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TICKED

The Battle Over Lyme Disease in the South

An exclusive e-single
by Wendy Orent

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Cover image: James Gathany/Public Health Image Library/CDC
ISBN: 978-1-62700-115-1
Discover Magazine
www.DiscoverMagazine.com
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Preface

In 2013, the Centers for Disease Control and Prevention (CDC) revised the stats on Lyme disease: Instead of the 30,000 cases reported annually to the agency, new patients with the tick-borne infection number 300,000 strong across the United States each year. The count is critical because untreated Lyme disease can result in devastating neurological and cardiac complications and long-lasting pain and fatigue. With CDC validation, hundreds of thousands of patients may get diagnosed and treated earlier, when the disease is easier to cure.

But the new accounting doesn't apply to the American South. Even as acceptance increases in much of the U.S., conflict mounts in states south of Virginia, where, the CDC says, Lyme disease is almost nonexistent. Contracting Lyme in Georgia and the Carolinas, in Florida and Alabama is less likely than getting hit by lightning, many experts contend. Risk of Lyme disease in Arkansas or Louisiana: virtually zero. Yet throughout the South, thousands of people report illnesses resembling Lyme disease, all after tick bites.

Now a growing body of evidence from research scientists and university labs suggests that Lyme disease — or at least something like it — could be present in the South as well. Researchers studying the issue say infection may be trickier to pin down in the South than in the North, where Lyme is caused by the bacterium Borrelia burgdorferi*, a spirochete transmitted by the bite of the blacklegged tick. In the South, the primary tick suspect is the aggressive, ubiquitous lone star (so-called for the splash of white on its back), which some entomologists contend may carry a cast of* Borrelia

species potentially involved in human disease.

New research on Southern tick-borne illness is controversial, falling far short of proof. But the matter is crucial, not only because of unexplained chronic health problems in the region but also because the lone star tick is on the move. It has been recorded in large numbers in recent years as far west as central Texas and Oklahoma and as far north as Maine. Adding to the complexity, novel spirochetes — one proven to cause a Lyme-like disease and others in need of investigation — have been reported around the U.S. (See appendices A and B.) When patients with suspected Lyme disease fail serological blood tests, an alternate tick-borne pathogen could be the cause.

For the three and a half years that this story was being reported, the CDC's Division of Vector-Borne Diseases in Fort Collins, Colo., repeatedly denied Discover*'s requests to visit top scientists there. After numerous phone calls and emails, our writer, Wendy Orent, was granted short phone interviews with three CDC experts. One of them did not specifically work on Lyme, and one was an epidemiologist who agreed to answer follow-up questions via email but never responded to any subsequent queries. After 18 months of vigorous lobbying, Orent was allowed to send a list of questions to microbiologist Barbara Johnson, one of the top experts in Lyme disease at the CDC's Fort Collins branch. Johnson answered questions via email and also responded to our researcher, Will Hunt, in the fact-check process. However, she declined to discuss the merits of new studies suggesting the possibility of spirochetal diseases spread by lone star ticks. "It is routine for CDC scientists to give detailed and explicit critiques in their scientific papers on*

subjects on which they have done research," Johnson explained to Hunt. "However, CDC staff should not make a review of another scientific work for publication in the popular press first, especially since such a review might be construed as an official position of the agency."

The CDC, in other words, refused to comment on published, peer-reviewed scientific evidence that contradicted its own stance. But we have not let the agency's silence stymie our report. Wide-ranging interviews with scientists on all sides of the issue helped Orent cast a critical, discerning eye on disturbing data coming out of the Southern United States.

CHAPTER 1

Dead Ringer

The hothouse ecosystem of the South hosts a complex nexus of parasites and microbes, including spirochetes like those causing Lyme in the North. But how many of these species infect us?

Kerry Clark never wanted to show that Lyme disease exists in the Southern United States by catching it himself. Clark, who calls himself the Tick Doctor, is a medical entomologist at the University of North Florida in Jacksonville. A slender, wiry man with a short shock of graying brown hair, he is most at home in a kayak on one of the ponds behind the heavily wooded Jacksonville campus. He jogs and lifts weights when he is well enough to do so.

Clark has spent years crawling through underbrush and kicking up leaf litter all over the South to collect ticks that transmit infections. He takes the ticks to his laboratory, cuts them open, pulverizes them and searches for DNA from germs that carry human diseases, two in particular. One is Lyme, caused by a twisting, spiral-shaped bacterium named *Borrelia burgdorferi* — a spirochete, like the bacterium that causes syphilis. The other disease is babesiosis, caused by the parasite *Babesia microti* — a pathogen similar in type and impact to the one that causes malaria. Ticks in the South also carry other diseases, including

Rocky Mountain Spotted Fever and ehrlichiosis, caused by two kinds of related bacteria. Despite innumerable tick bites, Clark says he never had a medical problem until the day he dragged for ticks in the town of Fayetteville, a leafy, middle-class suburb south of Atlanta. Clark was giving a talk on Lyme disease at a gathering of the Dougherty County Medical Society in Albany, Ga., where he met Fayetteville resident Liz Schmitz, president of the Georgia Lyme Disease Association. When he heard how many people from Schmitz's town had been bitten by ticks and how sick they were, he agreed to come up and have a look. Armed with his usual equipment — a long, white flannel cloth on a pole — he searched various properties, all of them abutting woodlands, dragging for ticks.

He didn't have to work hard. The landscape around Fayetteville is dotted with stands of trees interspersed with wide yards and family homes. In general, patchy woodland is the most hospitable habitat for Lyme disease, according to disease ecologist Richard Ostfeld of the Cary Institute of Ecosystem Studies in upstate New York. It is the patchiness that presents the danger. Small pieces of disconnected woodland are home to deer and field mice, rabbits and raccoons — all tick carriers. Continuous woodland houses more diverse communities of animals, naturally diluting the density of those hosting the ticks and therefore lowering the risk of tick-borne disease.

When Clark dragged his white cloth through the underbrush, ticks seemed to burst out, especially the hungry, aggressive lone star females with their distinctive white spots. He remembers one practically leaping from the drag cloth onto his finger. In less than

an hour, he collected hundreds of lone star nymphs (young ticks) and adults, as well as a couple of slower-moving adult blacklegged ticks.

And that, Clark guesses, is when an adult lone star tick nestled in his hair. He did not find it attached to his scalp for several days. Engorged from sucking his blood, it had already deposited whatever load of bacteria it had been carrying into Clark's body.

Since that day almost three years ago, Clark has been suffering from what he describes as intermittent pounding headaches, fatigue, odd twitches and "fuzziness." He reports that weeks-long courses of antibiotics make him feel better, but when he goes off the drugs, the symptoms return.

Other people from suburban communities around Georgia — and many other areas of the Southeast — report getting sick from what seems like tick-borne illness, too. A man in his 50s from Fayette County who prefers not to use his name developed severe neurological symptoms after a tick bite. Initially he had trouble walking; his right foot dragged, and he couldn't use his right arm at all. After visiting a series of neurologists, he was diagnosed with the lethal neurodegenerative disease ALS (for amyotrophic lateral sclerosis, also known as Lou Gehrig's Disease.) ALS gradually kills off motor neurons, causing progressive paralysis. It initially leaves patients weakened, then wheelchair-bound, then, within a few years, unable to eat or breathe.

The last specialist to render this sentence, at Emory University Hospital three years ago, told the man there was no treatment for his condition and sent him home to die. But after talking with Schmitz, he sent Clark samples of his blood for analysis. Using

polymerase chain reaction (PCR) testing, sometimes referred to as molecular photocopying, to amplify and then analyze fragments of foreign DNA in the man's blood, Clark found evidence of *Borrelia burgdorferi*, the pathogen that causes Lyme disease. Now on antibiotics, the Fayette County man says he feels better than he has in years. While he still uses a wheelchair and remains partially paralyzed, the rapid downward trajectory common to almost all ALS patients seems to have stalled.

When Clark ran PCR tests on the man, he also tested his own blood and found somewhat similar results. Clark himself had traces of two closely related species of *B. burgdorferi*. The first of these two genospecies was *B. burgdorferi* sensu stricto (meaning "in the strict sense"), the type associated with virtually all accepted Lyme disease in the United States. The Fayette County man had the same thing. (Bacterial genospecies are differentiated by divergent genetic codes. Another Lyme genospecies, *B. burgdorferi* sensu lato, meaning "in the broad sense," includes bacteria causing Lyme disease in Europe.) The second genospecies Clark found, though only in his own blood, was *Borrelia andersonii,* a Lyme spirochete thought to be restricted to rabbits. Both Clark and the man also tested positive for two different strains of the tick-borne, immune-suppressing parasite *Babesia microti*. The lab results seem clear enough: Both men were infected with Lyme disease or something very much like it, and babesiosis as well.

There is just one problem with this story: Many Lyme researchers, including some from the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention

(CDC), won't believe a word of it. There is little or no true Lyme disease anywhere in the South, say these experts, and what looks like Lyme disease is either a different, unknown condition or, quite possibly, an allergy to tick saliva.

They cite plenty of evidence: In the Northeast, where Lyme is endemic, the disease is spread by *nymphs* — the juvenile form — of *Ixodes scapularis*, commonly known as blacklegged ticks. But researchers agree that below Virginia's southern border, these juveniles, which can die in the Southern heat, are buried too deep in protective leaf litter to get at human hosts. Sturdier adult blacklegged ticks bite humans down south, but because of their large size, they're usually noticed and picked off long before they spread disease.

That leaves the aggressive *Amblyomma americanum,* the lone star tick, as the prime suspect in Southern tick-borne disease. The tick, which frequently bites people as well as other animals, has been scrutinized since the early 1990s, when researchers realized its bite sometimes caused an erythema migrans, often referred to as a bull's eye — a roundish, gradually spreading mottled red rash that looks similar and sometimes identical to the Lyme rash up north.

But how the rash is viewed varies greatly depending on geography. In the North, health authorities consider the rash so specific to Lyme disease that when a patient has the rash, no further testing is needed for a doctor to diagnose Lyme. But south of Virginia, where Lyme disease is deemed rare to nonexistent, the bull's-eye rash is said to indicate something else — a condition named STARI (for Southern tick-associated rash illness).

According to Barbara Johnson, a research microbiologist in the Division of Vector-Borne Diseases at the Fort Collins, Colo., branch of the CDC, and one of its top experts on Lyme disease, STARI is relatively benign, presenting with the rash and flulike symptoms of early Lyme but never advancing beyond these mild signs. It may not even be caused by a spirochete, Johnson says; the actual cause remains unknown. In the South, STARI patients with advanced Lyme-like symptoms are presumed to be suffering from another unknown condition or disease.

And this is where the CDC and researchers like Clark and his colleagues part ways: Clark agrees that "true Lyme" — the classic disease described in the Northeast as infection with *B. burgdorferi* sensu stricto transmitted by the bite of a blacklegged tick — is certainly less common in the South. But his research has revealed other, related spirochetes traveling through the ecosystem: inside the blood reservoirs of mammalian, reptilian and avian hosts; in the hard bodies of his ticks; and inside sick patients themselves. It is the lone star tick, Clark believes, that serves as a bridge, transmitting animal pathogens to us.

Yet until Clark has more proof, this is hypothesis at best. While strains of *Borrelia burgdorferi* can be found in the South, says Jean Tsao, a Lyme researcher from Michigan State University, the path from tick to human has never been confirmed. Instead, she maintains, the cycles Clark points to are "cryptic," meaning that the spirochetes move quietly among ticks and various animal host species but have no effect on human health.

Getting to the truth here is critical — especially to the hundreds or thousands of patients who believe they suffer from some form

of tick-borne illness acquired below the Virginia state line.

The confusion starts with the numbers. No one has any clear idea of how many STARI cases even exist because STARI, unlike Lyme, is not reported to state departments of health or to the CDC. Gary Wormser, an infectious diseases physician at New York Medical College and a recognized Lyme researcher who has studied the Southern rashes, says STARI is actually "pretty widespread in the Southeast and South Central part of the country." But Paul Lantos, an infectious diseases physician and Lyme disease expert at Duke University in North Carolina, insists that STARI is very rare. Hoping to quantify the risk, Adriana Marques, chief of clinical infectious diseases at NIH, launched a study of STARI in 2002. She managed to enroll only three suspected patients over 10 years and abandoned the work in December 2012; there are no published results to date. Public health researcher Marcia Herman-Giddens is the scientific adviser and health liaison for the Tick-Borne Infections Council of North Carolina, a research and advocacy organization. She says she can't believe anyone actively looking for STARI patients would come up with just three of them in a decade. Patients with Lyme or Lyme-like illness in the South likely number in the thousands, she argues.

Many of the patients Herman-Giddens refers to are seriously ill. I met with a group of them from all over Georgia, who, one by one, recounted their years of suffering. One man, a former military pilot, held up a binder two inches thick that detailed every symptom, doctor visit, medication and lab test he had over 15 years. Other people described the suffering of their children,

who, after tick bites, were hobbled by pain, facial tics, fatigue, behavioral difficulties and the inability to concentrate. One young woman, a bright and capable high-school student who scored a spot at the very competitive Georgia Technical Institute, suddenly found herself unable to study. Eventually bedridden, she could not attend classes and began to fail. Sustained antibiotic treatment from a sympathetic physician allowed her to raise her grades to a C level and she finally graduated, but she is still struggling.

No one denies these patients are really sick — but most of the local doctors they consult reject the idea that infection with *Borrelia* is the cause. Medical epidemiologist Paul Mead of the CDC's Division of Vector-Borne Diseases puts it like this: "Erythema migrans-like rash can certainly happen with *Amblyomma americanum* [lone star] ticks, particularly in the South … but it's not diagnostic of Lyme disease. … There are many things that make people really sick and chronically sick, and they have chronic symptoms, but [these symptoms are] not characteristic of Lyme disease."

And that leaves Southern patients who insist they have Lyme disease — or something much like it — angry and adrift. They say they have been allowed to slide into chronic illness every bit as serious and debilitating as untreated Lyme disease in the North, but because no one recognizes their illness, they are never treated, and they continue to suffer pain, joint swelling, headaches, heart inflammation, neurological damage and more.

CHAPTER 2

Legends of the North

How discovery of Lyme disease in the Northeast informed perceptions in the South.

The uproar down South might be best understood through the lens of history in the North. Almost 50 years ago Polly Murray, an artist and mother from Lyme, Conn., noticed a strange, seemingly inexplicable cluster of children suffering from juvenile arthritis, a rare and sometimes disabling condition, within a few blocks of her house, and even on her own street. She was sick herself, troubled by a slipping memory, fatigue and widespread pain — symptoms initially dismissed as psychological by doctors who could find no other explanation. But Murray persevered. By 1975, she had launched a relentless campaign to force doctors and scientists to figure out why so many in her town were afflicted with swollen knees and elbows, persistent fatigue, difficulty concentrating, headaches and rashes, among a host of other bizarre signs and symptoms of disease.

Although Lyme disease, under other names, had been described in Europe for a century, many public health experts initially believed the condition in Connecticut was unique. The CDC dispatched a rheumatologist to investigate the mysterious outbreak. Peering through the lens of his specialty, that investigator, Allen Steere of Yale, initially described a largely

rheumatologic syndrome notable for swollen knees and rash. This established a tenacious image of Lyme disease that has been difficult to dislodge. While Steere later included meticulous descriptions of neurologic and cardiac manifestations of Lyme in his reports, researchers and physicians nevertheless continued for years to view American Lyme disease — unlike European Lyme disease — as essentially rheumatologic.

Another line of concurrent research further limited perspective on the disease, by framing it as a phenomenon of the geographical Northeast. The person who erected that framework was Harvard entomologist Andrew Spielman, a pioneer who had spent years studying *Babesia*, the malaria-like parasite then found in Eastern offshore islands like Martha's Vineyard in Massachusetts and Fire Island in New York.

Babesiosis and Lyme (including whatever microbes cause them) were thought to move in tandem, sharing a womb of sorts inside *Ixodes scapularis*, the blacklegged tick. But as Spielman pursued his research, he became convinced that he was onto something different and new. By 1979, he was reporting that the vector for *Babesia* (and therefore Lyme) was not the reclusive *I. scapularis* but a peculiar cousin, a human-biting tick that he named *Ixodes dammini*, or the "deer tick." Spielman chose the nickname because adults of the species lived and mated on white-tailed deer, each of which can carry up to 2,500 ticks around its head and neck over the course of a single season. The distinction between the two ticks was crucial, Spielman insisted, since *I. scapularis* lived up and down the East Coast from Maine to Florida but *I. dammini* resided only in the North.

When NIH entomologist Willy Burgdorfer finally discovered the Lyme spirochete inside *scapularis* ticks from Fire Island, N.Y., in 1981, Spielman stepped up to say the ticks weren't *scapularis* at all, but *dammini*, the same ones carrying *Babesia* on the islands off Cape Cod.

With the Lyme pathogen identified by Burgdorfer, Spielman did went back to his field station on Nantucket Island to trace the two-year life cycle of Lyme in nature, from small mammals like white-footed mice, to ticks, to larger mammals like deer and — occasionally — people. In the first year of the Lyme cycle, he found, adult ticks fed and mated on the ears and hide of deer, laying eggs that dropped to the forest floor in late spring. The eggs hatched as larvae that were Lyme-free, since ticks do not pass disease on to their offspring. The uninfected larvae acquired *Borrelia* only after taking a blood meal from infected white-footed mice that had been previously bitten by other ticks.

In the second year of the cycle, infected larvae fell from the mice to the ground, growing into adolescent nymphs. The nymphs then "quested" — moving to the tips of long grass and brush, waiting for their next blood meal, a larger mammal, to wander by: a dog, a deer, or that accidental and dead-end host, a human. Spielman argued that the two-year cycle occurred exclusively in the North, where his *I. dammini* deer ticks lived. Crucially, the ticks' limited range restricted Lyme's range as well.

For more than a decade, that is how things stood. For Spielman, and many other scientists, the motto seems to have been *res ipsa loquitur* — the thing speaks for itself: no deer ticks, no Lyme disease.

But Spielman's triumphant discovery was short-lived: *Ixodes dammini* was torpedoed at the hands of Spielman's close friend, the entomologist and nationally-recognized tick expert James T. Oliver.

Oliver, ensconced at Georgia Southern University in Statesboro, was thinking hard about Spielman's *dammini* tick, its identification and its distribution. Today a tall, courtly Southern gentleman with high, defined cheekbones and a delicate frame, Oliver is known for building the National Tick Museum, perhaps the most extensive tick collection and library in the world. "When I started working in this area," he recalls, "I was told, point blank, Lyme disease was not in the South, and human Lyme disease could not occur there — there were no ticks and no germs." But immersed as he was in the comparative study of ticks, he was not convinced that the blacklegged ticks in the North and South differed much — or that *Ixodes dammini*, Spielman's discovery, represented a separate species from *Ixodes scapularis* at all.

Oliver had studied ticks for decades, beginning with his doctoral research at the University of Kansas in Lawrence in 1959. There, Oliver focused on tick and mite chromosomes and their role in determining gender and sex ratios. That research expanded into studies of tick and mite reproductive behavior and strategy, work that continued into the 1980s. Gradually, as the science of genetics matured, he switched his focus to genes. "No one was studying genes in ticks when I started this work," he says.

Oliver also had a long-standing interest in the relationship between ticks and their parasites — the bacteria and viruses that live inside them and, when transmitted to humans, cause disease.

He and Spielman collaborated on tick-borne diseases, becoming friends. “When I went to speak at Harvard, says Oliver, “I stayed at his house. He would stay with us when he came to Georgia.”

But the friendship ended in the early 1990s in their struggle over *Ixodes dammini* — a rift that roiled Spielman to the end of his life (he died of leukemia in 2006) and one that Oliver still regrets. Working with a team in his Georgia lab through 1989 and 1990, Oliver found that Northern deer ticks (*dammini*) and the ubiquitous blacklegged ticks found up and down the East Coast (*scapularis*) appeared to feed in the same way and showed no difference in what animals — rats, chickens or lizards — they chose to attach to. In 1992, he and collaborators examined genetic differences between Northern and Southern ticks, demonstrating that even those from widely separated areas like Georgia and Massachusetts were genetically too similar to be different species.

But that wasn’t enough. The traditional scientific designation of species involves breeding and fertility. Could the two types of *Ixodes* ticks, *dammini* and *scapularis*, mate and produce fertile offspring? Interbreeding several generations of Northern *dammini* and Southern *scapularis* in his laboratory, Oliver demonstrated that a series of matings produced reliably fertile offspring — a crucial test of species boundaries.

Oliver did confirm minor morphological variation between Northern and Southern blacklegged nymphs, but the only major difference between the ticks was *how they fed in nature*. “There is a difference in behavior for populations in Georgia or in Connecticut,” Oliver acknowledges. “*Ixodes scapularis* nymphs have many more hosts in the South, where they feed on lizards,

cotton mice, cotton rats, birds." Because they look for hosts down in the leaf litter material, away from the heat and away from potential human hosts, nymphal *scapularis* ticks rarely bother humans in the South," Oliver says, "but the adults are happy to bite on you. Is that genetics, or is it behavioral ecology?"

Oliver's experiments, which the overwhelming majority of entomologists considered the last word, blew up the idea that *dammini* was a new or separate species. The name *dammini* was dropped from the scientific literature, though the phrase "deer tick" continues to be used in the vernacular.

Despite Oliver's evidence, Spielman ignored the new consensus and kept using the term until he died. And his insistence that the ticks in the North and the South are fundamentally different still lies at the heart of the controversy over Southern Lyme. The consensus of entomology eradicated *dammini* as a species — and Lyme disease was even reported to CDC in Southern states like Georgia until 1989. But that did nothing to change the essential feeling among many experts that the cases weren't real — that ticks were still different enough to prevent Lyme in the South.

CHAPTER 3

Breaking All the Rules

Eventually researchers learn that Lyme disease can occur outside the Northeast.

It didn't take long for exceptions to Spielman's geographic rule to emerge.

On the heels of the Lyme spirochete's discovery, for instance, researchers realized that the two-year Lyme cycle charted in Massachusetts also occurred in the Midwest: Wisconsin, Minnesota and Michigan were recognized as home to the same mammal, tick and bacterial species that harbored Lyme *Borrelia* spirochetes in the Northeast, and endemic for the disease.

By 1985, University of California, Berkeley medical entomologist Robert Lane had demonstrated that Lyme *Borrelia* could be transmitted by *Ixodes pacificus,* a tick found only on the West Coast. So Lyme disease was recognized in California and parts of Oregon.

And in the South, many eventually realized that even if the nymphs hid in leaf litter, adult *scapularis* ticks could bite humans and cause the occasional case of Lyme.

It was 1998 when Mercer University entomologist Alan Smith was bitten by an adult *Ixodes scapularis* in the Piedmont National Wildlife Refuge, a forested area south of Atlanta that is home to several species of tick. He developed the characteristic rash, which

he regarded with aplomb despite a classic Lyme profile: prolonged feeding of an *Ixodes scapularis,* erythema migrans, low-grade fever and flulike symptoms. His physician initially wanted to treat him with antibiotics. "Oh, no, that's not necessary," Smith told the doctor. "The CDC says there's no Lyme in Georgia."

Within months Smith was nearly crippled with arthritis-like symptoms. His wife dragged him back to the doctor, and he went on antibiotics. He improved immediately. "There's definitely Lyme in Georgia," he says now with a laugh. And to his mind, there's no reason not to lay the blame on *Ixodes scapularis*, the culprit in Northern Lyme: "It's a lot of crap that blacklegged ticks don't ever bite people in the South."

According to a study Smith recently conducted, which has not yet been published, 6.9 percent of the adult blacklegged ticks he tested in various parts of Georgia were infected with *B. burgdorferi*. These types of infection rates may seem relatively low compared to the 15 to 50 percent of infected *scapularis* up north, but they are routinely challenged as too high.

Part of the disagreement stems from hewing to the Northern situation, where infection comes overwhelming from nymphs. Last year, Maria Diuk-Wasser of the Yale University School of Public Health published a massive, multiyear study mapping risk of infection with Lyme disease throughout the United States. She defined risk as the chance one could be bitten with an infected *Ixodes scapularis* nymph, the life stage most likely to cause Lyme infections in the Northeast. Diuk-Wasser's team managed to secure and analyze only nine nymphs from the entire South. Not surprisingly, none of those nine was infected with *Borrelia*,

reinforcing the notion of the South as a Lyme-free zone.

But the Yale researchers' ability to find just nine nymphs in the whole of the Southern U.S. may have been preordained by the study design itself. The problem, says Ostfeld, was the assumption that all *scapularis* nymphs ought to behave the same way wherever they're found. Diuk-Wasser and her team went out dragging for ticks in the South the way they do in the North, and in the same season. By high summer, Lyme season in the North, *Ixodes scapularis* nymphs in the South are never lying in wait on blades of tall grass, where they'd be in New Jersey or Connecticut. They're deep in the leaf duff and leaf litter, hitching rides on lizards and hiding from the heat.

Yet even if Yale's research design was flawed, that may be beside the point. Nymphs buried so deep they are inaccessible by dragging are also unlikely to bite humans and cause disease. But there is another tick in the South with implications for human disease — the lone star, named for the big white splash atop the female. It bites people. It exists in multitudes. And it is on the back of this fierce, ubiquitous, and rapidly spreading tick that much of the mystery of Southern Lyme-like illness rests.

CHAPTER 4

Show Me From Missouri

A country doctor from Missouri starts to question the Northern line — and brings James T. Oliver along for the ride.

Edwin Masters, a country doctor from Cape Giradeau, Mo., had no reason to doubt the conventional wisdom that the South was Lyme-free until 1988, when he was asked to give a talk on Lyme disease to a group of foresters. An active forester himself, Masters flung himself into the topic, spending a year collecting pictures of ticks and rashes in preparation for his talk. Suddenly aware of Lyme disease, he began to see signs of it in his patients. He saw erythema migrans rashes on their skin; he saw swollen joints; he listened to stories of fatigue, confusion, even paralysis. Masters began to suspect that some of the farmers, fishermen and hunters in his practice — many inexplicably suffering from extreme pain, immobility, inability to concentrate, meningitis (inflammation of the lining around the spinal cord and brain) and carditis (inflammation of the heart tissues) — could actually have Lyme disease. In fact, nothing in the array of symptoms he witnessed in his own practice, right down to the rash, distinguished his patients from Northeastern cases of untreated Lyme disease.

Masters began to take samples from his patients: blood and sometimes biopsies of skin from their rashes. He sent them to

a lab, and many came back positive on the then-current Lyme test, known as an ELISA, which looks for antibodies against the *Borrelia burgdorferi* pathogen in the blood. By 1990, he'd collected evidence from around 125 patients, reporting one and then the next to the Missouri Department of Health. He got no response.

Around the same time, the Georgia State Department of Health began receiving reports of Lyme disease from local doctors, too — four in 1987 and 715 total by 1989. That year, before the CDC stopped recording Lyme in the South, Georgia was ranked fourth nationwide for prevalence of Lyme.

Oliver was intrigued. After Ed Masters contacted him for help in 1993, he sent his postdoctoral student Tom Kollars to investigate. Kollars trapped animals at eight different sites, including a farm where two of Masters' patients had developed bull's-eye-shaped erythema migrans rashes along with arthritis, muscle aches and other Lyme-like symptoms after being bitten — almost certainly by lone star ticks. After trapping wild cottontail rabbits on that farm, he isolated from their blood what would prove to be five genetically distinct strains of Lyme *Borrelia*, including *Borrelia andersonii*, the spirochete later found in Kerry Clark's blood.

But as Oliver well understood, none of this proved that Masters' patients had Lyme disease. Oliver and his team could not find any evidence of *Borrelia* in either Missouri lone star ticks or Masters' human patients themselves. So they could not square the circle and prove that the lone star tick that bit Masters' patients actually transmitted a Lyme-like illness or, indeed, any spirochetal infection at all.

This frustrated Masters. "Jim," he said to Oliver, "you ought to

look at this differently. Epidemiologically, it makes sense."

It did make sense: The lone star ticks were there on the Missouri farm; the ticks had bitten patients living at the farm and suffering from Lyme-like illness; rabbits on the farm showed evidence of *Borrelia*. But where was the pathogen in the lone star tick? And where was the pathogen in the human patients themselves?

Indeed, like anyone else trying to prove a pathogen causes a disease, the Southern researchers had to fly on more than common sense — they had to connect all the dots. Often that can mean following the requirements for such proof established in 1890, at the dawn of the microbial age, by the great German microbiologist Robert Koch.

Applied to tick-borne disease, Koch's postulate stipulates that:

- The pathogen must be present in every single case of the disease.
- The pathogen must be isolated from an infected animal and then grown and sustained in culture.
- The pathogen must be injected back into an uninfected animal and shown to cause the disease.
- The pathogen must be isolated from the newly infected animal and grown in culture again.

Over the years, Oliver and many others tried dozens of different culture media, but no one could grow anything distinctive, either from patient samples or from lone star ticks. Either there was just too much contamination — too many other kinds of bacteria in the tick that crowded out the *Borrelia* spirochetes the researchers were trying to grow, or the elusive spirochetes were just too

fastidious; they had fussy culture requirements scientists didn't, and still don't, understand. That's not unprecedented: Scientists still can't culture the related spirochete that causes syphilis. (They test for that spirochete by looking for antibodies.) But it's maddening when you're trying to solve a riddle as difficult as the mystery of Southern Lyme. Nothing worked with lone star ticks until 2004.

First, in 2001, an entirely unknown *Borrelia* turned up in skin taken from the bite area of a patient with a lone star tick still attached. There was an erythema migrans rash around that bite. To test for a pathogen in the skin biopsy, researchers used PCR to analyze the spirochete's DNA. Instead of resembling Lyme spirochetes, the microbe seemed closer to so-called "relapsing fever" spirochetes, which cause a relapsing-remitting flulike disease. Even if it didn't prove that lone star ticks transmitted Lyme, scientists hoped that with the new species, named *Borrelia lonestari,* at hand, at least the elusive STARI agent had at last been found.

But culturing *B. lonestari* was also extremely difficult. Then in 2004, a graduate student named Andrea Varela-Stokes from the University of Georgia managed to grow it in embryonic tick cells — an expensive, difficult and time-consuming business. With large numbers of the spirochete in hand, researchers could finally inject it into mammals to see whether it caused a disease. But Varela-Stokes's cultured *B. lonestari* caused neither rashes nor any other symptoms in mice; no evidence of spirochetal infection could be found in the blood. Everyone, including the Missouri doctor Masters, reluctantly agreed that *B. lonestari* probably

wasn't the cause of Lyme-like illness in the South, or of any other human disease. The mystery of the lone star pathogen remained.

CHAPTER 5

Embracing the Complexity

Unraveling the twisted maze of myriad species and their relationships is key to understanding the nature of Southern disease.

The argument that there could be no Lyme-like disease in the South because the ticks and spirochetes there were different than those known to cause Lyme in the Northeast began to sound naïve to Clark, whose studies revealed a lush complexity everywhere he looked. In 1994 — while Masters was insisting his patients had Lyme disease and Oliver and the CDC were trying to isolate an infectious agent from lone star ticks — Clark was a graduate student in entomology at the University of South Carolina School of Public Health, doing a stint at the Wedge Plantation, a grand old estate turned research station in South Carolina. "It was the coolest place," he recalls. Old outbuildings and the barns and stables themselves were converted into a series of laboratories. Clark lived in half of The Cottage, a small house converted into two apartments; another graduate student occupied the other half. Sometimes, for weeks on end, they were the only people there.

Clark became an expert in trapping small mammals and culling their attendant ticks. Setting and retrieving traps was pleasant enough in the winter but hellish in late spring and summer, when

the yellow flies, biting gnats and saltmarsh mosquitoes invaded in clouds. There were two major hatching episodes for the pestilential flies: in May, and then again in July, when Clark tried to avoid the densely wooded areas where the flies were thickest. Unfortunately, that's where the ticks were, too.

Eventually, Clark learned to watch the phases of the moon. If you want to trap animals, the best times are during the new moon, when the nights are darkest, or in the one or two hours before the moon rises during the full moon. "The animals get out and get their business done in the dark," says Clark. "When the moon comes up, there's very little movement and it's hard to find anything." On a good day, he could lay 100 traps and secure five animals. He then carefully picked off the ticks, noting their species.

Telling the tiny ticks apart, even under the microscope, called for a lot of skill and experience. It was especially difficult when the tick was engorged and so swollen that its little features were all but absorbed: "Sometimes you'd have to clear them with a heated lactic acid solution to rid them of their last blood meal. You could tell that way," Clark says.

He didn't always stay on the Wedge Plantation. His graduate research took him to Mississippi and northern Florida as well, to try to understand the ecology of host and tick ranges and the degree of human disease risk in the South. What Clark came to believe, through his traveling and trapping, is that generalities like those put forth by Spielman and some other Northern researchers would never suffice. Different habitats produce different mammals, birds and lizards to host the tick, and thus, different

species and densities of ticks as well.

Clark studied this diversity from the ground. In northern Mississippi, where stands of white oak and hickory produce big crops of nuts, he had a far higher success rate trapping mammals than he did at the Wedge Plantation, where there was virtually no ground cover and the earth was pitted with little holes that served as burrows to hide the white-footed mice, one of the principal hosts for ticks. In Florida, he pulled *Ixodes scapularis* ticks off skink and glass-tailed lizards that live there in abundance; from wild mice and rats, he retrieved alternate *Ixodes* species, including *I. affinis* and *I. minor* — species not known to bite humans but capable of sustaining pathogens in the hothouse ecosystem of the South.

While Clark's specimens form a significant part of Oliver's famous tick library, the older scientist has carried out his own tick-gathering expeditions as well. He and his students, four or five of them at a time, drag for ticks over long weekends, going out once a month, trapping animals and shooting birds, all in the service of collecting whatever ticks they can find: *Ixodes scapularis, Amblyomma americanum* and others as well. The *Ixodes scapularis* nymphs gave them particular trouble.

"On St. Catherine's Island [off the Georgia Coast] we dragged an area that looked potentially prosperous, but we couldn't get anything," says Oliver. So they invented a new method of dragging that took *scapularis*' Southern reclusiveness into account: One student kicked and dragged his feet through the leaf litter, while another followed behind to collect ticks disturbed in the process. They found many adults, but few nymphs, which were buried

even farther down.

Validating the notion that *scapularis* wasn't much of a risk in the South, Oliver found it was hard to even keep these "weakly" ticks alive where humans roamed. "They can't stand a dehydrated environment," says Oliver. "When we brought them out of the field, no matter how tired we were, if we didn't put them in a humidified glass container very soon, we'd find them dead on the counter." The lone star ticks were a different matter. "You'd leave one on the counter, the next day it would be climbing the walls."

The more they uncovered, the more Clark and Oliver found that the explosive diversity of the South belied the simple narrative coming from the states up north. In the South, the sheer number of animal hosts, the diversity of ticks, and the ever-expanding number of *Borrelia* strains and genospecies those ticks contained made the situation far more tangled and knotty. In the South, says Oliver, there are simply more *Borrelia* that might cause Lyme or a Lyme-like disease. There are more hosts, including reptiles and birds. And the feeding behavior of ticks is more complex.

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It is July 2012, in the midst of the worst heat wave of the season, when Clark and I visit Oliver and his research partner, Natasha Rudenko, at their Statesboro lab. A ruddy, broad-faced, freely smiling woman, the Ukrainian-born Rudenko is headquartered at the Biology Centre in the Czech Republic. But she now spends several months each year working with Oliver to gain insight into the complex Southern ecology and its role in disease.

In order to help solve the mystery of Southern Lyme, Rudenko

and Oliver are working to classify all the *Borrelia* in the tick library they have amassed with Clark. So far, they say, they've found 300 Southern genetic strains of *Borrelia*, 57 of them so similar to the pathogen in the North that they have been classifiable as *Borrelia burgdorferi* sensu stricto. The scientists have also found, in a bird, DNA from the European genospecies *Borrelia garinii* (another member of the *Borrelia burgdorferi* sensu lato complex, and the strain that causes neurological Lyme disease in Europe); that makes Rudenko and Oliver wonder whether Lyme disease could spread across continents through bird migration. And they've found *Borrelia andersonii,* the rabbit-associated strain that Clark identified in his own blood.

Although *B. burgdorferi* sensu stricto is the most common strain Oliver and Rudenko have detected in nature, they've also found plenty of others. Some have been identified through PCR testing that targets tiny pieces of the spirochete's DNA. But if they want to prove the microbe's role in infection, taking testing to the next step — culture — is important as well. Rudenko, a master at culturing spirochetes, has also managed to cultivate many distinct strains and genospecies by growing them in glass dishes on a culture medium in the lab. Part of her success comes from her use of a particular medium developed in Slovenia that seems especially friendly to Southern strains.

Rudenko and Oliver send DNA from the spirochetes in those cultures for sequencing at a lab at the University of Washington in Seattle. Once they receive the sequence for any given segment of the spirochete back from Seattle, they compare it to other, known microbial species. If the new sequences fall too far from earlier

strains, the spirochete is classified as a new genospecies.

Using Rudenko's unique culture medium, the two have identified two new genospecies of the *B. burgdorferi* sensu lato complex: *Borrelia carolinensis* and *Borrelia americana,* detailed in a series of publications from 2009 through 2011. *Americana*, it appears, may cause human disease. Clark says he's found PCR evidence of it in samples he took from several human patients with severe Lyme-like illness and suspects it has worldwide distribution. He has found it in blood samples sent to him from patients in London, and Rudenko has found it in blood samples from a patient in the Czech Republic. But culturing and far more stringent evidence is required before its role in disease is proven, for sure.

The new spirochetes amplify the complexity of the ecological Southern cycles that Oliver and Rudenko have identified. In their publications, the two have described cycles involving lizards, birds (including the rufous-sided towhee, robins and cardinals), and small mammals (cotton mice, cotton rats, chipmunks, squirrels, woodrats, rice rats, rabbits and raccoons).

The ticks, too, are diverse: In addition to the ubiquitous *Amblyomma americanum,* the lone star tick, the most aggressive human-biting tick in the South, there is the familiar *I. scapularis*, and three related species *Ixodes dentatus, Ixodes affinis* and *Ixodes minor*. These last three species are commonly found on birds, rabbits or rodents; they rarely if ever bite people, but they keep their spirochetes cycling through a myriad other mammals, lizards and birds — and ultimately, other ticks as well.

None of this complexity proves the reality of Southern Lyme

disease in human patients, but it certainly underscores the point that any comparison to the Lyme situation in the North is moot. All of these complex ecological cycles, along with the different *Borrelia* spirochetes, the wide range of ticks and the spectrum of vertebrate hosts, mean that the neat picture in the North has, in the South, been blown apart into hundreds of fractured images.

Oliver views this complexity with a trace of sadness. If he had looked at even more *Borrelia* strains, more ticks, more hosts when he studied the patients in Missouri, it could have broadened his perspective and provided more answers, he says now. But with the help of Rudenko, he is making up for lost time.

Oliver believes the plethora of Southern *Borrelia* strains provides a clue to the spirochete's evolutionary history in North America. Usually, he observes, the areas where a pathogen shows the greatest genetic variability tend to be the places where it has existed longest, simply because it is in these areas that the germ has had the longest time to differentiate and evolve. He suggests that both *Borrelia* pathogens and the ticks that carry them are so much more diverse in the South because they have dwelled there so long, evolving together, finding new tricks to survive. It's possible that Lyme originated there and only later moved northward, up the coast.

CHAPTER 6

More Evidence Emerges

Kerry Clark's new DNA test could usher in a new age of research — but first others must replicate its success.

Borrelia spirochetes may be ancient inhabitants of the South, but Clark has come up with a new way to stalk them — by targeting tiny, elusive snippets of DNA in human blood. Using a new testing technique to capture these DNA fragments from several hundred Southern patients, he hopes he can prove that, both in lone star ticks and in sick people, *Borrelia* strains that cause human disease can be tracked down, analyzed and identified.

Clark's new test, if validated and confirmed by others, could represent a true advance over the standard PCR test for Lyme, which can easily fail to detect *Borrelia* infection. As Clark explains, a major problem with the PCR test, which searches for longer fragments of spirochetal DNA in the patient's blood, is that soon after infection, *Borrelia* pathogens settle into nerves, heart and collagen, the connective tissue found in muscles and joints. DNA that remains in the blood tends to break down quickly, especially in samples that have been cooled and shipped.

That being the case, it occurred to Clark that looking for even shorter bits of DNA might work better than seeking longer ones. Over the past several years, he has created primers, or sensitive strips of DNA, that target the short pieces he seeks in the blood.

Clark has created his primers to match bits from the gene that codes for part of the spirochete's flagella — tiny, whiplike structures protruding from the body of the bacterium that help propel it through the bloodstream. In particular, he has focused on targeting the gene coding for flagellin protein b, or flaB, which has proved to be quite distinct from one genospecies to the next. Each primer corresponds to a snatch of the flaB protein in a given genospecies, allowing Clark to see the range of spirochetes in his human blood samples and in lone star ticks.

The strategy has proved successful, yielding Clark far more "hits" than he had ever found using established *Borrelia* primers that bind to longer pieces of spirochete DNA.

Although Clark has not yet released all of his data, his latest article, published this June in the *International Journal of Medical Science*, documents distinct Lyme *Borrelia* in lone star ticks and in nine of 10 patients from Florida and Georgia. Among the finds: evidence of *B. andersonii* in three of the patients, *B. burgdorferi* sensu stricto (classic Lyme) in seven of them, and *B. americana* in two more.

Especially compelling are reports of two patients who managed to salvage the lone star ticks that bit them. Both the ticks and the patients had evidence of infection with *andersonii* and *burgdorferi*. A dozen control patients from the same areas without any symptoms showed no evidence of *Borrelia* DNA.

Clark's study represents the first published indication that the ultimate target of Southern Lyme research could be within reach: proof that *Amblyomma americanum*, the lone star tick, may actually transmit some form of Lyme *Borrelia*. "Our findings

suggest that some cases of STARI resembling Lyme borreliosis in the Southern U.S. may be attributable to previously undetected Lyme *Borrelia* strains, and thus represent cases of actual Lyme borreliosis rather than a separate disease entity or tick hypersensitivity reactions," his team wrote in the June publication.

CHAPTER 7

A Lyme Garden

Lyme-like disease has been documented in Mexico.
Texas researchers say they are seeing it, too.

If current research bears out, the South may not be a Lyme desert but a Lyme garden — an extensive one at that, with many and varied landscapes and ecosystems. A thousand miles away from the green vines and wet red clay of Statesboro, Ga., and the tranquil creeks outside Jacksonville, Fla., the town of College Station, Texas, lies baking in the sun. Neither the driest nor the hottest place in Texas, the campus looks like it has been dropped onto a savanna, a flat, grassy expanse broken by occasional clumps of short, spreading trees. Still, in this very different ecosystem, *Borrelia* strains also find a home.

Maria (known as Loles) Esteve-Gassent, a Spanish-born microbiologist and head of the Lyme lab in the School of Veterinary Science at Texas A&M, has been studying Lyme disease since 2004. What she's found in Texas using PCR to examine lone star ticks seems to corroborate the finds of Oliver and Clark: she has found a number of Lyme strains, including *Borrelia andersonii, Borrelia americana and* classic *Borrelia burgdorferi* in lone star ticks. Her research also reveals widespread *Borrelia burgdorferi* sensu stricto infection in Texas dogs, some of which show signs of arthritis and other health problems.

(Infections in dogs are "good sentinels for human infection," according to Paul Mead, a CDC Lyme epidemiologist.)

Like Clark, Esteve-Gassent has also found DNA evidence of *Borrelia burgdorferi*'s flaB gene in lone star ticks and is expanding her search in hopes of finding more *B. burgdorferi* genes in the lone stars — evidence that is essential if Clark's contention that the tick carries the spirochete is to be confirmed. Esteve-Gassent also reports she has found the Lyme spirochete in the lone star's close cousin, a tick called *Amblyomma cajennense*, which lives in abundance along the U.S.-Mexican border and in South America all the way down to Argentina.

On the day I visit Esteve-Gassent, a Mexican researcher named Guadalupe Gordillo-Perez is there as well. Gordillo-Perez has studied blood samples from people living across Mexico as part of a gigantic public health study sponsored by the Mexican government. Based on her analysis of 1,000 blood samples allotted to her as part of this study, Gordillo-Perez estimates that 1.1 percent of Mexican citizens are positive for different forms of *Borrelia burgdorferi* — and therefore, Lyme disease. In a recent study, she has found both *B. burgdorferi* sensu stricto and *Borrelia garinii*, known to cause Lyme disease in Europe, but thought not to exist in the Western Hemisphere (though Rudenko and Oliver have also identified g*arinii* genes in the Southern U.S.).

As a physician, Gordillo-Perez sees another hint that unusual forms of Lyme *Borrelia* may be lurking south of the border: strange skin lesions that look like skin cancers in patients diagnosed with Lyme. They resemble the lesions found widely among those with European forms of the disease. Gordillo-

Perez says she has detected *Borrelia* infection in both types of ticks, *Ixodes scapularis* and *Amblyomma cajennense*. Along with multiple ticks, she contends, many genospecies of *Borrelia* cause human disease.

The fact of the matter is that Gordillo-Perez and her colleagues in Mexico accept more fuzz around the edges than U.S. authorities, who restrict the definition of Lyme disease to the circumstances that mark the disease in the American Northeast, Midwest or West Coast: *Borrelia burgdorferi* sensu stricto spread by *Ixodes scapularis* nymphs. Like Clark and Oliver, Esteve-Gassent and Gordillo-Perez are more at home with the complexity of the disease terrain — the convoluted cycles among the rabbits, birds and lizards that break into people via the lone star tick or *scapularis* adults; the unusual strains, including *americana* and *andersonii*, and perhaps *garinii*; and the many flavors of *B. burgdorferi* that make the South such a heated mess.

"We aren't American; we haven't been indoctrinated with the same attitudes," says Esteve-Gassent. In Europe, she points out, there are at least five genospecies — members of the *Borrelia burgdorferi* sensu lato complex — known to cause human disease. "Why do Americans insist that there's only one kind of Lyme *Borrelia* that causes disease in the U.S., while there are so many in Europe?" she asks in exasperation. "It's a big country!"

CHAPTER 8

Framing the Debate

Putting evidence in context

To date, neither Clark, nor Oliver and Rudenko, nor Esteve-Gassent has succeeded in derailing the prevailing paradigm of the South as a Lyme-free zone. For one thing, Clark and Esteve-Gassent have not yet published all their findings. And Clark's recent study of positive PCR results for 10 patient samples will remain controversial until other researchers replicate the work, using his primers on blood samples collected from patients of their own. "Nothing is worth anything in science until it can be replicated," he says. Even then, the steps outlined by Koch — or a molecular substitute — would need to be invoked to prove infectious disease.

Since Clark's new work appears to open a door that has been shut for decades, I sought meaningful critique from some of the top Lyme disease experts at the CDC and elsewhere. But no one I approached was willing to comment on the paper outside the venue of the peer review. However, one of the foremost experts on molecular methods, Richard H. Ebright, Board of Governors Professor of Chemistry and Chemical Biology at Rutgers University and laboratory director at the Waksman Institute of Microbiology, comments, "The paper appears generally sound." He notes one possible criticism: In principle, he says, a shorter

primer "could result in cross-reaction with nucleic acids other than the target nucleic acids and therefore could have resulted in false positives." However, Ebright adds, the specificity of the sequences Clark reported appears to have ruled that out. Indeed, Clark's findings in lone star ticks and human patients do in fact fall into precise *Borrelia* genospecies: *B. burgdorferi, B. andersonii, B. americana* and *B. carolinensis.* False positives or contamination would be unlikely to elicit such a specific spread.

The biggest weakness in the case for Southern Lyme transmitted by the lone star is that, despite Clark's DNA "hits," no one has yet grown a colony of any kind of disease-causing *Borrelia* spirochetes in a petri dish. Since STARI is by definition associated with the bite of a lone star tick, not being able to culture a disease agent from lone stars despite innumerable attempts suggests to many serious scientists that there is nothing to find. "The evidence so far is that we can't find any pathogen — and we've looked," says New York Medical College's Wormser. "So far, every study has come up empty."

The CDC's Barbara Johnson agrees: "The consistent inability to grow the bacteria that cause Lyme disease from the skin or blood of STARI patients is one piece of evidence that indicates that STARI is not Lyme disease," she says in an email. "People also have tried, without success, to isolate a microorganism from STARI patients in other ways, such as by culturing specimens in the presence of tick cells. These results mean that we do not know what causes STARI."

Johnson, who has been conducting a study (still unpublished) on STARI, feels that it is "not likely" to be caused by a spirochete,

and indeed, may not be an infection at all. Based on the evidence so far, including from her own research, she suspects positive Lyme antibody test results for STARI patients are either false positives, caused by exposure to other, non-Lyme-causing spirochetes, or are unlucky souvenirs of travel to an area where Lyme exists. "A person who grew up in the Northeast, but now resides in the Southeast, could have had a Lyme disease infection that resolved and also have STARI now," she says. For this reason, the CDC advises both Southern state health departments and physicians considering a Lyme diagnosis to discount bull's-eye rashes in Southern patients unless they have travelled to the North, the Midwest, or another known Lyme zone.

Herman-Giddens, the North Carolina public health researcher and Lyme patient advocate, sees Johnson's logic as circular. Since patients without a travel history to endemic areas are considered necessarily negative for Lyme and regarded as either false positives or as STARI cases, they aren't part of any official count. That keeps the Southern states' case numbers so low that they appear to be virtually Lyme-free. In other words, Southern states aren't considered endemic for Lyme because the disease isn't reported there; and Lyme disease isn't reported in the South because Southern states aren't considered endemic.

This pattern is changing in Herman-Giddens home state of North Carolina, where three counties are now considered endemic by the CDC. But elsewhere in the South, physicians uncertain about the possibility of infection and worried about side effects of antibiotics are reluctant to treat patients with bull's-eye rashes.

Duke University infectious diseases pediatrician Paul Lantos, who had a substantial background in Northern Lyme disease before he moved South, breaks down the whole issue like this: Because lone star ticks cannot transmit Lyme, STARI cannot be Lyme disease. What is STARI? "That is not known, Lantos says, "but if it is not Lyme disease, then what relevance do Lyme treatment options have for STARI? None. If STARI, in fact, ought to be treated similarly to Lyme, then it is a coincidence. Just as, coincidentally, you can treat pneumonia or urinary tract infections with doxycycline, ceftriaxone or amoxicillin, which are the antibiotics for Lyme, depending on circumstances."

Lantos argues that antibiotics are sometimes used to treat STARI just because Missouri doctor Masters, an influential figure, insisted on it. "That's the weakest possible reason," he says. He thinks the risk of side effects from several weeks of antibiotics outweigh the risk of STARI. In one case, he and a child's mother, herself a physician, jointly decided to forgo antibiotics for a 5-year-old girl who sported an erythema migrans rash — but no accompanying Lyme-like symptoms — acquired in North Carolina. Several months later, the child remains well.

Put in this simple fashion, the issues seem clear: No one has ever been able to prove definitively that lone star ticks transmit *B. burgdorferi* sensu stricto or any other *Borrelia* strains; and some cases of Lyme-like illness don't seem to need treatment.

Yet nothing is simple here. For instance, although some STARI cases prove not to advance beyond a bull's-eye rash, that does not mean that all cases are noninvasive. As Marques, the NIH researcher who studied STARI and abandoned her project

without publication, acknowledged in an email, researchers still don't know "the full spectrum of the disease."

In fact, "rash-only" Lyme disease is common in the Northeast as well. According to infectious diseases physician and Lyme expert Benjamin Luft of the State University of New York at Stony Brook, only certain strains of *Borrelia burgdorferi* in the North cause invasive Lyme in which the spirochete leaves the skin and moves into the body to cause systemic disease. The rest of the strains cause a simple erythema migrans rash that will never spread beyond the skin. To date, physicians have no readily available way of telling which is which, but in the North it is accepted clinical practice to treat all patients with the rash as if they will develop invasive disease.

And then there is the argument, put forth by the CDC's Johnson as well as by Lantos, that lone star ticks contain a "Borreliacidal" element. She and others, including Wormser, the expert from New York, cite several experiments showing that most — though not all — Lyme Borrelia germs are killed after being bathed for 48 hours in lone star tick saliva (fewer than 13 percent survive, according to one study).

But it is worth noting that these laboratory strains were all once carried by *Ixodes scapularis*, the Lyme tick vector of the Northeast. As the Czech Republic-based Rudenko points out, there is no reason to believe that strains specifically adapted to *lone star* ticks would be killed by *lone star* saliva. Any strains adapted to lone star ticks will, by definition and of necessity, have evolved to withstand the elements that kill germs normally vectored by *Ixodes scapularis,* a different tick species. It's an

intense adaptive struggle among ticks, hosts and spirochetes, and that struggle can and will move the biology of germs vectored by different ticks in very different directions.

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In 2007, two years before he died of diabetes at age 63, Masters spoke at a conference on Lyme in the South held at the Duke University School of Medicine. He acknowledged that no one had been able to culture any infectious agent that might have been the cause of STARI in his patients. He listed every medium that had been tried; the list seemed endless. In the video of his talk, Masters looked weary and discouraged. But he never abandoned his fundamental belief that his patients were sick from either Lyme or a Lyme-like illness that demanded antibiotic treatment. "Absence of proof is not proof of absence," Masters insisted, to the end of his life.

As they wait for scientific validation, Schmitz, the Georgia support group leader, and Herman-Giddens, the community health liaison, field call after call from desperate patients whom almost no one believes — not their doctors, not the CDC. That disbelief compounds the patients' isolation and misery, not to mention the difficulty of finding doctors who will care for them. At the patient group I attended, one young woman insisted she'd rather have cancer: "At least then, I'd be recognized as having a real disease, with a real treatment," she said.

Meanwhile, nothing has changed for the Fayette County man, the presumed ALS patient treated for Lyme disease after Clark

tested his blood. He sits in his wheelchair, taking his antibiotics, struggling to move, struggling to stay alive.

The resolution can only come from more science. If Rudenko confirms Clark's PCR tests, perhaps using additional gene targets. If she or Clark manage to grow *Borrelia* cultures out of human and lone star tick samples, then even the fiercest skeptics will have to recognize that what Masters said is true: that alternate Lyme *Borrelia* strains represent a significant threat to human health and that Lyme-like illness deserves Lyme-like treatment.

Until then, patients who get a diagnosis of STARI will have little recourse to that treatment, and those with "true Lyme" in the South — vectored by the bite of an adult *scapularis* tick — will continue to be dismissed as the bitter controversy over Southern Lyme rages on.

Appendices

SPIROCHETE NATION

From Connecticut to California, Alternate Microbes Might Be at the Root of Contested Lyme-like Disease

Appendix A

CALIFORNIA CHETES

By Laith Agha

On a chaparral-covered mountainside above Hopland, a pit-stop town of 750 on the northern outskirts of California's wine country, Robert Lane drags a white flannel cloth over a patch of fallen oak leaves. Wearing a blue cap and spectacles, Lane is searching for nymphal Western blacklegged ticks, the prime vector for spreading *Borrelia* species. "This is the key habitat," Lane says. "It's well-shaded, you've got an abundance of trees, and the leaves are deciduous." After the first drag, two poppy seed-size nymphs appear on the flag.

A hundred miles south, in his Berkeley lab, Lane and his staff will examine these parasites to see whether they are carrying *Borrelia burgdorferi*, the agent of Lyme disease, or any one of several other spirochetes that might be making people sick. Lane says the newly found *Borrelia* — along with others he might yet encounter — could explain why some people with Lyme-like illness never test positive for the disease.

Lane, a medical entomologist at the University of California

in Berkeley, has spent nearly 40 years studying ticks and the diseases they spread in the Western U.S. While Lyme disease was originally considered an East Coast problem (and still is in some medical circles), Lane and his team established in 1985 that it can be transmitted to humans by ticks in the Western United States, where ecosystems are far more ecologically diverse. From the tick (*Ixodes scapularis* on the East Coast and *Ixodes pacificus* out west) to the kind of deer to the rodent species involved, everything is different out west but the spirochete itself.

By 1992, Lane and Humboldt State University ecologist Richard Brown suspected they had isolated alternate spirochetes as well, but they weren't sure. "We thought it might be a highly variable single species," Lane says. Then in 1998, French molecular biologist Danielle Postic confirmed their hunch. They, indeed, had found a different species, *Borrelia bissettii*, which is known to cause Lyme disease in Central and Southern Europe. (Two other *Borrelia* species — *B. azelii and B. garinii* — are more common causes of Lyme disease in Europe.)

Since then, Lane and his colleagues have continued to discover more *Borrelia* species in California's coastal ranges. The next spirochete they isolated, *Borrelia miyamotoi*, also had a track record on another continent. Found by Lane's research team in 2005, *B. miyamotoi* was originally discovered a decade earlier in Japan, and then turned up in Connecticut in 2001. (Read Appendix B for *B. miyamotoi* news.) In 2007, Lane and colleagues discovered *B. californiensis* and "*Borrelia* genomospecies II" — a placeholder name until the species is fully described. In 2010, Natasha Rudenko, the entomologist from the Czech Republic,

isolated *Borrelia americana*, yet another spirochete that must be investigated as a cause of Lyme-like disease in the West from a Northern California tick.

And that's not all. Lane has come across several other possible *Borrelia* species, now under evaluation in his lab. "We have probably three or four genospecies that we've discovered in the last several years that we're hoping to introduce as new to science, or at least new to North America," Lane says.

Does the bounty of new species explain some of the contested patients who claim they have Lyme disease, but do not pass the approved tests? One hint for Lane comes from *B. bissettii*, which has shown up in blood tests of three Mendocino County residents. While Lyme-like symptoms were not diagnosed for any of these three, Lane says, "it is evidence that one of the other 17 or so named *Borrelia burgdorferi* genospecies can infect people in North America.

"Every time we discover a new spirochete," says Lane, "we ask the question, 'Does it infect people?'" If so, does it cause Lyme-like symptoms, and what are the implications for diagnosing and treating people with these other forms of disease?

Appendix B

COUSIN IN CONNECTICUT: BORRELIA MIYAMOTOI, THE RELAPSING-REMITTING LYME-LIKE DISEASE

By Breanna Draxler

Peter Krause has seen plenty of patients with Lyme disease. He also has seen his fair share of cases where classic symptoms suggest Lyme, but tests for the disease-causing bacterium, *Borrelia burgdorferi*, come back negative. The Yale tick-borne diseases expert says that in some cases, a related and recently discovered disease may be to blame.

Borrelia miyamotoi elicits symptoms similar to its better-known bacterial cousin with two trademark exceptions: Patients do not get a bull's-eye rash, and they come down with relapsing fever. Krause discovered the first cases of the disease in a study of Russian patients in 2011.

"That was the first report that people became aware of this disease in humans," Krause says. Researchers suspect that the disease — still too new to be named — exists "anywhere in the world that hard-bodied ticks are transmitting Lyme."

But tracking down a disease based on generic symptoms like fever and fatigue is tricky.

"The best way to do this is not to wait around for a case, but to test sera [blood] from people who live in an area where there is a

lot of tick-borne illness," Krause says. New England, where Krause has been studying tick-borne disease for 20 years, certainly fits the bill. "I have freezers full of serum," Krause says.

Krause thawed out about fifty of these blood samples from studies done in Rhode Island and Massachusetts. His analyses confirmed the presence of *B. miyamotoi* beyond Russia's borders: One percent of healthy patients and 3 percent of patients with Lyme-like symptoms tested positive for the antibody against the bacteria.

If you're positive on this, it is strongly suggestive of *miyamotoi* infection, Krause says. The results, published in the *New England Journal of Medicine* in January 2013, give researchers a pinhole-size window into the behavior and prevalence of this previously unknown disease.

Whereas Lyme-causing bacteria usually invade fixed tissues like the skin, joints, nervous system and heart, *B. miyamotoi* is found predominantly in the bloodstream. The immune system works to design an antibody specific to the bacterium's protective protein coat, and a fever develops. But just as the antibodies start gaining on the bacteria and the person's health improves, the spirochetes pull a costume change.

"They have a cassette of genes that can design a whole new suit of armor," Krause says. Clad in this unrecognizable new coat, the antibodies no longer work. The bacteria quickly multiply, and the fever returns as the immune system scrambles to come up with a corresponding antibody. Krause says that in other types of relapsing fever, patients can relapse as many as 10 times over the course of a year before the combined remnants of each of the

antibodies is enough to finally overcome the evolving infection.

The cyclic nature of the disease could make it frustrating for undiagnosed patients who go untreated. Despite having found a partial answer, Krause says there are an enormous number of questions yet to be answered about the newfound *B. miyamotoi* disease.

One characteristic, Krause has learned, gives *B. miyamotoi* a real leg up: While ticks cannot pass Lyme spirochetes to tick offspring, *B. miyamotoi* is transmitted from female to larvae through eggs. Thus, the relapsing fever can be spread by the bite of larval ticks as well as the customary nymphs.

Krause may be one of the world's foremost experts on *B. miyamotoi* disease, but diagnosis remains a major hurdle for him and the rest of the health care community. Existing methods of looking at blood smears under a microscope fall short, so a number of labs are developing tests that Krause thinks will be widely available soon.

"This will take a long, long time to fully understand, but a start has been made," Krause says.

Appendix C

GUIDE TO LYME REPORTAGE

By Wendy Orent

The most serious diseases have been designated "reportable" by the CDC, which tracks cases per year of infections such as plague, leprosy, HIV and Lyme. The agency has set up fairly stringent criteria to determine what constitutes a real case.

If you live in Connecticut and show up in a doctor's office with a spreading circular red rash and a history of tick exposure, you're a confirmed case of Lyme disease, reportable to the state.

If you have the rash but no evidence of tick bite or tick exposure — but still have signs of systemic Lyme disease, say, meningitis or arthritis — your blood must be tested for the case to be confirmed. Confirming the case requires passage of the CDC's two-tier testing: First, you need to test positive or equivocal on the ELISA, which detects antibodies against Lyme proteins. Then you need to test "CDC positive" on a Western blot, which shows the specific *amounts* of antibodies against 10 proteins the agency deems most dominant as the spirochetal infection spreads. If your tests match the requirements, you're a confirmed case.

Any patient whose case does not exactly match the CDC's protocols is considered a *suspect,* or a *probable,* case and not counted. In August 2013, the CDC estimated that 10 times the number of reported cases actually have Lyme — 300,000 cases per year.

All this holds true only in "endemic" areas: the Northeast, Upper Midwest, California and parts of Oregon. And it refers only to what the experts refer to as true Lyme caused by *Borrelia burgdorferi,* not any suspect or confirmed Lyme-like disease.

Erythema migrans rashes in the Southern U.S., however, indicate STARI. Positive Lyme lab tests are only false positives, and you still have STARI. And STARI is not reportable.

Wendy Orent is an anthropologist based in Atlanta (she holds a Ph.D. from the University of Michigan) who writes about nasty diseases, whether they're natural outbreaks or biological weapons threats. She wrote a book called *Plague: The Mysterious Past and Terrifying Future of the World's Most Dangerous Disease* (Free Press, 2004) which looked at the natural and unnatural history of this unpleasant infection. A new e-book edition of *Plague* will be released by Simon and Schuster in fall 2013.

Orent also co-authored a memoir with Igor V. Domaradskij, the co-designer of the entire Soviet bioweapons program (*Biowarrior: Inside the Soviet/ Russian Biological War Machine*, Prometheus Books, 2003). Orent's favorite preoccupation is the evolution of infectious disease and understanding how animal diseases evolve to become human threats. Her articles have appeared in *Discover*, the *Los Angeles Times*, *The Washington Post*, the *New Republic*, *Proto* and *Natural History Magazine*, among other publications. She taught science journalism for three years at Emory University.

Orent's e-single on Lyme disease in the South for *Discover* has been in preparation for over three years.

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