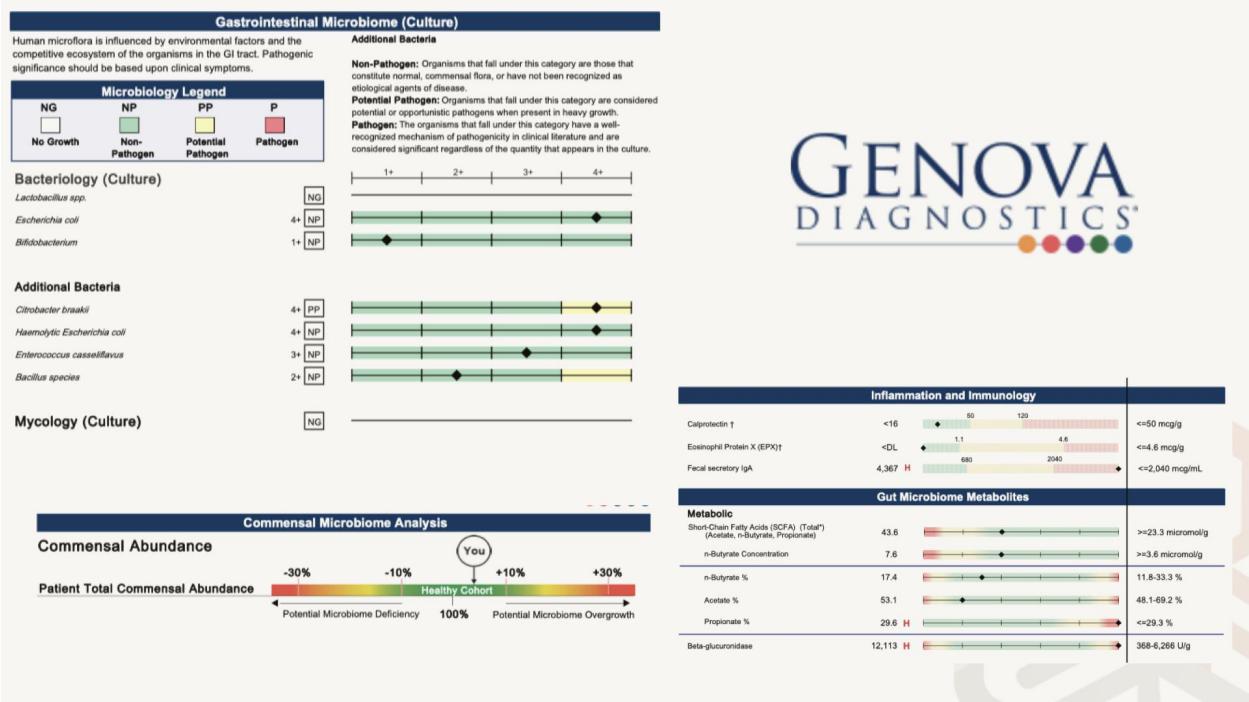
Here's a DSL GI-MAP report showing *Klebsiella* under the opportunistic bacteria section. So this is a follow-up test after an antimicrobial protocol, restoration protocol, [autoimmune protocol] (AIP) diet, and other valances that were corrected. As you can see, the intestinal health markers have improved significantly, as did his symptoms. Before this, he had really low elastase, high secretory [immunoglobulin A] (IgA), the occult blood was still there, and his beta-glucuronidase

was really high. But his *Klebsiella* markers persisted. So we ran [an] HLA-B27 test and he tested positive through Labcorp. So, at this stage, I don't think he has ankylosing spondylitis, but we sent him for imaging to rule it out. And we'll know more once we get those results back. But I will note that in most cases, the patients that I've tested that are positive for *Klebsiella* go on to be negative for HLA-B27. But I think it is important to keep that on your differential, especially when there is presence of joint pain and autoimmune conditions.

[For] *Klebsiella,* like we mentioned, only a small percent of people with the gene end up developing ankylosing spondylitis. So the question is, does this suggest that there must be some other environmental trigger that is associated with *Klebsiella* and these autoimmune conditions? Dr. Alan Ebringer at Middlesex Hospital in London suspected that this trigger might be microbial in nature. He found that the *Klebsiella* has molecules resembling [the] HLA-B27 blood group, a normal resident, but can become overgrown. The elevated levels of antibodies to *Klebsiella* have been found in ankylosing spondylitis patients, especially during flare-ups. So the theory is that the body makes antibodies to *Klebsiella* but also attacks HLA-B27. And this phenomenon, as many of us have heard, [is] known as molecular mimicry.

So, if we use the antimicrobials to kill *Klebsiella*, that has worked. The likelihood of reinfection is high. And Dr. Ebringer discovered another solution by accident. One of his patients wanted to lose weight, and Dr. Ebringer suggested a low-carb diet, which also by default [is] completely lacking [in] starch. So indirectly, this low-starch diet was tested, and then more indirectly, or more purposefully, was tested on hundreds of patients. It was shown to be pretty effective at keeping *Klebsiella* at bay. Still using antimicrobial treatments, but also doing a low-starch diet for people who have *Klebsiella* and suspicion of autoimmune-related conditions.

Next on our list is *Citrobacter freundii*. This is a facultative aerobic gram-negative bacilli of the *Enterobacter* ACA family. [It's] a pretty close relative of the better known food poisoner, *Salmonella,* often found in soil, water, sewage, food, and intestinal tracts of animals and humans. It's also known to be the cause of a number of nosocomial infections of the respiratory tract, urinary tract, blood, and many other normally sterile sites in patients. [It] represents about 29 percent of all opportunistic infections, and some subspecies are even more pathogenic than others.



This is a 43-year-old male with complaints of chronic cough, acid reflux, sinus infections, and insomnia. You can see that his commensal abundance was similar to other “healthy cohorts” and levels were mostly within normal range. But he did have quite a high dysbiosis score of eight because of this potential pathogen, like *Citrobacter freundii* and high levels of fecal beta-glucuronidase.

As a reminder, high levels of fecal beta-glucuronidase can indicate unfavorable metabolic changes in the colon. Beta-glucuronidase may also indicate dysbiosis, and interference with phase two detoxification involving glucuronidation. So I would call this moderate pathogenic dysbiosis and would use an antimicrobial protocol to address the dysbiosis and likely some support for his detox pathways to lower that beta-glucuronidase. In this particular situation, we have additional supportive markers of dysbiosis. But I did question the four plus *Citrobacter* because, as we’ve discussed previously, culture-based results don’t show abundance, and when we have a potential pathogen, it’s important to take this into consideration.

If possible, I would recommend following up with PCR or whole genome testing to assess abundance. Or if you have other pathogens you’re targeting, then moving forward with an antimicrobial protocol would make sense because I think, again, we’re looking at risk versus reward. But I may be a little bit more hesitant to use prescriptions to treat this “potential pathogen” based [on] a culture test.

Now, let's discuss *Helicobacter pylori* (*H. pylori*). *H. pylori* is a bacteria that's associated with stomach and duodenal ulcers. It's also thought to increase the risk of gastric cancer, which is the second most lethal cancer. For decades, doctors thought that stress, spicy foods, and smoking caused ulcers almost exclusively. But in the early '80s, a couple of researchers in Australia, Warren and Marshall, discovered the *H. pylori*, the bacterium, and proposed it as a cause of ulcers. I think many people have heard this story, but we'll summarize it quickly. As the story goes, when they first introduced this idea at a medical conference, they were laughed off the stage and no one really took them seriously. But they continued to work for many years on the theory. And in fact, I think we all know that one of them ended up ingesting it and swallowed a vial of *H. pylori*, purposely infecting himself, developed an ulcer, and then treated it successfully with antibiotics.

Even after that, it took many years for the dominant paradigm to accept this new theory that ulcers were caused by *H. pylori*. But eventually, Warren and Marshall went on to share the Nobel Prize in Medicine, so their efforts were vindicated. Just a little anecdotal side note on how difficult it really can be to change the dominant paradigm in medicine and how long it can take. Today, it's well understood that *H. pylori* is a primary factor in causing ulcers. We also know that stress and other factors play a role. So it's not that *H. pylori* is the only cause. But you'll see why when we talk about it more that *H. pylori* has been referred to as the most successful pathogen in human history. [It's] not as deadly as the bacteria that caused tuberculosis or cholera or the plague, but it infects more people than the others I just mentioned combined.

H. pylori migrated out of Africa, along with our ancestors, and it's been intertwined with our species for at least 200,000 years with prevalences as high as one out of two people globally. It's often contracted via fecal contamination, oral to oral, and even family interinfection are common modes of transmission.

David Y. Graham
Gastroenterologist

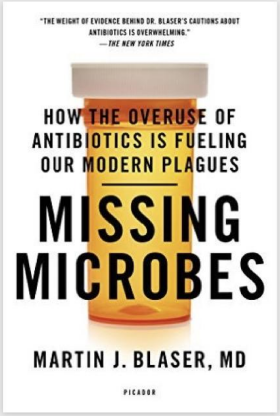
> The **only good** *Helicobacter pylori* is a dead *Helicobacter pylori*.

Image Source: https://www.bcm.edu/research/office-of-research/debakey-awards/recipients/graham-david

A consensus for many years, as stated on this slide by gastroenterologist David Graham in 1997, [is] that the only good *H. pylori* is a dead *H. pylori*. But Dr. Martin Blaser, a physician and researcher, developed the first blood test to identify *H. pylori* and started to wonder how an organism that's as old as humans survived so long if it only caused harm. It doesn't really make sense [from] an evolutionary perspective.

So he began to gather evidence suggesting the *H. pylori* is not always harmful. And it may even be helpful in some circumstances. In the beginning of the 20th century, and even now in the developed world, pretty much everyone has *H. pylori*. But today, just 5 percent of children in developed countries have it. In the [United States] and Canada, it's estimated that about 30 percent of adults on average have *H. pylori*, and 20 percent of adults under the age of 30 and 50 percent of adults over the age of 60. The relationship between *H. pylori* and cancer is also well established. But it's important to note that young people rarely develop cancer either.

Helicobacter pylori **may not be all bad…**



Dr. Blaser's research shows that *H. pylori* has beneficial functions that actually begin in infancy. So if a baby acquires it early on, it appears to protect against the development of allergies and asthma. The decline in *H. pylori* prevalence matches the increase in the prevalence of these conditions in kids in many different countries, and there's some evidence that maybe even eradicating *H. pylori* could alter metabolism in a way that predisposes toward obesity. *H. pylori* seems to decrease ghrelin and increase leptin. And of course, ghrelin and leptin are important satiety hormones, and they also help to regulate appetite. There's also some evidence that eradicating *H. pylori* may increase the risk of [gastroesophageal reflux disease], esophageal cancer, and Barrett's esophagus.

Martin J. Blaser

> One day **doctors will purposely infect** kids with *Helicobacter pylori*.

Image Source: https://www.martinblaser.com

Blaser believes that on a population basis, *H. pylori* is likely benign or even beneficial. In fact, he thinks that one day, doctors will purposely infect kids with *H. pylori* in order to protect them against allergies [and] asthma, and provide immune regulation. So only in some cases, does it become a pathogen and have adverse effects.

So what makes the difference? As usual, I think it's the context and the host environment. This is a theme we'll come back to over and over and over again. Another important factor is the timing of acquisition. In animal studies, where *H. pylori* has been shown to play a protective role, it's only the case that the animals are infected early on in life, like shortly after birth or during infancy. And the studies also suggest that the earlier someone is infected, the more protective it is, and the later it arrives, the more inflammation it causes and the more it predisposes [people] to conditions like gastric cancer.