



TAKING AIM AT LYME DISEASE

Efforts are under way to develop a vaccine and better diagnostics for **OUTDOOR THREAT**

BARBECUES, CAMPING, hiking, and biking in the woods—the many outdoor rituals and activities of summer are in full swing. Memorial Day picnics soon give way to Fourth of July fireworks and August heat.

But by autumn, tens of thousands of people in North America and Europe—especially children, but many adults, too—will have had their summer idyll shattered by contracting Lyme disease, a serious bacterial spirochete infection spread to humans by the common deer tick.

Thousands more people will not even realize that they have been infected with the Lyme disease organism, *Borrelia burgdorferi*. Infection occurs when a tick carrying *B. burgdorferi* finds a human host, begins to feed, and transmits the bacteria.

Over the 30-year period of 1982 to 2012, about 400,000 cases of Lyme disease in the U.S. were reported to the Centers for Disease Control & Prevention. However, the disease is likely to be underreported. Some people estimate there could be as many as 600,000 cases per year in the U.S., growing at about 6% each year.

Some 70% of infected individuals become aware they've contracted Lyme disease after they develop a bull's-eye skin rash called erythema migrans. Other symptoms include joint, heart, and central nervous system problems. Symptoms may take months or years to erupt, and the damage to health may become chronic and permanent.

There is an urgent need to prevent infection, through a vaccine, and to improve diagnosis of the disease. C&EN examines this in two stories. First, we report on the struggles accompanying vaccine development, which are social and scientific.

Without a vaccine, the emphasis is on new diagnostic tests, the topic of the second story. Current tests only detect antibodies formed in response to the infection, which may take several weeks for a person to develop. Negative results are common in the early stages

of the disease when antibiotics are most effective. But new ways to measure infection quickly are showing signs of progress. ■

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A SHOT AGAINST LYME DISEASE

As Lyme disease cases spread, a **NEW VACCINE** is being developed amid worries about a hostile reception

WILLIAM G. SCHULZ, C&EN WASHINGTON

LYME DISEASE HAS BECOME so widespread that it is today's number one vector-borne illness in the U.S., according to the Centers for Disease Control & Prevention (CDC). Over the past 10 to 15 years, it has become of increasing concern to state and federal public health officials as infection hot spots have radiated from a few northeastern states across to the Midwest and all the way to the Pacific coast. Every state in the continental U.S. has reported at least one case of Lyme.

To combat Lyme disease, public health experts say, there is urgent need for a safe and effective vaccine. This is especially true, they say, for high-risk groups—young children and adults who work or spend a great deal of time outdoors.

"The fact that there is no vaccine for an infection causing some 20,000 annual cases of Lyme is an egregious failure of public health," says Stanley A. Plotkin, an emeritus professor of medicine at the University of Pennsylvania and a consultant on vaccines to the drug industry.

But hope for a new vaccine may be on the horizon. An investigational Lyme disease vaccine being developed by Baxter International has shown promise in clinical trials in Europe. Baxter published clinical trial data on safety and immunogenicity for the new compound in May (*Lancet Infect. Dis.* 2013, DOI: 10.1016/S1473-3099(13)70110-5). Yet work is moving slowly, and vaccine specialists say the caution is driven by a backlash from antivaccine groups that sank earlier Lyme treatments.

The Baxter vaccine prompts an antibody response by making use of a surface protein from the organism that causes Lyme disease, *Borrelia burgdorferi*. The protein is called bacterial outer-surface protein A (OspA), but Baxter spliced the

vaccine version together from variations found in the four *B. burgdorferi* species implicated in human disease. The protein was genetically altered to delete a fragment of the protein, the OspA1 epitope, that is implicated in the type of arthritic condition that can occur in Lyme disease. Researchers replace it with the related OspA2 epitope to eliminate the possibility of any cross-reactivity with human proteins that might cause disease symptoms.

As is the case with many human vac-

that the vaccine—after three primary inoculations and one booster shot—will "induce substantial antibody titers against all targeted species of *Borrelia*, the causative agent of Lyme disease," the May study says. "The novel multivalent OspA vaccine could be an effective intervention for prevention of Lyme borreliosis [Lyme disease] in Europe, and the USA and possibly worldwide."

Baxter says the next step is an expanded safety and immunogenicity study, but plans for these further clinical studies "have not yet been finalized."

THE HOPE for Baxter's vaccine, however, comes with a big dose of historical caution. In the late 1990s, two Lyme disease vaccines were approved in the U.S. by the Food & Drug Administration. One of those, ImuLyme, which was developed by Sanofi forerunner Pasteur Mérieux Connaught, never made it to market.

The other vaccine—Lymerix, developed by SmithKline Beecham, now GlaxoSmith-

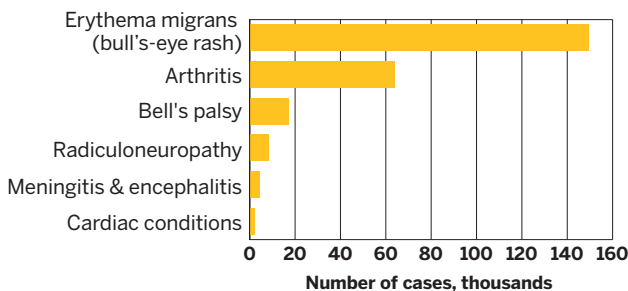
Kline—was put on the market in 1998 without much fanfare. The marketing approach for this product targeted potential patients at the expense of gaining buy-in from physicians, say experts in the infectious disease community who monitored or were involved in the Lymerix debut.

Much worse for GSK, the company was hammered with a false claim that Lymerix caused arthritis, a claim that gained traction in the echo chamber of some Lyme disease patient advocacy groups. The vaccine was

pulled from the market in 2002 because of declining sales and a number of class-action lawsuits that were filed on the basis of still more false claims, says Gregory A. Poland of the Vaccine Research Group at the Mayo Clinic and other Lyme disease specialists. One claim was that Lymerix itself could give people Lyme disease.

Today, GSK does not respond to inquiries about Lymerix. It is against this backdrop that Baxter's potential vaccine is being developed.

MULTIPLE SYMPTOMS People with Lyme disease can suffer from myriad health problems.



NOTE: Study of 213,515 patients; some presented with more than one symptom.
SOURCE: CDC

cines, Baxter's formulation contains an aluminum adjuvant that reduces side effects and boosts the human immune response to the OspA antigen. In safety and immunogenicity testing, Baxter compared vaccine versions with and without the adjuvant. The vaccine with the adjuvant performed better both in terms of the elicited immune response and the reduced incidence of side effects such as fatigue, headache, and injection site reaction.

The clinical studies to date demonstrate

"We have to explain why a vaccine is safe and effective and that it's not going to cause Lyme disease."

"We keep hearing about the Baxter vaccine, but there is no progress toward licensure," Poland says. Given the continuing rhetoric from the antivaccine camp, he asks, "Why would any company invest in a product with no demand and lots of nega-

because of patient compliance. So far, the Baxter Lyme vaccine candidate—as Lymerix before it—requires multiple doses. Also, they say, a profitable vaccine will need to be approved for use in both children and adults because children rep-

Lyme disease becomes critical, medical experts say. Most diagnoses of Lyme disease are made courtesy of the unmistakable bull's-eye rash around the tick bite that occurs in some 70% of infected patients.

For others, a constellation of symptoms including visible tick bites, flu-like illness, and arthralgia, move doctors toward a Lyme diagnosis. Laboratory tests confirm the presence of Lyme, but often after a lengthy time.

The disease can mean serious illness and weeks of treatment depending on how far the ailment has progressed. Early-stage treatment includes oral administration of antibiotics such as doxycycline, amoxicillin, or cefuroxime axetil. In later stages of the disease, treatment often includes intravenous administration of powerful compounds such as ceftriaxone or penicillin over a period of several weeks. There is no evidence for a chronic form of infection, but many experts say there is some evidence for chronic conditions such as pain and fatigue, and cardiac and neurologic/psychiatric problems that might result from an infection that is not treated early.

There are some people, medical experts say, who have probably suffered more than one bout of Lyme disease, particularly those who live in high-risk areas of the upper Midwest and

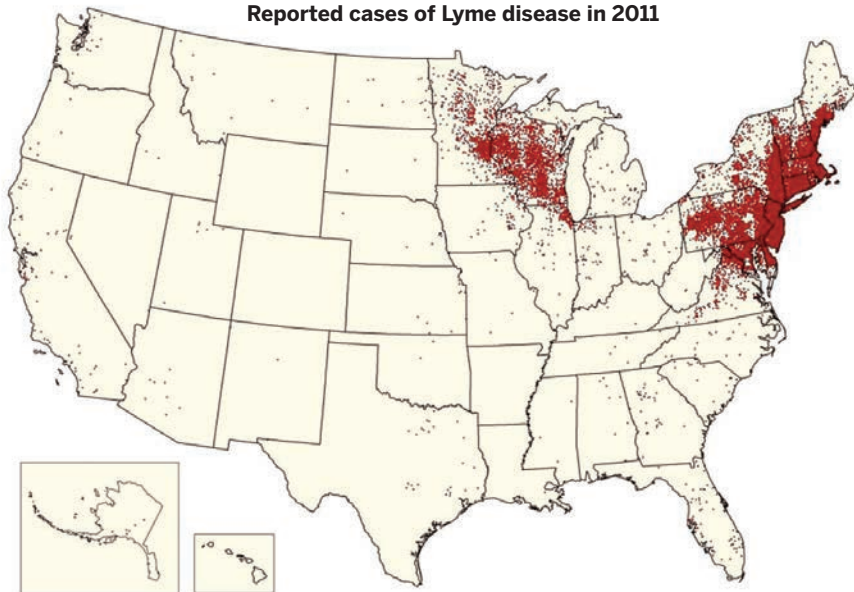
northeastern states. There, and in other areas of the U.S. where Lyme is spreading, field mice and exploding populations of white-tailed deer sustain a growing reservoir of *B. burgdorferi*. Ticks pick up the bacteria from these animals and subsequently transmit it to humans.

That's why another approach to fighting Lyme disease spread is a so-called reservoir vaccine, explains C. Ben Beard, chief of the Bacterial Diseases Branch in CDC's Division of Vector-Borne Diseases. A group of researchers in New York state reported in 2006 that an oral vaccine for mice protected 89% of those immunized (*Vaccine* 2006, DOI: 10.1016/j.vaccine.2005.08.089). But Beard says methods of delivering adequate oral doses of vaccine to wild mice to break the Lyme disease cycle may prove difficult.

In the absence of a vaccine, preventive measures will continue to be a top priority for CDC, Beard says. "We do everything in relation to that goal," he notes. "It's a challenge, yes, but impossible, no." ■

ON THE MOVE Lyme disease was once concentrated in a few northeastern states, but now the upper Midwest is a hot spot and cases have been reported in states throughout the continental U.S.

Reported cases of Lyme disease in 2011



NOTE: One dot placed randomly within each county of residence for each confirmed case. **SOURCE:** CDC

tives" in terms of potential lawsuits and bad publicity?

Lymerix was not a grand slam in terms of efficacy and ease of vaccination, Poland says, "but it was something." Nonetheless, he says, opposition groups were able to destroy the vaccine in the public mind and in the marketplace.

But Plotkin, who has been a consultant for Baxter, believes that times have changed and so, too, have the chances for bringing a successful Lyme disease vaccine to market. He believes that both CDC and FDA understand that any Lyme vaccine they might approve will need a stronger endorsement from them than what the GSK vaccine got. Likewise, he says, public health officials and drugmakers will have to emphasize physician buy-in—especially with the pediatricians, family doctors, and other practitioners who see patients for routine health matters.

For the vaccine itself, Plotkin and others say, a treatment that requires multiple inoculations and boosters is problematic

resent the highest risk group for contracting Lyme. The Baxter drug has not been tested for safety and efficacy in children.

AS FOR ANTIVACCINE or hostile Lyme disease patient advocacy groups, "engaging people from the start is the thing to do" to win widespread public acceptance, Plotkin says. "We have to explain why a vaccine is safe and effective and that it's not going to cause Lyme disease."

On the other hand, Poland, who studies vaccine response in children and adults and has written about the history of Lymerix, is skeptical. He says the denialists and conspiracy theorists have not gone away. "They aren't interested in debating science," Poland says. He points to the disproven claim that the MMR (measles, mumps, rubella) vaccine causes autism as an example of another case where an assertion not backed up by scientific evidence has been forwarded as fact.

Without a vaccine or other effective preventive measures, early diagnosis of

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DISEASE AGENT

B. burgdorferi, spirochetal bacteria that cause Lyme disease, are difficult to detect directly because only a small number are present in a patient's blood. The average length of the bacterium is 20–30 μm .

ACCELERATING LYME DISEASE DIAGNOSTICS

New measurement technologies bring hope for **EARLY-STAGE DETECTION**

BRITT E. ERICKSON, C&EN WASHINGTON

WHEN TRACY LAMBETH woke up one day with extreme back pain, she brushed it off as a pulled muscle. But after several weeks, the pain did not go away. She was tested for Lyme disease, even though she did not have a bull's-eye rash—a common sign of the illness—or any recollection of a tick biting her. The results were negative. Tracy, then 26 and living in Pennsylvania, was told she had fibromyalgia and was sent home without any treatment.

One-and-a-half years later, Tracy was bitten by a tick and developed a bull's-eye rash on the back of her knee. She was hospitalized for four days. “We think I had the bacteria in my system, and this next tick bite just made everything worse,” she says.

Tracy soon discovered that she was infected with the bacterium that causes Lyme disease—*Borrelia burgdorferi*—as well as a tick-borne parasite called *Babesia microti*. She was prescribed a cocktail of antibiotics, which she took for five years. Today, 17 years later, she still suffers from extreme pain and has about 60% of the energy of an average 43-year-old.

Tracy's doctors believe that if she had been diagnosed with Lyme disease and treated with antibiotics when she suspects she first got the disease, she probably would not have any symptoms today. Her story is not uncommon. The standard test for Lyme disease recommended by the Centers for Disease Control & Prevention (CDC)—an immunoassay followed by a Western blot—does not work well during the first few weeks of the disease when antibiotics are most effective.

Researchers have been working to develop a more effective diagnostic test for Lyme disease for more than a decade. Although no approach has been shown to be more sensitive and more specific than the standard two-tiered test, advances in measurement technology may soon change that.

The current test has many shortfalls. It doesn't detect bacteria because bacterial levels in Lyme disease are low. Instead, it detects antibodies in a patient's blood produced in response to *B. burgdorferi*. The approach is prone to false-negative results because it can take several weeks

before a person produces such antibodies.

The rate of false positives is also a problem for the test. Other diseases can produce a similar immune response, so it is common for people to be misdiagnosed with Lyme disease when they actually have some other ailment.

Nor do available tests give doctors the ability to know whether a patient has been cured of Lyme disease. When patients finish their prescription of antibiotics, there is no test to determine whether the bacteria remain in their body.

ANOTHER PROBLEM is that ticks often carry more than one pathogen that cause Lyme-like symptoms. So even if *B. burgdorferi* is the most commonly known tick-borne pathogen, patients may need several different kinds of antibiotics to treat their infection. The current test does not differentiate between the organisms.

The National Institutes of Health spends about \$26 million annually on research to improve the understanding and detection of Lyme disease. The agency is currently funding about 60 Lyme disease research grants, of which about a dozen are focused on developing diagnostics.

In some cases, people are trying to identify better targets, such as bacterial peptides, that could be used to detect a host's response to *B. burgdorferi* under the same conditions as the CDC-recommended test, says Joseph J. Breen, a program officer who oversees Lyme disease grants at NIH's National Institute of Allergy & Infectious Diseases. The challenge is to find enough of the peptides to get a strong response.

Some surface antigens on *B. burgdorferi* are produced only at low levels during infection, so they can't be measured, Breen notes. The key is to find peptides that are expressed at that right time during infection, he says.

A peptide-based test that relies on multiple peptides would be easier to automate and interpret than the currently used test for Lyme disease, Breen explains. It would also be more specific for *B. burgdorferi* and thus reduce the number of false positives. However, because it is an antibody-based test, false negatives would still be a problem during the first few weeks of the disease.

To get around the false-negative prob-

“Finding more sensitive and specific diagnostics is the linchpin to breaking the gridlock on Lyme disease.”

lem, researchers have been trying to develop a Lyme disease test that detects a host's T-cell response, which produces immunomodulating proteins called cytokines. Such a response occurs much earlier than the B-cell response that makes antibodies. Until recently, however, the tools for measuring T-cell response were not specific enough to Lyme infection, Breen says. "You couldn't tell if the body was developing a T-cell response to something that was Lyme or something else."

With the help of advanced microarrays and DNA-sequencing technologies, there is now "some hope that we could have a way to measure a T-cell-based response," Breen points out. Such methods could be combined with new ways to look at the pathogen itself to get enough specificity to understand an early response, he says. The key is to identify which cytokines are specific to Lyme disease and produced early.

ONE OF THE GROUPS working with microarrays for Lyme disease detection is being led by Charles Chiu, director of the Viral Diagnostics & Discovery Center at the

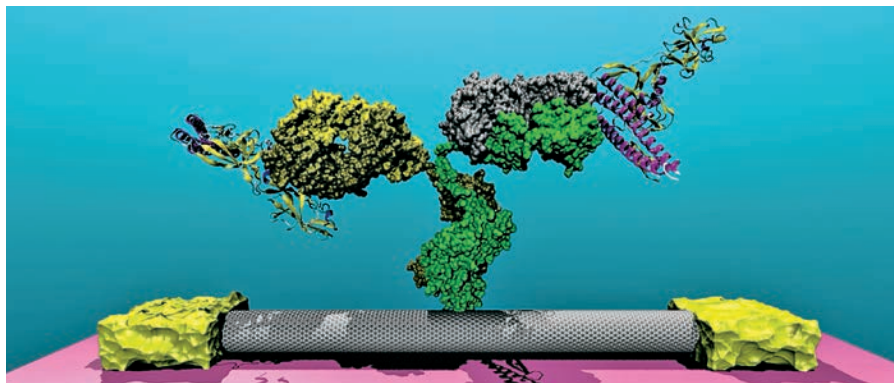
University of California, San Francisco. Chiu and colleagues have expanded a microarray for detecting novel viruses to tick-borne pathogens. The so-called TickChip is a 60,000-probe array that can detect diverse strains of bacteria, parasites, and viruses from a blood sample.

Another approach to detecting Lyme disease that looks promising is the detection of small-molecule biomarkers—such as fatty acids, amino acids, nucleotides, and lipids—in serum or urine samples by liquid chromatography/mass spectrometry. Such biomarkers reflect the rapid

NANO-BOUND
As shown in this illustration, antibodies bound to carbon nanotubes can detect proteins from bacteria that cause Lyme disease.

change in metabolites associated with disease state, says John Belisle, a professor of bacterial genetics and physiology at Colorado State University. Belisle began applying metabolomics to Lyme disease detection about two years ago.

Emerging technologies such as nanotechnology are also providing potentially novel ways to detect Lyme disease. A research team led by A. T. Charlie Johnson, a professor of physics at the University of Pennsylvania, is developing a system that uses monoclonal antibodies bound to carbon nanotubes to detect proteins from *B.*



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burgdorferi. The proteins bind to the antibodies, changing the electrical conduction of the nanotube. Preliminary results from a protein-spiked buffer solution are promising, Johnson says.

"We could distinguish down to about 1 ng/mL, which we thought was pretty good compared to what we knew about commercially," Johnson notes. He is confident that his group can boost the sensitivity of the approach by engineering the antibody.

HUGE STRIDES are also being made in using imaging to diagnose Lyme disease, says James W. Serum, a retired chemist and measurement expert who spent much of his career working for instrumentation company Hewlett-Packard (now Agilent). Serum, along with several members of his family—Tracy Lambeth is his daughter—has been affected by the disease. He organized a National Institute of Standards & Technology workshop on Lyme disease detection earlier this month to help accelerate the development of more effective diagnostics.

Advances in imaging are being driven by more effective contrast agents, Serum notes. One promising agent, he points out, is being developed by Niren Murthy, a professor of bioengineering at the University of California, Berkeley.

Murthy is testing the feasibility of attaching a maltodextrin molecule, which is a food source for bacteria, to a typical imaging agent used in positron emission tomography. When bacteria eat the sugar, they would also ingest the agent and thus could be imaged. Murphy plans to work with researchers he met at the NIST workshop to obtain samples to test his method.

Getting biological samples from Lyme disease patients to validate tests like this can be difficult. One effort to address this challenge is being led by David Roth, a Lyme disease patient and managing director in the Blackstone real estate group, a private equity firm in New York City.

Roth realized the need for such a repository when he started talking to the X Prize Foundation about managing a competition for novel Lyme disease diagnostics. The X Prize Foundation is a nonprofit organization that manages public competitions to spur technological development.

An X Prize competition seemed like a terrific way to leverage the private market and focus the research, biotech, and venture capital community on the problem of inherently flawed Lyme disease diagnos-

tics, Roth explains. The challenge to setting up the competition is that to test the tests, researchers need lots of samples, he notes.

Roth is also the cochairman of the Tick-Borne Disease Alliance, a nonprofit dedicated to increasing funding for research on tick-borne diseases. He is working with the Bay Area Lyme Foundation, in California, to explore options for creating a repository

of samples from Lyme disease patients.

"Finding more sensitive and specific diagnostics is the linchpin to breaking the gridlock on Lyme disease and other tick-borne diseases," Roth says. Like many other people with Lyme disease, Roth was diagnosed four months after he got the disease, when antibiotics are less effective. As a result, his symptoms persist today. ■

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