Subcommittee on Africa, Global Health, and Human Rights
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Global Challenges in Diagnosing and Managing Lyme Disease—Closing Knowledge Gaps

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The Need For Better Diagnostics For The Direct And Early Detection Of Lyme Disease And Other Tick-Borne Illnesses.

Lyme disease, caused by the tick-borne bacteria *Borrelia burgdorferi*, is the most commonly reported vector-borne infectious disease in North America. The number of yearly cases reported to the CDC has steadily increased since 1982 when case reporting began, with 20,000-30,000 cases now reported each year [1,2]. It is estimated that in endemic areas of the United States the disease is underreported. In addition, Lyme disease is also endemic to Europe and Asia. Other tick-borne illnesses, such as the protozoan parasite *Babesia*, are found worldwide, and in many parts of the world, such as Africa, Babesiosis is frequently mistaken for Malaria. In regions with Lyme disease other tick-borne pathogens are typically present leading to the risk of co-infection with Lyme disease.

Early Lyme is the period of time immediately following infection when the first symptoms of Lyme disease occur. If promptly diagnosed and correctly treated, outcomes for early Lyme disease are generally considered to be excellent. However, if undiagnosed and/or untreated, Lyme *Borrelia* can cause long-term chronic infections with the bacteria spreading to parts of the body such as the nervous system, the joints, or the heart where they can cause serious illness with the potential for long-term damage to the infected individual. As a result, infection with Lyme *Borrelia* can result in a wide range of disease symptoms and corresponding treatments, **Figure1**. To prevent these serious consequences of infection, Lyme disease is best treated early: at the first sign of Lyme symptoms. The challenge is that the symptoms of early Lyme infection are varied and frequently mistaken for other illnesses. The most typical symptoms for early Lyme disease include the Bull's eye rash also called eyrthema migrans (EM). However, these Bull's eye rashes are present in only a little over half of the Lyme infections[3,4]. Furthermore the Bull's eye rashes can be quite varied in their presentation so that the typical family physician may not recognize them as being Lyme disease even in endemic areas. Other symptoms of Lyme disease are typically Flu–like: fever, fatigue and headache. As a result early Lyme disease can be difficult to clinically diagnose by physicians who are not Lyme disease specialists.

Due to highly variable symptoms of Lyme disease and the benefits to treating the infection early before the bacteria can spread to other parts of the body, a diagnostic is needed that can detect the bacteria during the critical early part of the infection. As many patients, even after treatment, continue to suffer from a variety of Lyme disease symptoms, a diagnostic is also needed that can show that the infection has responded to the treatment.

The current diagnostic for Lyme disease does not test for the Lyme *Borrelia* bacteria directly, but rather looks to see if the patient's immune system has developed antibodies against the Lyme *Borrelia* bacteria (the two-tiered serological test). The two-tiered serological test looks for *Borrelia* antibodies in the patient's serum. There are several problems with the two-tiered serological test.

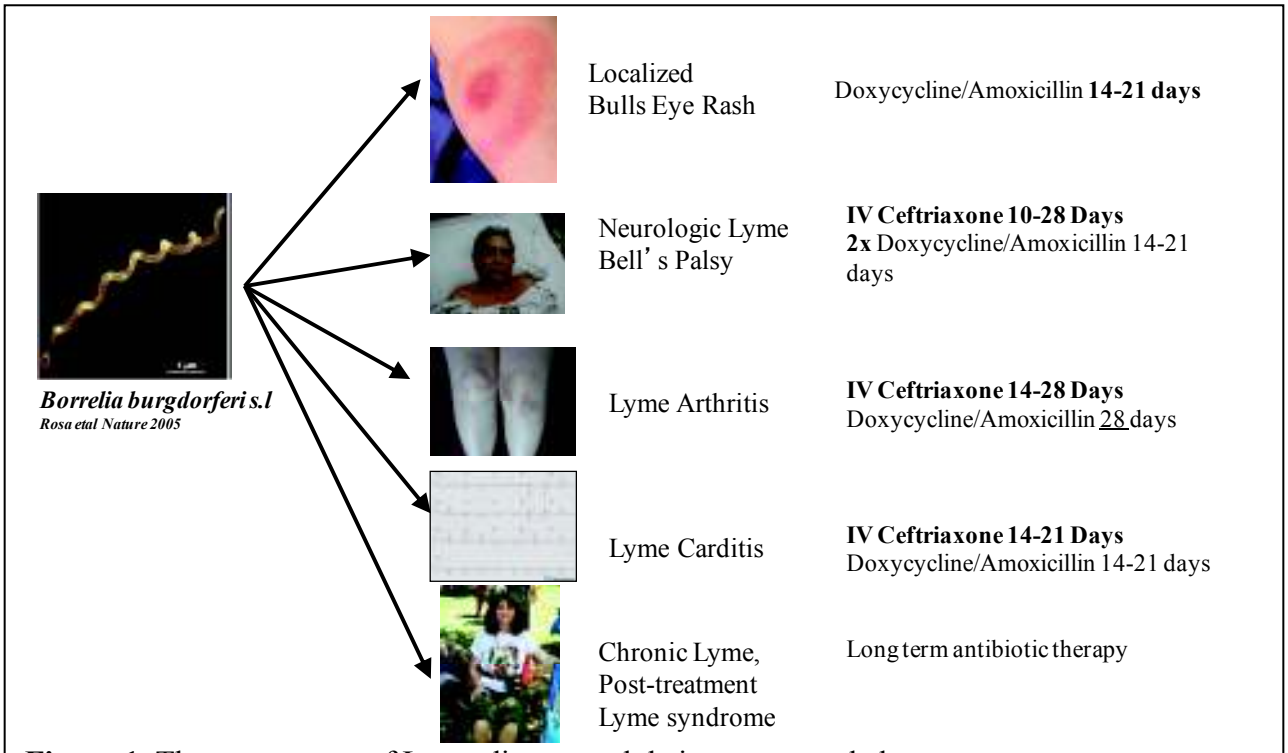

Figure 1. The many types of Lyme disease and their recommended treatments.

For most Lyme patients it can take three or more weeks of infection with the Lyme *Borrelia* bacteria for the immune system to develop sufficient response to test positive by the two-tiered serological test, thus treatment could be delayed during the critical early period of the Lyme infection and lead to the infection spreading to more sensitive and harder to treat parts of the body such as the nervous system or the joints.

Secondly, the interpretation of the two-tiered serological test results can be subjective with essentially the same test from the same patient being performed by two separate testing labs reporting opposite results. Results of this can lead to the treatment of patients who may not have Lyme disease but rather suffer from other illnesses.

Thirdly, once a person has had Lyme disease they will continue to test positive even after treatment due to the fact that the immune system remains active against the Lyme *Borrelia*

bacteria long after the infection has been cured. As a result there is no test that can show that the treatment for Lyme disease has cured the infection. Because of this fact there is controversy over how much treatment is needed to cure Lyme disease with some physicians treating with antibiotics for a couple of weeks while other physicians recommend treatment with antibiotics for months to years. Overuse of antibiotics can contribute to antibiotic resistance and may put the patient at risk for opportunistic infections, such as fungal infections, *Clostridium difficile*, etc.

The ideal diagnostic test for Lyme would directly detect the Lyme *Borrelia* bacteria in the blood from a patient while they were still early in the infection and identify active infection prior to the development of antibodies. Direct molecular tests for the Lyme *Borrelia* bacteria, such as those employing the polymerase chain reaction (PCR), look for the DNA of the bacteria and thus are not dependent on the patient's immune response. Furthermore, a direct molecular test by virtue of detecting the Lyme *Borrelia*'s DNA will only test positive while the bacteria are present, thus having the potential to be used to measure response to antibiotic treatment.

However, in the past, PCR based tests for Lyme disease have suffered from too low a sensitivity for their clinical use. This is due to the fact that there are very few Lyme *Borrelia* bacteria circulating in the blood stream in patients with Lyme disease. Importantly, this does not mean that there are NO Lyme *Borrelia* bacteria circulating in the blood stream. Studies have shown direct molecular detection of *B. burgdorferi* by PCR can be enhanced by culturing the blood specimen in growth medium prior to testing for Lyme *Borrelia* by PCR. This finding indicates that there is the presence of Lyme *Borrelia* bacteria in the bloodstream but that it's below the limits of detection by PCR [5]. In the past, direct PCR based assays for Lyme disease did not have the benefit of current advances in sample preparation [6] or DNA amplification techniques that could enable the detection of the very low levels of Lyme *Borrelia* bacteria in the bloodstream.

We have previously applied our direct molecular test based upon broad-range PCR and electrospray ionization mass spectrometry (PCR/ESI-MS) for the detection of a wide range of vector-borne pathogens such as *Ehrlichia, Rickettsia*, Powassan virus, *Babesia, Anaplasma* and canine heartworm [7,8,9]. The basis of this detection and identification is a multi-locus broad range PCR followed by the determination of the mass of the amplicons using automated electrospray ionization mass spectrometry. From the masses of the amplicons, the numbers of DNA base pairs A's, G's, C's and T's in each PCR amplicon are determined. By analysis of the base compositions of amplicons from all primer pairs, the organisms present in the sample can be identified from a database of all known base count signatures and quantified [10,11]. This technique has the advantage of rapidly identifying pathogens, genotyping pathogens and can identify new genetic variants.

To address the need for a better and sensitive diagnostic test for early Lyme disease we have improved the sensitivity of PCR/ESI-MS by several means. First we employ an assay that consists of eight independent targets to test for the Lyme *Borrelia* bacteria. This way we have eight chances of finding the bacteria's DNA in the blood specimen. Secondly we use a larger volume of blood in the test thereby increasing the chances of finding the Lyme *Borrelia* bacteria in a given specimen. Thirdly we employ a sensitive DNA amplification technique prior to the PCR to increase the amount of *Borrelia* DNA to levels above the limits of detection of PCR. Initial results of this approach have been very encouraging. In a recent study of 21 patients with

serologically confirmed early Lyme disease we detected early Lyme disease in 62% of the blood specimens collected from the patients at their first doctor visit [12].

Many pathogenic bacteria come in various strains and the strain may determine the risk of exposure and severity of disease. For example there are many genotypes or variants of *E. coli*, many of which are harmless but some can cause serious illness. Similarly there is a wide range of *Borrelia* species and genotypes world-wide that can cause Lyme disease. Our sensitive direct molecular test also yields a genotype for the Lyme *Borrelia* bacteria. These genotypes represents genetics differences in the bacteria that can be used to better understand roles of genetics differences and the type of illness in humans. Knowing the role of *Borrelia* genotypes for human illness may be critical in knowing how best to treat the infection. For example here in the United States, we have identified over 80 different genotypes of Lyme *Borrelia* in ticks. However when we look at the *Borrelia* genotypes found in patients with Lyme disease we find a much smaller representation of genotypes. This finding suggests that some genotypes may be more pathogenic for people. Furthermore, some genotypes could be associated with what type of Lyme disease the bacteria might cause: neurological Lyme or Lyme arthritis. A better understanding of the role of *Borrelia* genotypes and illness could help to direct the physician on how aggressively to treat the infection.

What's needed, Where are the gaps?

We believe increased government funding and research are needed in three key areas.

1) We believe that we need sensitive molecular tests that directly detect the DNA of the Lyme *Borrelia* bacteria and are not dependent upon the immune response. These tests would enable detection of Lyme disease earlier in the infection before the bacteria are able to spread throughout the body. A direct molecular test would also enable the physician to monitor the response to treatment. Though we have demonstrated the feasibility of a sensitive molecular test for early Lyme disease more work is needed to improve the sensitivity further and to get such a test approved by the FDA.
2) Better understanding of the role and causes of post-treatment Lyme and an understanding of why a significant number of Lyme disease patient's symptoms do not resolve with treatment. A sensitive direct molecular diagnostic test may be instrumental to understanding the causes of these symptoms.
3) Increased research into the roles of *Borrelia* genotypes and Lyme disease in humans. Studies are needed from many geographical regions looking at the prevalence of *Borrelia* genotypes in ticks, skin biopsies of patients with Eyrthema migrans, and other forms of Lyme disease.

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