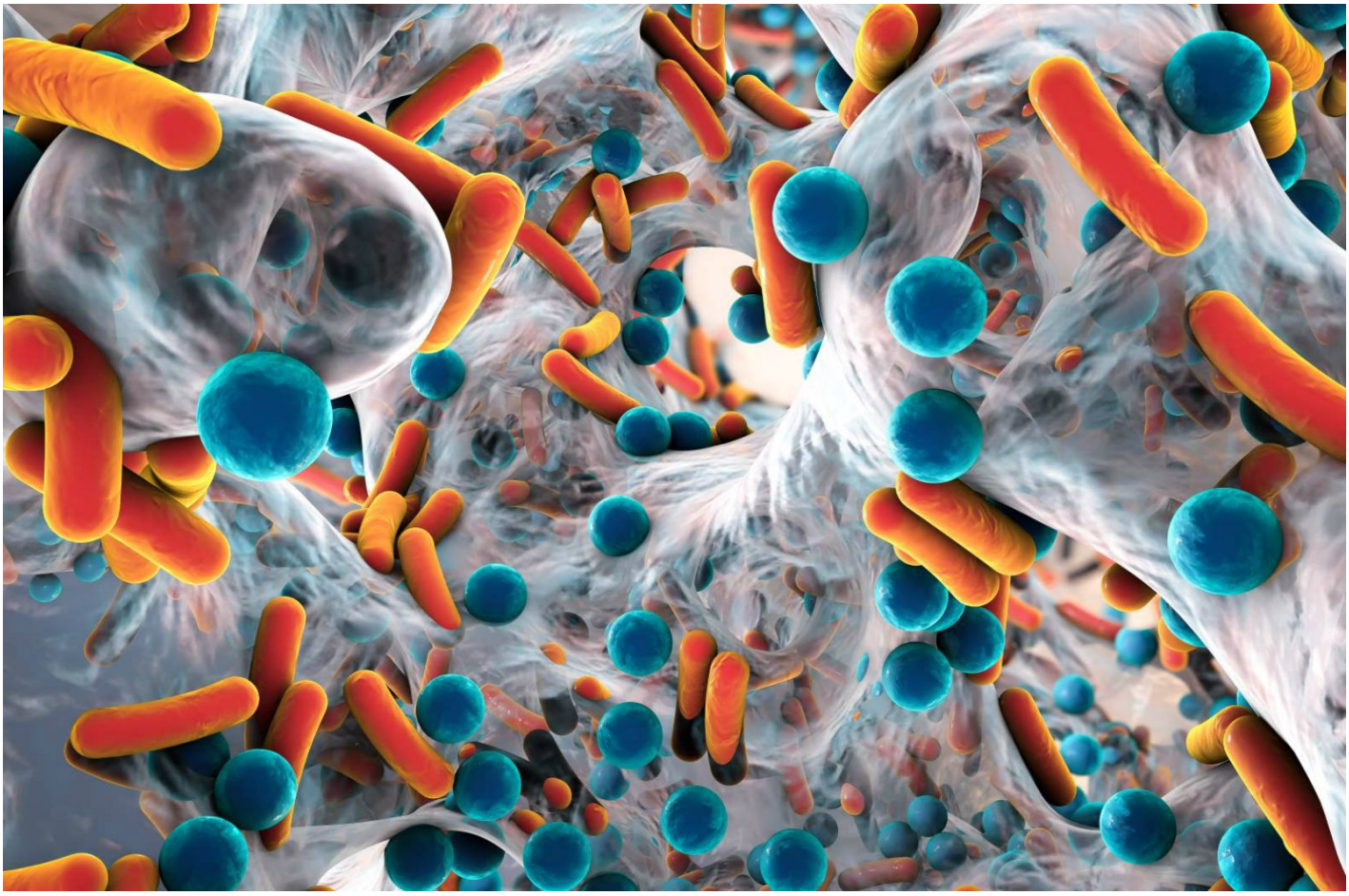
Improved Diagnostic Test Targets Hard-To-Detect Bacteria

North Carolina State University



By the time Edward Breitschwerdt was in 9th grade, he knew he wanted to become a veterinarian. After turning that childhood dream into a degree, Breitschwerdt, D.V.M., expected to spend his career taking care of the local animals in Maryland, where he had grown up on a small farm. Instead, he has dedicated years to teaching young veterinarians, practicing internal medicine and performing infectious disease research that has implications for global health. His research has led to a diagnostic test that dramatically improves the detection of *Bartonella* — a bacteria that infects a range of animals. In humans, Bartonella infection has been documented in patients with a startling range of chronic diseases, including rheumatoid arthritis, fibromyalgia and multiple sclerosis.

> *Bartonella has wreaked havoc on humans for centuries — it infected as much as 20 percent of Napoleon's army. The trench fever epidemic from World War I has also been attributed to a*

> *species of Bartonella that's still prevalent in homeless populations.*

A Microorganism with Many Disguises

Scientists have discovered more than 30 *Bartonella* species so far, and about half of those cause disease. These bacteria are primarily transmitted by tiny insects like ticks, fleas, lice and biting flies — and also through the bites and scratches of other animals, such as cats (cat scratch fever is one disease example).

Because *Bartonella* are slow-growing bacteria that hide within cells, they often elude detection. When conventional testing is used on a patient's blood sample, the result too often is a false negative. Patients can have many common symptoms like persistent headaches, chronic fatigue or muscle pain. "It doesn't have a signature," says Anupama Ahuja, Ph.D., licensing associate at North Carolina State University's Office of Technology Transfer. As a result, many patients infected by *Bartonella* are misdiagnosed or not diagnosed at all.

The Search for Fertile Ground

In the early 1990s, Breitschwerdt's research laboratory at North Carolina State University's College of Veterinary Medicine studied vector-borne infectious diseases like those carried by fleas, ticks or mosquitoes. "As time went on, my research laboratory went from putting 90 percent of effort into other known vector-transmitted organisms to putting 90 percent of our research effort into *Bartonella*."

Bartonella are known as fastidious bacteria. Basically, they are picky about where they live, and often need a complex set of nutrients to grow to detectable levels in patient specimens. In 1993, Breitschwerdt isolated the first *Bartonella* species from a dog with endocarditis (an infection of the heart valve), but subsequently wasn't able to culture the bacteria from other dogs thought to be infected.

His research lab attempted to culture it using mammalian cell-based media — that's what microbiologists always did if a type of bacteria was pathogenic for mammals. But *Bartonella's* comfort zone seemed to be in insects, the most common carriers of the bacteria. With that in mind, someone suggested a different approach: Instead of using media derived from mammals, why not use insect cells? "No one had ever asked that question in that manner," says Breitschwerdt. "Those two biochemical compositions — mammals vs. insects — are extremely different." This novel culture method worked.

With co-inventor Sushama Sontakke, Ph.D. (and support of internal funds from North Carolina State University), Breitschwerdt led the development of BAPGM — shorthand for Bartonella alpha Proteobacteria Growth Medium. It is a medium made to support the growth of insect cells and optimized chemically to enhance growth of *Bartonella*. Subsequently, Ricardo Maggi, Ph.D., research associate professor at the College of Veterinary Medicine, led efforts to optimize the medium for growing *Bartonella* and other fastidious bacteria.

Because it significantly improved the detection of *Bartonella*, BAPGM upended conventional wisdom that *Bartonella* didn't cause a chronic bloodborne infection. Some researchers, like Breitschwerdt, had suspected that *Bartonella* could remain in patients' bloodstream for many years. But no one could really prove it, until BAPGM came along. "We are demonstrating that there are individuals with chronic symptoms that are persistently infected with *Bartonella* in their bloodstream," he says. "I think the rest of the world is starting to catch on to the fact that could be a big deal."

Testing the Business Potential

In 1999, North Carolina State University saw that BAPGM filled an unmet need and filed a patent application. Not

everyone saw BAPGM's potential, though. That became clear after Breitschwerdt approached several major diagnostic companies, in an effort to find a corporate partner. Even with his long list of credentials — including his role as former president and subsequently the chairman of the board of the American College of Veterinary Internal Medicine — he could not convince any companies to invest.

Breitschwerdt had no plans to start a company. But, his oldest son had enrolled in a class that required the development of a business plan as a project. He led a team that featured BAPGM. To turn that plan into reality, Breitschwerdt and his co-founders secured a company inception loan from the North Carolina Biotechnology Center. The funding allowed Galaxy Diagnostics Inc., based in Research Triangle Park, N.C., to obtain an exclusive license for the BAPGM technology from North Carolina State University and open its doors in 2009.

"The tech transfer office played an important role in getting the technology patented," says Amanda Elam, Ph.D., president of Galaxy Diagnostics. "They don't patent everything … they do their own due diligence.That's an important step in the process, early on in the innovation."

The Office of Technology Transfer continues to be involved with the advancement of Galaxy Diagnostics.The company was included in the 2012 inaugural class of NC State Fast 15 program, a venture launch support and mentoring program run by New Venture Services in the Office of Technology Transfer.The program helped Galaxy Diagnostics build connections in the growth capital community.

The test offered by Galaxy Diagnostics is called Bartonella ePCR. The e refers to enrichment — that's BAPGM's role, and it's critical when diagnostically dealing with a slow-growing bacteria like *Bartonella*. "You're looking for minute amounts of foreign bacterial DNA in a patient sample," says Elam. By giving *Bartonella* more fertile ground to grow, BAPGM increases the chance of identifying the bacteria with the use of PCR (polymerase chain reaction) — a highly sensitive technology that can make millions of copies of a DNA sequence. Previous conventional *Bartonella* PCR tests only detected about 20 percent of cases. With BAPGM, now 80 percent of infected individuals can be diagnosed.

In 2009, the company launched *Bartonella* ePCR testing services for the veterinary market.Two years later, it began offering testing services for physicians. The company and its four employees are developing a test kit that could be used in hospitals around the world.

Before they receive a *Bartonella* diagnosis, patients often see more than a half-dozen medical specialists and can end up with a spectrum of diagnoses, ranging from arthritis to serious neurological problems, like seizures and loss of feeling or motor ability in arms and legs. The symptoms of cat scratch disease (caused by *Bartonella henselae*) can closely resemble lymphoma. Elam recalls talking with one physician who said the ability to make a cat scratch disease diagnosis meant he could send a patient home with antibiotics, instead of a referral to an oncologist and the possibility of two years of chemotherapy.

Says Breitschwerdt,"Doctors, whether physicians or veterinarians, can't diagnose and treat what they don't know exists." For many years, he has taught veterinary students, interns and internal medicine residents that, "The kindest form of therapy is an accurate diagnosis."

Identifying More Culprits Behind Chronic Disease

It can take quite a while for the medical importance of a microorganism to gain widespread acceptance. Breitschwerdt points to *Helicobacter pylori*, a bacterium known to cause most gastric ulcers in people. After that disease association was first proposed by researchers in Australia, it still took many decades to change the belief that ulcers were caused only by stress.

Using BAPGM, ePCR has already enhanced understanding of the scope of *Bartonella* infection. "With all the case studies they've collected and published, I feel like every correct diagnosis is a life changed," says Ahuja. Symptoms can be debilitating — but it's not just physical distress that's alleviated with correct diagnosis. "Many of these patients are already struggling to convince even their physicians that the symptoms are real," she says. "Even people around them are saying, 'It's all in your head.'"

Accurate testing could do more than alleviate patient suffering. It may also have a dramatic effect on healthcare costs. For U.S. rheumatoid arthritis patients alone, some estimates show annual healthcare costs could exceed $8 billion, and costs of other rheumatoid arthritis consequences (such as lost work productivity) were $10 .9 billion. Even if *Bartonella* only accounted for a small percentage of rheumatoid arthritis cases, it could save the healthcare system a lot of money.

Breitschwerdt readily acknowledges the big "if" in that scenario. The more complex a disease, the less likely a single factor (or bacteria) is solely responsible for the patient's symptoms. That's why he emphasizes the need for more research to establish that *Bartonella* actually causes or contributes to the development of certain chronic diseases. With ePCR, that research is now possible. He also suspects the usefulness of BAPGM may extend beyond *Bartonella*. "In the future, I think what we will ultimately find that BAPGM is going to allow us to find other bacteria in humans and animals that cause chronic intravascular infections that we didn't know existed."

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