Pathogen Discovery: West Nile and Beyond

W. Ian Lipkin, MD

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Genome projects and high throughput methods for profiling gene expression using cDNAs and oligonucleotides have revolutionized biology by providing tools for simultaneous assessment of thousands of nucleic acid sequences. Methods for cloning nucleic acids of microbial pathogens directly from clinical specimens offer new opportunities to investigate microbial associations in diseases predicted to have an infectious basis.

The power of these methods is that they can succeed where methods for pathogen identification through serology or cultivation may fail due to absence of specific reagents or fastidious requirements for agent replication. Over the past decade the application of molecular pathogen discovery methods resulted in identification of novel agents including Borna disease virus, Hepatitis C virus, Sin Nombre virus, HHV-8, Bartonella henselae, and Tropherema whippeli, and facilitated implication of Nipah virus and West Nile virus as significant causes of human morbidity and mortality. This talk will review strengths and weakness of current and proposed technology as well as revisions of Kochs Postulates required in this new era of molecular microbiology.

Pathogen discovery process - detecting newest news. Unexplained death (Ca, CT, MN, Or) proj

Prime of life who got sudden illness & encephalitis is a ?

Lipkin — Debora Asgnes, Tracy [illegible], Sub proj w/ 50-20%

8/23 - Cluster of [illegible] neuro.

9/7-9

Bronx 300

dogs

Samples

USDA, Ames

9/3 - 1gm

9/13-15 -

Sept 24

Preskel

CDC/Ft Collins [illegible]

300 animals [illegible]

West Nile Virus: A Global Hitchhiker

John Anderson, PhD
Connecticut Agricultural Experiment Station

West Nile virus (WNV) occurs naturally in Africa, Asia, and Europe. An outbreak of WNV occurred in New York City and surrounding areas in 1999. Humans, birds, and horses died. The first viral isolations from mosquitoes were made from *Culex pipiens* and *Aedes vexans* collected on the Stamford/Greenwich, CT border on September 14, 1999. These viral isolates and those from an American crow and a Cooper's hawk in Connecticut were shown to be similar to one another and to be West Nile virus. Virus isolates from birds, mosquitoes, and horses in New York and viral RNA recovery from tissues of brain from humans were similarly identified as WNV and to be virtually indistinguishable from a WNV isolate from a goose that died in Israel in 1998. Seven people died from WNV infections in New York in 1999; 62 people were clinically diagnosed with infection. The virus was identified in four states: New York, Connecticut, New Jersey, and Maryland.

During the winter of 1999/2000, WNV was isolated from hibernating mosquitoes in New York by the CDC. During the summer and fall of 2000, WNV reappeared in the New York City area. Two people died, and 21 humans were diagnosed with infection. However, the virus spread to 12 states and the District of Columbia. The virus was detected throughout most of New York State, the southern counties of Vermont and New Hampshire, and as far south as North Carolina and Virginia.

The virus has been isolated or detected from about 75 species of birds and possibly 13 species of mosquitoes in the USA. In Connecticut, WNV was isolated from July into the first week of November and had spread to all eight counties. Genetic differences were observed among 33 WNV isolates from Connecticut birds and a striped skunk at 11 positions along a 921-nucleotide sequence yielding 10 unique isolates. Relatively large numbers of one group of isolates that differed from WNV isolates studied in 1999 were identified. This group had a thymine instead of a cytosine at position 654 of the sequence and was recovered from six species of birds and a striped skunk.

Ehrlichiosis in the Northeast

Louis Magnarelli, PhD
Connecticut Agricultural Experiment Station

Ticks transmit bacteria that cause ehrlichiosis in humans and domesticated animals. Based on clinical findings, serologic results, DNA evidence, or isolations, the following occur in northeastern United States: *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia phagocytophila* genogroup organisms, and *Ehrlichia risticii*. *Ehrlichia canis* and *E. risticii* primarily affect dogs and horses, respectively.

Ehrlichia chaffeensis and *E. phagocytophila* genogroup organisms are human pathogens, but the latter cause disease in horses and may infect cattle in the United States. Human granulocytic ehrlichiosis (HGE), caused by *E. phagocytophila* or a closely related bacterium, is most prevalent in Connecticut. During the period 1995 - 2000, there were 420 confirmed and 839 probable cases of HGE reported in Connecticut. *Ixodes scapularis* nymphs and females are the chief vectors. DNA of *E. chaffeensis* was recently detected in adult *Amblyomma americanum* (lone star ticks) from western Connecticut and Prudence Island, Rhode Island. Further surveillance and additional laboratory testing for human and veterinary ehrlichial infections are needed.

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Babesiosis, Ehrlichiosis, Lyme(-like) Disease Variations

Ed Masters, MD
Regional Primary Care Physicians

Human babesiosis in the U.S. had been associated in the Northeastern and upper Midwestern states with Bebasia microti and Ixodes Scapularis ticks, in California and Washington State with WA1-type piroplasms and in Southeast Missouri with a related but distinct intraerythrocytic piroplasm MO1, which is genetically similar to B. divergens. Human monocytic ehrlichiosis (HME) caused by Ehrlichia chaffeensis has been associated with Amblyomma americanum (lone star) ticks in the Midwest and south central states. Human granulocytic ehrlichiosis (HGE), caused by Ehrlichia phagocytophylia-like organism has been found in the upper Midwest and Northeast. Ehrlichiosis has also been found in California.

Atypical Borrlelia burgdorferi has been found in Missouri ticks and a new species, Borrlelia lonestari, have been discovered in lone star ticks. Additionally, erythema migrans cases have been documented in the lower Midwest and other borrelia-appearing spirochetes that stained with H5332 have been observed in lone star ticks. There is growing acknowledgement of the presence of a lone star tick-vectored borreliosis in the South. This disease has not been fully characterized and has been called Lyme, Lyme-like, STARI (Southern Tick Associated Rash Illness by the CDC) and even Masters' Disease (Rich et al, J Clin Microbial, Feb. 2000, p. 494-497). Furthermore, ticks simultaneously harboring Borrelia and Babesia as well as Borrelia and Ehrlichia have been reported.

Are there parallel paths of pathogenicity between the lone star and Ixodes Scapularis ticks? Geographical and temporal histories of the evolving knowledge and awareness of Babesiosis, Ehrlichiosis and Lyme and/or Lyme-Like disease variations are compared, along with the disparate geographic clustering.

Bartonella in Humans

Lesley Fein, MD, MPH
Mountainside Hospital

Bartonella (Catscratch fever) has recently been isolated from the blood and spinal fluid of patients with tick-borne illnesses. In addition, ticks located on the property of some of these patients were also found to test positive for Bartonella PCR.

Another, independent study, has reproduced the finding of Bartonella in *Ixodes Scapularis* ticks. Bartonella has been reported in multiple studies to be associated with neurologic involvement. This presentation will include case reports of 6 patients who have tested positive for Bartonella PCR, results of tick testing and a review of the literature.

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Evidence for *Borrelia burgdorferi* as an Etiologic Agent in Morphea

Andrew Franks, Jr., M.D.
New York University School of Medicine,

Morphea: An Infectious Disease

- Localized
- Generalized
- Guttate (LSA)
- Linear (coup de sabre)
- Parry-Romberg syndrome

Morphea: An Infectious Disease

Acrodermatitis chronica atrophicans

- **European Borreliosis**
- Bb ***afzelii***
- Bb ***garinii***

Morphea

- **North American Borreliosis**
- Bb ***sensu stricto***
- Bb ***garinii***
- Bb ***afzelii***

Morphea: An Infectious Disease

- Erythema migrans
- Acrodermatitis chronica atrophicans
- Morphea / localized scleroderma
- Lichen sclerosis et atrophicus
- Lymhadenosis benigna cutis

Morphea: An Infectious Disease

- 82 patients entered
- 14 men, 68 women
- 27 years (2 - 61)
- Skin biopsy and/or 'skin score'
- Autoimmune serology (SER)
- Lyme western blot (WB)

Morphea: An Infectious Disease

Interpretation:

WB: *'any bands present'*
'all bands negative'

Morphea: NYU Protocol *Results*

- 61 patients completed study
- 34 had clinical improvement
- ***WB*** frequently seroconverted
- No patient had 'Lyme disease'
- ***WB+SER-*** were likely to respond

Criteria to confirm the causal role of an organism in the pathogenesis of a disease (Koch's postulates, 1877)

- The organism must be isolated from every patient with the disease
  - Caveat: diseases can have more than one etiology such as peptic ulcers caused by *H. pylori* or NSAIDs
- The organism must be isolated free from all other organisms and grown in pure culture *in vitro*
- The organism must cause the disease in a healthy, susceptible animal
- The organism must be recovered from the inoculated animal

Investigation of *Borrelia Burgdorferi* as a cause of morphea

- Antibody studies: ELISA, indirect immunofluorescence (IIF), and western blot (WB)
- Histologic and immunohistochemical studies
- Culture of organism from morphea lesions
- PCR studies to isolate and subtype BB from morphea lesions

BB antibody (Ab) studies

- 28 studies have examined sera of morphea patients for the presence of BB Ab
  - 15 by indirect immunofluorescence, 17 by ELISA, and 5 by western blot

- BB Ab was detected in 20% (123 of 617) of samples studied
  - 20% by IIF, 17% by ELISA, 18% by WB

BB antibody (Ab) studies

- 14 of the 28 studies had healthy control groups
  - Seropositivity ranged from 0-34%
  - In 5 of these 14 studies, morphea patients were seropositive more often than controls (23-50% vs. 0-14%)
- 9 of the 28 studies evaluated antibiotic use prior to biopsy.
  - 48% of patients (92 of 192) had been previously treated
- The single American study (Rochester, MN) found no significant difference in seropositivity between morphea patients and controls (1 of 25 morphea vs. 1 of 50 healthy patients)

Histologic Studies
Cell-culture studies
PCR studies

- PCR has the advantages of
  - High sensitivity, especially with nested PCR
  - Less time and labor intensive than cell culture
- Primary disadvantage is ease of contamination with non-lesional DNA

PCR studies

- 14 studies of 254 patients have used PCR in attempts to detect BB in morphea lesions
- BB DNA has not been detected in the 5 PCR studies of American patients (115) with morphea
- 4 studies have successfully amplified BB DNA from morphea lesions

Methods of positive PCR studies

Why have PCR studies given conflicting results?

- The Fujiwara study indicates that Bb strain may be important for pathogenesis since only *B afzelii* and *B garinii* were amplified

- Lack of evidence for BB in American morphea patients could be due to
  - The predominance of *sensu stricto* strains in the U.S.
  - Relatively low rates of endemic BB infection in regions studied (Detroit, St. Louis, New Orleans, Houston)

PCR study pitfalls

- 7 of the 10 negative studies did not quantify template DNA before beginning PCR amplification
  - thus there may not have been enough DNA in the PCR reaction for successful amplification

- Many patients may have taken anti-borrelial antibiotics prior to biopsy
  - In the 2 studies that provided data, 27 of 61 (44%) had taken antibiotics prior to biopsy

PCR study pitfalls

- The primers used in negative studies may not have been specific or sensitive enough
  - Must amplify as little as 5 copies of BB genome
  - Great genetic diversity of BB makes selection of specific primers crucial to PCR success

- 4 of the 10 negative studies did not perform confirmatory southern blots
  - It is easy to miss small amounts of DNA on an ethidium bromide gel

Conclusions

- Evidence for BB as an etiologic agent in morphea includes:
  - Histology and immunohistochemistry
  - Cell culture
  - PCR

Conclusions

- Several studies, including the 6 American studies, have found no evidence of BB in morphea lesions

- *B afzelii* and *B garinii* strains of BB may be more important than *sensu stricto* in pathogenesis of morphea

- Some negative studies have been limited by flaws in study design

Conclusions

- All patients with Morphea are candidates for a *therapeutic trial* of antibiotics regardless of serologic test results.

- IgM & IgG Western Blots should be obtained prior to and after treatment.

- Conventional Lyme Tests including OD, IF, Elisa, PCR, etc. are not currently useful when evaluating Morphea.

- The risks with antibiotics are minimal and the results may be dramatic.

A call for tissue!

- Please call 917-816-2714 when a new, untreated morphea patient is in your office

- A consent form & instructions for obtaining a 4mm punch biopsy for PCR will be faxed to you.

Presentations April 23, 2001 Monday

21st Century Lyme Disease

Daniel Cameron, MD, MPH
Internist, Epidemiologist, New York

PREMISE: 21st century Lyme disease patients are in need of care rendered in a community-based setting which is convenient, driven by "best practice" protocols, delivered by primary-care professionals, supported by the newest information technology, and outcome-driven.

METHOD: 20th century classic peer-reviewed studies will be compared with our practice — a practice in a community driven by "best practice protocols, supported by a surveillance data base and evaluated by an outcome-driven Lyme Disease Retreatment Study.

OUTCOME MEASURES: Outcomes for those patients with seronegative presentations, recurrences, persistent illness, and retreatment will be compared.

RESULTS: Both our practice and numerous papers presented in professional journals on Lyme disease recognized problems with seronegative presentations, recurrences, persistent illness and retreatment. In contrast, treatment guidelines from other sources have not recommended treatment beyond 3—4 weeks, and often limit treatment to cases presenting with specific symptoms such as erythema migrans rash, arthritis, heart block or meningitis. The majority of guidelines for chronic Lyme disease were based on consensus and a nonsystematic literature review. Consensus-based guidelines, while less time-consuming to develop, lacked crucial information and resulted in a wide variety of outcomes.

DISCUSSION: 20th century Lyme disease treatment guidelines failed to address emerging problems with seronegative presentations, recurrences, persistent illness and retreatment. Changes to guidelines and consensus papers to reflect community-based, "best practice" protocols would best be accomplished by using the newest technologies and outcome studies. Until these can be improved, the challenge to the practicing physician is to maximally utilize available modalities of treatment to prevent progression to chronic Lyme disease.

Cardiac Manifestations of Lyme Disease

Kornelia Keszler, MD
Yale University School of Medicine

Neurologic Lyme Disease

Patricia Coyle, MD
SUNY at Stony Brook School of Medicine

Lyme Encephalopathy: Definition and Differential Diagnosis

Brian Fallon, MD, MPH
Columbia University College of Physicians & Surgeons

Definition: Lyme encephalopathy is a term that is used loosely to describe the late manifestations of cognitive problems experienced by patients with Lyme disease. A review of the literature on Lyme disease provides little guidance on how best to objectively identify patients with encephalopathy. In some reports, objective deficits are based on cognitive complaints substantiated by intrathecal antibody production, other CSF markers, or other markers of brain disease (SPECT, MRI). In other reports, the subjective cognitive complaints must be confirmed by objective neuropsychological testing.

The criteria for the determination of impairment on neuropsychological testing, however, vary widely with some researchers comparing scores to national norms, without consideration of a person's premorbid level of ability. Other researchers compare the patient's scores to his/her expected performance based on a test which is presumed to be a stable measure of premorbid intellectual functioning. The advantages and disadvantages of these approaches will be discussed. The multi-domain approach used in defining HIV encephalopathy will be discussed. Finally, using data collected during the first year of the Columbia-NIH Lyme study, the sensitivity and specificity of several different methods will be addressed.

Differential Diagnosis: Because the causes of cognitive dysfunction are many, the clinician needs to ascertain that the patient with chronic symptoms does not have another disorder that has not yet been identified. The leading alternative diagnoses will be reviewed, focusing on key differentiating aspects.

Cognitive Remediation: A Proposed Intervention in the Treatment of Patients with Lyme Encephalopathy

Leo Shea, PhD
New York University School of Medicine

The use of cognitive remediation as a therapeutic intervention has proven beneficial in work with brain injured patients. It's adaptability for use in other neurological illnesses and injuries has emerged over the past decade. This presentation will address the theoretical underpinnings of cognitive remediation, the historical approaches used, and newer techniques specifically related to Lyme patients.

It will include the presentation of data based on a year-long study of 9 Lyme diagnosed patients who were tested on measures of attention, concentration and speed of processing prior to the intervention of IV antibiotic treatment, while on I.V. antibiotics and three months after cessation of I.V. antibiotics. Discussion will include the nature of the training tasks provided over the course of the study, patient ratings of cognition, behavior, emotion, and physical stamina, clinical observation and the overall results achieved.

Pediatric Neurologic Lyme Disease

Dorothy Pietrucha, MD
Jersey Shore Medical Center, Meridan Health System

A. Involvement of the Central Nervous System

Patients may present acutely with headache, blurry vision, double vision, confusion, irritability, fever, and stiff neck. Chronically, they may be encephalopathic, have lingering headache, personality change and depression. Patients who present acutely may have aseptic meningitis with pleocytosis and elevated protein in the spinal fluid. Occasionally, there may be lesions on the MRI and about twenty percent of patients may have abnormal EEGs.

Increased intracranial pressure with an opening pressure over 200 mm/H2O is seen much more often in children with Lyme disease than it is in adults.

The CSF pleocytosis usually improves. Frequently, it may improve even without treatment but certainly if the patient is treated with antibiotics, this will clear. The increased intracranial pressure responses to medication such as Diamox and seizures should be treated with anti-convulsant medications. The lesions on the MRI may remain or may disappear with time.

The most common lingering problem that patients have as a result of involvement of the central nervous system in Lyme is the encephalopathy, which the children call "brain fog." These children complain of persistent headache and fatigue. There may be personality change, irritability, and frequently, depression.

The impact academically is most significant. These children have fall-off in academic performance, difficulty learning new material, problems with short-term memory, word finding and a number of them have lost reading skills. Frequently, these children may present with a picture of ADD or may have an underlying ADD or ADHD that is made worse by Lyme. Incidentally, children with Tourette's have also had a worsening of their tics when they become ill with Lyme disease symptoms.

This Lyme encepholapathy merits special attention because it has a significant impact both educationally and economically. These children may require home instruction, necessitating a parent to stay home from work to be with the child. These children have to have tutors come to the home. When they return to school, many frequently need a shortened school day and continued home instruction. Many have to be classified as other health impaired and receive on-going services such as Resource Room, etc. A number of these children need to be placed on medication for their short attention spans and distractibility, as you would treat any other ADD patient. Frequently, the depression has to be treated both with medication and counseling.

In addition to the impact on theses children educationally, there is a social burden due to the fact that they cannot participate in extracurricular activities and lose contact with peers.

B. Peripheral Nervous System

Patients may present with a sudden onset of weakness. It may be facial weakness, weakness of an extremity or an ascending weakness or paralysis. There may be pain, a burning sensation in the extremities, numbness, tingling and myalgia.

Children do not have involvement of the peripheral nervous system as frequently as they have involvement of the central nervous system. The most common peripheral nervous manifestation in children is Bell's Palsy with a sudden onset of a facial palsy. Rarely, there has been involvement

of an isolated extremity or even an isolated nerve involvement, such as the peroneal nerve. Patients have presented with a picture very typical of Guillain-Barre and many children do complain of a burning sensation, numbness, tingling and this is a mild sensory neuropathy. There have been cases of children presenting with muscle pain, weakness and elevated CPK.

In addition to treating their Lyme disease with appropriate antibiotic therapy, these patients may require physical therapy, anti-inflammatory medication and analgesia. Patients presenting with a picture typical of Guillain-Barre should be treated like any Guillain-Barre patient, keeping in mind that if the underlying cause is Lyme disease than that, too must be treated. Overall, the prognosis for children to show a complete recovery from involvement of the peripheral nervous system in Lyme disease is very good, probably better than in the adult population. Bilateral Bell's Palsy has certainly been seen in Lyme disease children.

Over the years, I have treated many children with Lyme disease. I have noticed that in the very young child, one of the more common complaints has been irritability and personality change that the parents notice in the preschooler with Lyme disease. The older child frequently will have depression with their encephalopathy and this cannot be ignored. It has to be treated with counseling, anti-depressants, etc. Many times, children who have no documented peripheral neuropathy or documented myositis still have a lot of aches and pains and fatigue and sometimes involving them in a physical therapy program has been beneficial.

The patient should also have a complete eye examination done if they have any complaints whatsoever referable to the visual system, such as blurry vision, eye pain, tearing, etc. Although hearing loss has been documented in adults with Lyme, it occurs rarely in children but certainly if the child is complaining or there is any suspicion of a decrease in hearing, then formal audiologic testing should be done to document this.

Fortunately, the vast majority of children who have involvement of the central and/or peripheral nervous system improve, although for some it may be a longer protracted course and they may be symptomatic for weeks, months and, in some cases, even years. There are, however, a small percentage of patients with Lyme disease who go on with a chronic encephalopathic picture and they, of course, have involvement of other organ systems as well. This population continues to be symptomatic. They may have remissions and relapses but unfortunately, in this small population they do not show significant improvement and are unable to continue with their pre-morbid educational programs and/or career goals and continue to require on-going care. The question, of course, is whether these patients have persistent infection, whether they have infection with another organism or whether they have developed some type of "autoimmune" or post-infectious process that is not well understood that leaves them so debilitated. This population deserves a great deal of attention from the medical community. A lot more needs to be done to determine exactly why these patients continue to be so sick and more rational therapeutic approaches are needed to improve their quality of life.

It is important for any treating physician caring for a child with Lyme disease to keep in mind that the nervous system is frequently involved in Lyme disease but that the neurological disorders occur frequently regardless of Lyme. Seizures, migraine headaches, Attention Deficit-Hyperactivity Disorder, tic disorder, other behavioral problems, and learning disabilities are all seen in the pediatric population. One should not assume that every child who presents with any of the above has Lyme disease as the cause since there may be other reasons for the neurologic problem and Lyme may be a contributing or coincidental factor. It takes a comprehensive evaluation of the patient and clinical experience to help sort out the patient's problems, where Lyme fits and what approach should be taken therapeutically.

Gastrointestinal Manifestations of Lyme Disease

Martin Fried, MD
Jersey Shore Medical Center

Lyme disease is one of the causes of gastrointestinal distress in children and adolescents who present with multi systemic organ complaints. Children and adolescents with Lyme disease may present with heartburn, reflux, abdominal pain, diarrhea, blood in the stool and/or incontinence of bowl movements. The heartburn associated with active Lyme disease does not completely resolve with antacids or H2 blocking medications if the infection remains untreated. In those patients with an active Lyme infection and abdominal pain, the symptoms may diminish with antacids, antispasmodic medication or acid blocking medication but they also will not resolve until the infection is treated. Ongoing symptoms after the infection has been treated may be due to immune mediated chemical and these symptoms usually occur at a lower level of discomfort in the absence of the infection.

Conditions such as mouth sores and psoriasis have accompanied the gastrointestinal symptoms and they alert the physician to the immune system mediated nature of the disease process. When Lyme disease elicits immune system chemicals in the gastrointestinal tract, its symptoms and presentation could mimic that of other immune system mediated diseases such as Crohn's disease and ulcerative colitis. It may be difficult to clinically distinguish between Lyme disease, Crohn's disease and Ulcerative Colitis because they may each present with similar symptoms. Performing a polymerase chain reaction test for the Outer surface protein A of *Borrelia burgdorferi* on biopsies from the gastrointestinal tract establishes the presence of Lyme disease in the gastrointestinal tract.

LD Retreatment Study: A Double-Blinded Placebo-Controlled randomized Trial Evaluating the Efficacy and Success of Oral Antibiotics for Recurrent Seropositive and Seronegative Lyme Disease in Adults

Daniel Cameron, MD, MPH
Internist, Epidemiologist, New York

PROJECTION: While the evidence for the beneficial effects of antibiotic use for persistent and recurrent Lyme disease is compelling, firm experimental evidence in support of the hypothesis that long-term antibiotic treatment of Lyme disease is effective is needed. Pilot studies completed to date have enabled our practice to proceed to a full-scale definitive randomized, double-blind, placebo-controlled clinical trial.

PATIENTS AND METHODS: We calculated from results of our more than 2,000 patient Lyme disease surveillance database that a 70% success rate in the oral antibiotic group and a 35% success rate in the placebo group would require 108 patients in both the seropositive and the seronegative arm to detect a 5% significance with a 10% type II error even if allowing for 20% loss to follow-up. The rationale for oral antibiotics, requirements of previous success, exclusion of patients failing Amoxicillin therapy, inclusion of both seronegative and seropositive patients, 90-day treatment duration, placebo, and 2-to-1 ratio of treatment to placebo presented to the Institutional Review Board will be explored.

RESULTS: The Lyme Disease Retreatment Study (LDRS) was approved by the IRB in January 2001 and will be launched in the spring of 2001. Two hundred sixteen recurrent Lyme disease patients (108 in the seropositive, 108 in the seronegative group) will be enrolled into a single-investigator, randomized, double-blind, placebo-controlled trial to ascertain the efficacy and safety of oral antibiotics versus placebo in recurrent seropositive and seronegative Lyme disease.

DISCUSSION: The LDRS trial will introduce innovative epidemiologic methods attuned to the particular features of chronic Lyme disease biology and epidemiology. The LDRS envisions completing this clincial trial to help move new treatment strategies more rapidly from the primary-care setting into peer-review journals.