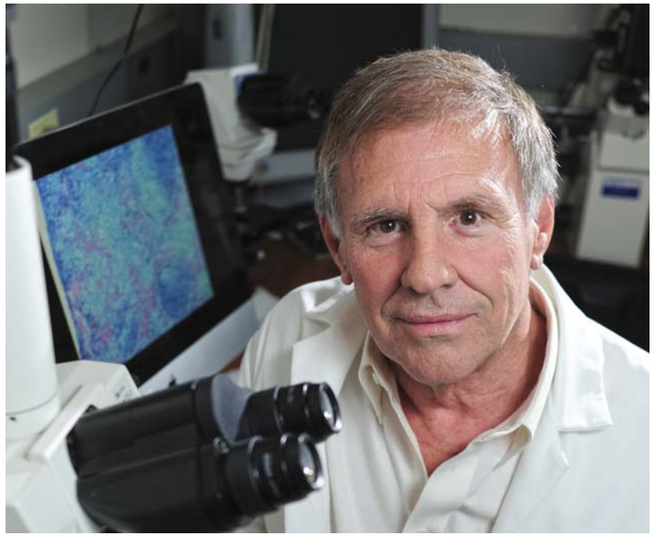
BY LIZA BURBY

How Stony Brook scientists broke the Lyme disease case

While the world is fascinated with infectious diseases such as the Ebola outbreak and the Enterovirus D68, it is worth noting that Stony Brook faculty were at the forefront of identifying the cause of the most common tick-borne infectious disease in the United States — Lyme disease. Finding the cause of an infectious disease is like solving a mystery; scientists and detectives have a lot in common. As with any good sleuth, a scientist gathers facts, analyzes data and investigates evidence. Some leads go nowhere, while others result in major discoveries, just like one that took place at Stony Brook decades ago. The case: Discovering the cause of Lyme disease. The investigator: Researcher Jorge L. Benach, PhD.

Benach is a SUNY Distinguished Professor, chair of the Department of Molecular Genetics and Microbiology and director of the Center for Infectious Diseases at Stony Brook. In the late 1970s he was one of several researchers who began to study ticks after noticing a spike in the number of Rocky Mountain spotted fever cases on eastern Long Island.



JORGE L. BENACH, PHD

> “Jorge’s seminal work really helped us to understand the pathogenesis and transmission of this important disease.” — PRESIDENT STANLEY

The bacterium *Rickettsia rickettsii* causes the disease, which can be transmitted by the bite of the dog tick and other infected species. Because untreated *Rickettsia* could be fatal, Benach knew that an early and accurate diagnosis and treatment were crucial.

At the same time he was immersed in *Rickettsia* research, Benach and other scientists noted the rise of two other diseases in the region. One caused a skin rash along with flu-like symptoms and affected the nervous system, heart and joints. It was first reported in Europe in the early 20th century but recognized in the United States in 1975 when Allen Steere, MD, and colleagues at the Yale University School of Medicine identified a cluster of patients in Connecticut with similar symptoms — rash at the site of a tick bite, followed by rheumatoid arthritis. The illness? Lyme disease, named after the town where it was first detected in the United States.

The second disease, babesiosis, is a parasitic blood infection similar to malaria. Benach suspected a link between ticks and babesiosis and in 1978 launched a formal study, along with Willy Burgdorfer, PhD, a zoologist and microbiologist at the National Institutes of Health’s Rocky Mountain Laboratories in Hamilton, Montana, under whom he had trained. Benach and his colleagues set out to collect a variety of ticks from their natural habitats — grassland for dog ticks, brush for deer ticks. They dragged a white cloth over the vegetation and collected about 400 specimens for culture.

“At that time we were looking for the *Babesia* organism and to see if it was associated with Lyme disease,” Benach said.

He suspected that *Babesia* was linked to the dog tick but was surprised to find quite the opposite: It was the deer tick, which was abundant on Shelter Island and the South Fork, that carried the organism. Even more important, it was the species implicated in Lyme disease.

What Benach and Burgdorfer learned is that ticks carry a number of organisms in their guts just like humans do, and deer ticks in particular carry several pathogens, including the corkscrew-shaped bacterium known as a spirochete — the organism that causes Lyme disease.

In 1982 the medical community honored that groundbreaking discovery by naming the spirochete *Borrelia burgdorferi*. The findings were published in *Science*, which thrust Stony Brook University and the School of Medicine into the limelight and instantly made Benach a pioneer of Lyme disease research.

"Lyme disease is one of those fascinating stories of an emerging infectious disease that has not been recognized until very recently," said Stony Brook President Samuel L. Stanley Jr., MD. "Jorge Benach's seminal work really helped us to understand the pathogenesis and transmission of this important disease."

A Succession of Discoveries

Unraveling the medical mystery of what causes Lyme disease quickly led to a succession of other discoveries for Benach. He learned that it's easier to extract spirochetes from a skin biopsy than from blood and that *Borrelia* tend to infect tissues such as the skin, heart and joints.

"We cultured the blood of a large number of patients with Lyme disease, but the *Borrelia* were only cultured from two patients," he said.

Despite the low yield, the finding was significant — it confirmed the etiology of Lyme disease. The study was published in *The New England Journal of Medicine* in 1983. That same year Benach, along with Edward Bosler, PhD, a medical entomologist, published another study, this time in *Science*. The team identified the main source of *Borrelia* — the field mouse.

The discoveries didn't end there. In 1984 Benach worked with Barry Lissman, DVM, a veterinarian in Suffolk County, to characterize canine Lyme disease and isolate spirochetes from sick dogs — a finding that created the field of veterinary borreliosis. A year later, Benach learned just how dangerous deer ticks can be — some patients contract both Lyme disease and babesiosis, both of which can be carried by the same tick.

Diagnostic Testing

Now that the organisms for causing Lyme disease were isolated, Benach said the next priority was to develop diagnostic serology to detect the presence of antibodies to *Borrelia burgdorferi*.

He teamed up with Marc G. Golightly, PhD, who oversees the

Lyme Disease Research at SBU

1978

Jorge Benach studies Rocky Mountain spotted fever, a disease carried by the dog tick, on Shelter Island and the South Fork.

Benach begins to study a new disease, babesiosis.

1982

Benach and Willy Burgdorfer discover *Borrelia burgdorferi*, the organism that causes the Lyme disease infection. The study is published in *Science*.

1983

Benach conducts the first isolations from patients and confirms the etiology of Lyme disease. The study, published in *The New England Journal of Medicine*, also discloses that *Borrelia* favor infecting tissues such as skin, heart and joints.

In a study published in *Science*, Benach and Edward Bosler, PhD, identify the field mouse as the main source of *Borrelia*.

1984

Benach and Barry Lissman, DVM, characterize canine Lyme disease and isolate spirochetes from sick dogs. These discoveries open the field of veterinary borreliosis.

Benach and Marc Golightly create an enzyme-linked assay to diagnose Lyme disease in patients' blood tests.

1985–86

Benach discovers that some patients can have concurrent Lyme disease and babesiosis, as the agents for both diseases are carried by the same tick.

Raymond Dattwyler creates an antibiotic treatment for Lyme disease, Ceftriaxone, also known as cephalosporins, which becomes the gold standard for treatment.

1987

The Lyme Disease Laboratory at Stony Brook opens to meet increasing patient load and the need to develop better treatment regimens.

1990

University Hospital Laboratory becomes known as a national reference lab for Lyme disease due to accuracy of diagnostic tests created by Benach and Golightly.

1997

The Benach lab discovers that Lyme disease agents use the fibrinolytic system to spread throughout the body. The study is published in *Cell*.

2001

Benjamin Luft, Dattwyler and microbiologist John Dunn, PhD, at Brookhaven National Laboratory, develop a more rapid diagnostic test. Their findings are published in the *Archives of Internal Medicine*.

2002

Luft and his team solve the structure of the major outer surface proteins of *Borrelia*, enabling them to see how the structure looks.

Stony Brook licenses the technology and patent of the first bioengineered vaccine to Baxter International.

2010

Luft and his team of medical researchers determine the genetic blueprint of 13 strains of the bacteria that cause Lyme disease.

The Benach lab in collaboration with Erwin London, PhD, biochemistry, discovered the presence of cholesterol-bearing lipid rafts — unique structures that are useful for the organism to move from ticks to humans. The study is published in *Cell Host & Microbe*.

2012

Luft sequences the entire genome of 22 *Borrelia* variants, providing the entire library of all the variations occurring in this organism.

Clinical Immunology Laboratories at Stony Brook, who in 1983 began to study immune responses in Lyme disease.

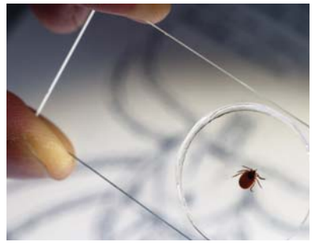
"The diagnostics for Lyme disease were few and far between and not very good," said Golightly, who is a professor and director of the Flow Cytometry Core Research Facility at Stony Brook University Hospital.

"Jorge asked if I'd be interested in taking over some of the diagnostics to free him up to do other things. So I agreed, and we worked together on a test for the clinical side and started up doing diagnostic tests," said Golightly.

In 1984 Benach and Golightly made a kit from scratch. It was an enzyme-linked immunosorbent assay (ELISA) — a test that uses antibodies and color change to identify a substance. Having an accurate Lyme disease test is crucial, Benach said, because it reduces the chance of false positive results.

In 1990 Benach and Golightly learned how accurate the ELISA test was. In a paper titled "Serologic Tests of Lyme Disease Interlaboratory Variability," published in *Archives of Internal Medicine*, a study followed 18 Lyme disease specimens that were sent to nine different reference labs, including University Hospital. The study looked at the accuracy of the labs' diagnostics in confirming Lyme disease in the specimens.

"We were the only lab that got 18 out of 18," said Golightly. "The result was that Stony Brook



DEER TICKS CARRY THE CORKSCREW-SHAPED BACTERIUM THAT CAUSES LYME DISEASE.

University Hospital became a national reference lab for Lyme disease. Everyone wanted to send their specimens to us." He added, "At one time we were doing 30,000 specimens a year."

Increasing Patient Needs

While Benach and Golightly were working on the diagnosis and clinical manifestations of Lyme disease, other Stony Brook scientists began to study therapeutic options. To meet increasing patient load and the need for better treatment regimens, in 1987 Stony Brook opened its Lyme Disease Laboratory with Raymond Dattwyler, MD, professor of medicine, as director. Under his leadership, a successful regimen of antibiotic treatment for Lyme disease, Ceftriaxone, also known as cephalosporins, became the gold standard for treatment worldwide.

One of Dattwyler's colleagues was Benjamin Luft, MD, who is currently an Edmund D. Pellegrino Professor of Medicine and medical director of the LI-CCE Board Certification Internal Medicine—Infectious Diseases Training and Medical School Residency Training VA Medical Center. Luft said that because Lyme disease can take months to diagnose correctly, and even then it is often misdiagnosed, it leaves patients open to serious arthritic, cardiac and neurological complications. In 2001 he and Dattwyler, along with microbiologist John Dunn, PhD, at Brookhaven National Laboratory, created a rapid diagnostic test for Lyme disease that reduces the time it takes to get results from about a week to less than 30 minutes. They published their findings in the *Archives of Internal Medicine*.

From 1994 to 2002 Luft's other goal was to develop a vaccine to prevent Lyme disease. That meant solving the structure of the protein. He said one of the main challenges was to discover a method that was effective on all species. To do so, Luft collaborated with Dunn to use Brookhaven's National Synchrotron Light Source, a sophisticated machine that allows for the study of the atomic structure of proteins.

"By using it, you know where every atom is located within that protein, so it's extraordinarily powerful," Luft said. "We solved the structure of the major outer surface proteins of *Borrelia*. This is a very important discovery because you can actually see how the structure looks."

They focused vaccine development on the most abundant *Borrelia* outer surface protein found when the spirochete bacteria reside in ticks. Using the scaffold of this protein, called OspA, they bioengineered a set of unique OspA proteins not found in nature.

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>
> — KENNETH KAUSHANSKY, MD, MACP

This technology and patent was licensed to Baxter International in 2002 with the hope it would be the first safe and effective vaccine in protecting against every known strain of Lyme disease. The vaccine is still in the clinical stages.

Luft was still looking for the building block to develop more accurate and effective diagnostic tests, therapeutic agents and vaccines. In 2010 he and a team of medical researchers determined the genetic blueprint of 13 strains of the bacteria that cause Lyme disease. By 2012 they had sequenced the entire genome of these 22 *Borrelia* variants.

"By doing this we were able to identify every gene and every protein that the *Borrelia* organism could ever produce anywhere," he said. "We were able to get the entire compendium of proteins. We had the entire library of all the variations occurring in this organism. This is a tremendous tool."

What started as research to discover one organism more than 30 years ago by Benach has led to better diagnosis, better treatment, and perhaps one day soon, better prevention of Lyme disease. All this is possible because of the collaboration between departments here at Stony Brook University and the Hospital.

DR. BENACH AT WORK IN HIS LAB.

"The understanding of the origin, the diagnosis, and the ultimate treatment of Lyme disease and babesiosis is a tour de force accomplishment for team science," said Kenneth Kaushansky, MD, MACP, Dean of the School of Medicine and Senior Vice President of Health Sciences at Stony Brook University.

"As the problems we face in biomedical science continue to grow in complexity, their solution will require the skills and insights of multiple basic researchers, translational scientists and clinicians," continued Kaushansky.

"That is our goal at Stony Brook Medicine, to bring together the very best and brightest, so that the whole will be greater than the sum of its parts. Of course, we also know that it will always take the genius of a Jorge Benach to set the solution in motion."

When it comes to Stony Brook's role in Lyme disease, "we've had a good run. People need to know how involved this institution has been. This is really a great story but there's probably more to be done," Benach said. "Lyme disease is worldwide and we are helping patients all the way from Japan, Siberia, Europe, through North America. In the '80s we started to work on the microbiology of the organism. We went from clinical/epidemiological research to more bench research — 140 papers later, and we're still doing it."