

Luck, Labor, and a Labrador Retriever: A Veterinarian's Journey into *Bartonella* Research

By Samantha Lin

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Dr. Ed Breitschwerdt is the recipient of the 2017 AKC Canine Health Foundation's Asa Mays DVM Award for Excellence in Canine Health Research

To anyone who has conducted scientific research, the task can often feel as fruitless as digging for a needle in a haystack. But what if you're not sure the proverbial needle even exists? Is it worth searching for? To Dr. Ed Breitschwerdt and his team at North Carolina State University (NCSU), it certainly was.

In the early 1990s, the AIDS epidemic led to the discovery of two new *Bartonella* species—prior to that point, there was no knowledge of infection of any animal in North America, including humans, by this bacterial genus. Then in 1992, Dr. Russell Regnery, a researcher at the Centers for Disease Control and Prevention (CDC), discovered an association between a novel bacterial species, *Bartonella henselae*, and cat scratch disease in people, the first evidence that the *Bartonella* genus had any zoonotic potential at all—that is, that it could be transmitted between animals and people.

These findings piqued the interest of Breitschwerdt, a friend of Regnery's and a veterinary internist at NC State. Breitschwerdt had been looking at vector-borne diseases since completing his internal medicine residency at the University of Missouri. While an internist on the faculty at Louisiana State University, located in an area of the United States swamped with bugs capable of carrying organisms pathogenic to dogs, his focus narrowed to vector-borne infectious diseases.

"In Louisiana, because of the constant warmth and moisture, there are a lot of vectors—mosquitoes, ticks, fleas, in particular, and a lot of vector-borne infectious diseases," Breitschwerdt said.

At NC State, Breitschwerdt's laboratory had already been studying other vector-borne diseases of dogs such as Rocky Mountain Spotted Fever, and as researchers began discovering *Bartonella* in other species, Breitschwerdt and his graduate students joined the race to find the bug.

Bartonella is notoriously difficult to find in a species for which it is not adapted. Unlike many well-understood bacterial infections, *Bartonella* is a "stealth" pathogen, meaning it can escape detection by conventional laboratory techniques due to adaptations that allow the infection to persist inside a variety of cells. Cats were one of these adapted hosts, so a number of investigators, including Breitschwerdt, were able to isolate *Bartonella* from cats, but dogs were a different story. Because of this, Breitschwerdt needed something that no amount of time in the laboratory could confer: a little luck.

A few months after Regnery's discovery of the association between *Bartonella* and cat scratch disease in humans, a Labrador Retriever named Tumbleweed was referred to NC State's School of Veterinary Medicine, Internal Medicine service with an endocarditis (inflammation of the heart) unresponsive to treatment. The referring veterinarian had diagnosed Tumbleweed with systemic lupus erythematosus and started her on immunosuppressive therapy. As Tumbleweed's condition continued to worsen, the more immunosuppressive drugs she received.

When Breitschwerdt saw Tumbleweed's worsening endocarditis, he immediately thought of the growing literature on *Bartonella*, where a number of human cases were associated with endocarditis. So despite the previous supposition that dogs were not infected with *Bartonella* and the lack of success finding *Bartonella* in dogs, he and his graduate student (Dorsey Kordick, PhD) tested Tumbleweed for the bacteria. The resulting isolate, grown by Kordick from Tumbleweed, was the first isolate of *Bartonella* from a dog by anyone, anywhere in the world. Furthermore, it was a species of *Bartonella* previously never seen in an animal or a human (*Bartonella vinsonii* subsp. *berkoffii*).

"We were very lucky [that the referring veterinarian was treating for lupus], otherwise it may have delayed knowing that dogs were ever infected with *Bartonella* for years," Breitschwerdt said. "At the same time, luck comes to a prepared mind."

His discovery of a new *Bartonella* species eventually shook the infectious disease world, when the novel *Bartonella* found in Tumbleweed's blood was identified as a human pathogen. Work in the subsequent years in humans at Duke University Medical School and the Mayo Clinic confirmed what Breitschwerdt's lab had inadvertently discovered: immunosuppressing patients for treatment of autoimmune diseases facilitated identification of this pathogen; however, finding *Bartonella* remained a Herculean task in immune competent dogs and humans.

As researchers around the world started isolating many *Bartonella* species from animals (bats, rabbits, and squirrels to name just a few) over the ensuing years, the stakes surrounding *Bartonella* continued to grow. Working with the special pathogens group at the CDC, Breitschwerdt and his team attempted to characterize this new species of *Bartonella* but found it problematic to isolate out of immune competent dogs. They ran into a double-edged sword: the bug was not only hard to detect in infected dogs, but even after detection, it was challenging to grow in culture to further study.

After 10 years of trying to use conventional microbiological culture techniques to grow the bacteria, Breitschwerdt and a research postdoctoral scientist in his laboratory (Dr. Sushama Sontakke) were struck by a unique idea. Instead of using conventional culture techniques that used mammalian cell components for growth, they created a new culture technique. This technique used media that supported the growth of insect cells based upon the hypothesis that the *Bartonella* might grow better on such a media due to its properties as a vector-borne pathogen. A few years later, the self-described "farm boy from Maryland" was the co-inventor of US Patent 7,115,385 B2, for cultivating fastidious—or difficult to culture—microorganisms.

From humble roots—Breitschwerdt recalls his mentor in veterinary school at the University of Georgia recommending he pursue a rotating internship and residency after graduation, and his response of “What’s an internship?”—as an internist, he has certainly had an illustrious career. From being awarded the most prestigious honor a faculty member at NC State can receive in the 2013 Holladay Medal, to publishing more than 120 peer-reviewed publications on *Bartonella* alone, Breitschwerdt would be well within his rights to hang up his lab coat after 30 years of research. But he’s not done yet.

Breitschwerdt and his collaborators received an AKC Canine Health Foundation grant last year to tackle the other hurdle to studying *Bartonella* in dogs: antibody detection. While laboratory techniques used to diagnose many bacterial infections work by locating antibodies to the microorganism produced by the infected host, *Bartonella* is unique in that many infected animals, including dogs, horses, and humans, do not produce a detectable antibody response. So, Breitschwerdt’s team is hoping to take a three-pronged approach to develop techniques to enhance serological diagnosis of Bartonellosis centering around detecting specific antibodies induced by the bacteria.

Creating a diagnostic assay that can effectively detect *Bartonella* infection will be crucial to understanding the epidemiology, disease spectrum, and pathogenesis of this microorganism. Indeed, without such a test, Breitschwerdt notes that it is impossible to fully characterize the reach of Bartonellosis, an issue that he hopes will be mitigated with the completion of this work. However, detection of these stealth bacteria remains challenging and there is always more work to be done.

“It takes decades to change medical understanding and to shift paradigms from ‘we didn’t know this organism exists’ to ‘this is an important...zoonotic organism that can cause disease in cats, dogs, horses, and humans,’” Breitschwerdt said. “The reality is that I think *Bartonella* poses a substantial risk to the human-companion animal bond, a reality that I do not like to contemplate.”

Treatment and prevention options for Bartonellosis are still limited. While some infections clear with use of long-term antibiotics, other affected individuals—humans and dogs alike—remain persistently infected or have relapses, necessitating further research and collaboration between institutions working on *Bartonella*.

Infectious diseases are at the heart of the One Health concept, which represents the confluence of human, animal, and environmental health, and no one is more adamant about the importance of researchers embracing One Health in solving these issues in the future than Breitschwerdt.

“One Health is all about teams working together outside their silos to benefit society, and animals, and the planet we live on,” Breitschwerdt said. “The younger generation has a lot to

do. The simple problems were solved by my generation and the generations before me. But the really complicated problems we've left for the young people."

Even with all of the success that studying *Bartonella* has lent his career, Breitschwerdt refers to the journey as an emotional roller coaster. On top of the difficulty of studying a species incredibly challenging to isolate, he has dealt with scientists around the world looking to discredit his work for the last 20 years simply because it was done at a veterinary college rather than a prestigious human infectious disease research center.

One of Breitschwerdt's 2012 publications on *Bartonella* in *Emerging Infectious Diseases* prompted acerbic letters to the editor in the same leading infectious disease journal questioning his work.

"It's been absolutely depressing at times, when you know you're way out there on a limb, and that it wouldn't take much to fall," Breitschwerdt said. "We could make a mistake in the laboratory and publish something inaccurate—it happens, not intentionally, and ultimately, that would result in discrediting every manuscript we've published on *Bartonella*."

Perhaps one of the most valuable assets in a prolific researcher is tenacity, and Breitschwerdt has it in spades. He has persevered through all of the criticism of skeptics, and part of the reward comes through his email inbox, where he receives letters from people touched by *Bartonella*, acknowledging the impact his work has had on them, their pets, or their children.

One of the most validating experiences came last year, when French researchers published a manuscript in *Emerging Infectious Diseases* on *Bartonella* activity in humans in France, including infections by *Bartonella* species reported for the very first time. In the discussion of their findings, the researchers recognized Breitschwerdt's work, confirming the work, which they and others had previously questioned and affirming that the laboratory was generating valid results while being questioned by researchers around the world. During moments like these, Breitschwerdt sees his relationship with *Bartonella* research changing from "a curse...to a gift."

Breitschwerdt and his collaborators have come a long way since the 1993 discovery that originated with a dog named Tumbleweed, though the trials still continue. Like many other researchers, he still struggles for grants, and convincing human physicians and veterinarians alike of the importance of *Bartonella* is an uphill battle. But if his appearances at the National Academy of Sciences taking a stand for funding for both veterinary and infectious disease research are evidence of his determination, Breitschwerdt is planning to continue the battle for years to come.

"No one, whether you're an MD, a PhD, a DVM...or a private citizen, has a license on knowledge," Breitschwerdt said. "I say that inasmuch as everyone has a right to knowledge, and everyone has, in my opinion, an obligation to generate new knowledge, particularly those of us who are professionals."

Today, Breitschwerdt's lab remains as busy as ever, a team of scientists always on the path to generate new knowledge. As *Bartonella* is still relatively young in the infectious disease research world, there is still much to uncover in the pathogenesis and epidemiology surrounding this mysterious bug. And even with a patent and over 500 scientific publications under his belt, the farm boy from Maryland is still on the hunt for new needles in this ever-growing haystack.

About the author: Samantha Lin is a second year veterinary student at North Carolina State University. Her interview with Dr. Breitschwerdt took place in July 2017.

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