Journal of Spirochetal and Tick-borne Diseases

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Tick-Borne Diseases

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Dedicated to:
''Science and Art in Spirochetal and Tick-Borne Diseases''

Information for Authors and Editorial Policy

The following guidelines are in accordance with the ''Uniform Requirements for Manuscripts Submitted to Biomedical Journals'' and International Committee of Medical Journal Editors (the ''Vancouver Group'') statement, agreed at the January 1993 Meeting.

The *Journal of Spirochetal and Tick-Borne Diseases* publishes quarterly reviews and original work studies about any aspect of spirochetal and tick-borne diseases. The primary purpose is to broaden our understanding of spirochetal and tick-borne diseases. Special focus is given to Lyme borreliosis (also known as Lyme disease), as the most prevalent spirochetal and tick-borne disease. The clinical topics may involve all medical disciplines, nursing, and pharmacy, as well as the social, ethical, and biological features of spirochetal and tick-borne diseases.

The Journal is composed of two major sections. One section is devoted to the review of a specific topic that is established by the Associate Editors, and a special guest editor is invited to coordinate the development of up to six manuscripts relating to the specific topic. The second section of original works is composed of unsolicited manuscripts that are subsequently reviewed by the Review board, as well as external reviewers, depending upon the potential for conflict of interest within the editorial panel and the potential interest by the readership.

Expeditious review of all manuscripts is carried out with a projected response time of not more than four weeks. Rejected manuscripts are usually returned to authors within six weeks. Decisions about potentially acceptable manuscripts may take somewhat longer.

The Journal will publish material defined within the categories described below.

Reviews

Each issue includes a series of articles on the state of the art on a topic related to spirochetal and tick-borne diseases. The articles represent invited presentation by authorities in the field on topics related to spirochetal and tick-borne diseases, with an emphasis on Lyme borreliosis.

Each manuscript should present a comprehensive state-of-the-art analysis and should be accompanied by an abstract of 300 words or less summarizing the major points.

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Original articles of 5000 words or less may be submitted to the editorial office. Each article should be accompanied by an abstract of 300 words or less describing the findings of the original research. All articles will be peer reviewed within a three-week periods with subsequent notification to the authors within five weeks of submission. Submitted articles may relate to any area of spirochetal and tick-borne diseases.

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Specific clinical case reports describing a unique approach to Lyme disease and other related disorders in the area of diagnosis or treatment may be submitted for review. An abstract of 250 words or less should accompany the text.

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Letters to the Editor in the form of correspondence related to material published in the Journal or some aspect of Lyme borreliosis and other spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the Journal will be referred to the author of the original work for a response. Letters to the Editor should be submitted in duplicate, typewritten and double-spaced, not exceeding 400 words of text and a maximum of five references. Letters should have no more than three authors, and all should sign a letter. Please include a word count. Receipt of letters is not acknowledged, but correspondents will be notified when a decision regarding publication is made.

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Editorials may be published, usually at the solicitation of the Associate Editors, but unsolicited submissions that relate to an unusual topic of interest exceeding the usual designation of correspondence, i.e., 1000 words or less will be considered.

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The topical photographic section will be a regular feature. Photographs pertinent to articles presented in the *Journal* as well as other photographs related to any aspect of spirochetal or tick-borne diseases will be considered for the publication. The guidelines for the submission are designated in **Illustrations.**

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The Journal asks authors of research articles to disclose at the time of submission any financial or other arrangements they may have with a company whose product figures in the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, the editors will usually discuss with the authors the manner in which such information is to be communicated to the reader.

Because the essence of reviews and editorials is selection and interpretation of the literature, the Journal expects that authors of such articles will not have any financial or other interest in a company (or its competitor) that makes a product discussed in the article. Potential authors who have questions about these issues should contact the Editor-in-Chief.

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Journal of Spirochetal and Tick-Borne Diseases
Lyme Disease Foundation
1 Financial Plaza
Hartford, CT 06103
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Manuscripts containing original material are accepted with the understanding that neither the article nor any part of its essential substance has been or will be published or submitted for publication elsewhere before appearing in the Journal.

All manuscripts should be submitted with a cover letter indicating the category for which the manuscript should be reviewed. Copies of any closely related manuscripts should be submitted to the Editor along with the manuscript that is to be considered by the Journal. A cover letter, signed by all authors, should identify the person (with the address and telephone number) responsible for negotiations concerning the manuscripts; the letter should make it clear that the final manuscript has been seen and approved by all authors and that they have taken due care to ensure the integrity of the work. Manuscripts should include a title page, abstract, and text with tables, illustrations, and references below. For the integrity of the published material, manuscripts describing clinical aspects of Lyme borreliosis must disclose: criteria for patient enrollment into the study and criteria for defining ''successful'' or ''nonsuccessful'' Lyme borreliosis treatment. Manuscripts without these requirements will be automatically rejected.

Titles and Author's Names

With the manuscript, provide a page giving the title of the article; titles should be concise and descriptive (not declarative). Also include a running head of fewer than 40 letter spaces; the name(s) of the author(s), including the first name(s) and academic degree(s); the name of the department and institution in which the work was done; the institutional affiliation of each author; and the name and address of the author to whom reprint requests should be addressed. Any grant support that requires acknowledgement should be mentioned on this page.

Abstract

Provide on a separate page an abstract of not more than 300 words (original and review articles) or 250 words (case report). This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed, the results, and what the authors conclude from the results.

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All material should be typed double spaced. Standard sequence of methods and materials, results, and discussion should be employed with tables and figures numbered in the order in which they are cited in the text.

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Submit tables typed double spaced and provide a heading for all columns with a comprehensive title on separate sheets.

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Journal of Spirochetal and Tick-Borne Diseases

Topic review for upcoming issues:

March 1994

Overview of Spirochetal and Tick-Borne Disease
Guest Editors: *Martina H. Ziska, M.D. and Richard C. Tilton, Ph.D.*

June 1994

International View on Spirochetal and Tick-Borne Diseases
Guest Editors: *Willy Burgdorfer, Ph.D., M.D. (Hon.) and Klaus Weber, M.D.*

September 1994

Aspects of Lyme Borreliosis Pathogenesis
Guest Editor: *Claude F. Garon, Ph.D.*

December 1994

Clinical Manifestations of Lyme Borreliosis: An Enlarging Spectrum?
Guest Editor: *Kenneth B. Liegner, M.D.*

March 1995

Focusing on the Tick-Borne Diseases in North America:
Relapsing Fever, Ehrlichiosis, and Babesiosis
Guest Editor: *Tom G. Schwan, Ph.D.*

June 1995

Treponemal Biology and Pathogenesis at the Cellular and Molecular Level
Guest Editor: *James N. Miller, M.D., Ph.D.*

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Editorial content: Topics relating to understanding disease mechanisms and the application of better diagnostic techniques and treatment strategies for all individuals suffering from spirochetal and tick-borne diseases.

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Journal of Spirochetal and Tick-Borne Diseases

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The *Journal of Spirochetal and Tick-Borne Diseases*
wants to encourage potential authors
to submit manuscripts
to all sections of the Journal.
Manuscripts without requirements, designated in the ''Information to Contributors,'' will be automatically rejected.

CALL FOR PHOTOGRAPHS

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for publication in the Photographic Section of the Journal.
The Editor-in-Chief should be contacted for further details.

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JOURNAL OF SPIROCHETAL AND TICK-BORNE DISEASES

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Editorial

We welcome you to Volume 1, Number 1 of the *Journal of Spirochetal and Tick-Borne Diseases,* the first Journal devoted solely to all aspects of spirochetal and tick-borne diseases. The increasing prevalence and significance of these diseases in the society has been recognized and reflected in the *Journal*. The purpose of the *Journal* is to encourage basic and applied research on a broad range of topics related to understanding disease mechanisms, applying better diagnostic techniques and treatment strategies for all individuals suffering from spirochetal or tick-borne diseases.

There have been many concerns among the medical journal publishers and some authors about the research integrity and unbiased review process of the published scientific articles. In order to guarantee the highest quality of the peer review process, the *Journal of Spirochetal and Tick-Borne Diseases* incorporated the statement of the International Committee of Medical Journal Editors from January 1993 as part of its editorial policy. These guidelines reflect the *Journal's* commitment to initiate open scientific discussion in order to further our understanding of disease processes and to insure the scientific integrity.

An exciting year is planned with topics of interest to all of us involved in the various aspects of spirochetal or tick-borne diseases. The *Journal* will expand to the following regular sections: Reviews, Peer Reviewed Articles, Case Reports, and Letters to the Editor. The special photographic section will be a regular feature. Every physician and researcher is encouraged to submit a manuscript and/or photographs. **The *Journal of Spirochetal and Tick-Borne Diseases* is the platform to demonstrate and share your clinical experiences and research results with your colleagues.** Your active support is needed.

We have a long way to go. The *Journal* will be a new experience for all of us. We hope that our readers will find the Journal medically interesting and educationally beneficial. The Board welcomes your suggestions as well as unsolicited commentaries, reviews, or letters to the editor for informational purposes or for publication.

Martina H. Ziska, M.D. and Richard C. Tilton, Ph.D.

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Paul E. Lavoie, M.D.: An Appreciation

The first issue of the *Journal of Spirochetal and Tick-Borne Diseases* is dedicated to Paul E. Lavoie in recognition of his distinguished service to the Lyme Disease Foundation (LDF). Paul has participated actively in the LDF as a frequent speaker at its national conferences, as a Board Member since 1990, and as the Chairman of the Scientific Medical Committee since 1991. His papers at LDF conferences have contributed to our understanding of the clinical spectrum of Lyme disease in the far-western United States and dealt with such controversial issues as the failure of published (i.e., short-term) antibiotic regimens for treating Lyme disease.

Paul has been equally active in other medical and scientific societies. He has attended all five international conferences on Lyme borreliosis and presented papers or posters at the second through fifth conferences. He also presented 17 papers or posters at 13 western regional or national meetings of the American College of Rheumatology since 1982; all but two of these presentations concerned Lyme disease or other borrelial infections. Moreover, he has regularly attended and participated in the International Northwestern Conference on Diseases in Nature Communicable to Man since the mid-1980s. He has been invited twice by the National Institutes of Health to participate in State-of-the-Art Conferences on Diagnosis and Treatment of Lyme Disease and once by the Centers for Disease Control and Prevention to participate in a special conference to discuss the national surveillance case definition for Lyme disease.

He has held several academic medical appointments in San Francisco, California, including those at the University of California, California Pacific Medical Center, Fort Miley Veterans Administration Hospital, and St. Mary's Hospital. During his tenure at the University of California at San Francisco, he rose from Clinical Instructor of Medicine in 1973 to Clinical Professor of Medicine in 1993. Further, he has published 25 abstracts, book chapters or articles so far, many of which were co-authored with other physicians or scientists. Highlights of these publications include the description of several of the initial cases of Lyme disease recognized from California (with J Campagna, NS Birnbaum, and DP Furman) and the first description of acrodermatitis chronica atrophicans, a dermatologic manifestation of late Lyme disease, from western North America (with AJ Wilson and DL Tuffanelli).

Perhaps Paul's greatest contribution, however, has been his devoted effort to bridge the gap between the clinician and the biomedical community. During his 21 years of clinical practice in internal medicine and rheumatology, Paul has treated literally hundreds of patients afflicted with Lyme disease, many of whom had difficult-to-treat late-stage manifestations. He spends many hours with each of his patients, listening to their complaints and treating them with compassion and kindness while devising individualized treatment regimens. Extensive experience taught Paul that many patients with late Lyme disease could not be cured with standardized antibiotic regimens. Therefore, he developed long-term antibiotic regimens that brought considerable relief to, as well as significant reduction of symptoms for, many patients who failed to respond to short-term therapies for the disease. He communicated his findings routinely at local, national, and international meetings that, though often controversial, induced many physicians to at least reconsider their approach for treating patients with late manifestations of Lyme disease. In recognition of his contributions, The Lyme Disease Resource Center and the Lyme Disease Foundation awarded him the Distinguished Physician Award in 1993 and established an annual award in his name.

Both of us have had the pleasure of knowing Paul for about a decade and consider it a privilege and an honor to dedicate the first issue of the Journal to our friend and colleague. We can think of no one more deserving of this accolade than Paul who contributes so much to the LDF and his profession.

Note added in proof: we were greatly saddened to learn of Paul's death in Mill Valley, California on 23 January, 1994.

Robert S. Lane and Willy Burgdorfer

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Overview of the Pathogenic Spirochetes

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The spirochetes are a unique group of bacteria that can be distinguished morphologically from most other bacteria based on their large size and helical or corkscrew-shaped appearance. They also possess flagella that are internal rather than extracellular like most other motile organisms. Spirochetes usually cause diseases that are nonacute upon initial exposure of the host to the infectious agent, but they can have devastating consequences on the human body later on, in the absence of curative antibiotic therapy.

The sexually transmitted treponemal disease, syphilis, has been with us for many centuries, perhaps as far back as Biblical times, whereas the tick-borne borrelial-caused illness, Lyme disease, has only been recently described as a serious clinical entity. Both of these pathogens can cause a variety of multi-system disorders that can be easily confused with other infectious or noninfectious illnesses. Early diagnosis of syphilis and Lyme disease is essential for successful antibiotic treatment and prevention of chronic debilitating sequelae. Other spirochetal infections caused by *Borrelia, Leptospira*, and treponemes are uncommon in developed countries but still can cause significant morbidity in various other parts of the world.

All spirochetal infections rely heavily on serologic techniques for verifying or establishing the diagnosis. Nonmedical preventive measures include avoiding contact with (a) the appropriate insect vector; (b) an infected sex partner or body fluid; and (c) contaminated eating utensils or skin lesions. Vaccines for human use are unavailable except for experimental ones now being tested for the immunoprophylaxis of Lyme disease.

Key words: Pathogenic spirochetes, *Borrelia*, *Treponema*, *Leptospira*, Lyme disease, syphilis, spirochetal infections

This article is meant to be a minireview of the pathogenic spirochetes, highlighting their unique basic biological properties and the important clinical, pathologic, and diagnostic entities and immune phenomena associated with the diseases caused by them. Major emphasis will be placed on the causative agents of Lyme disease (*Borrelia* (*B.*) *burgdorferi*) and syphilis (*Treponema* (*T.*) *pallidum*), since these represent the most common spirochetal diseases in North America and have generated the most interest and discussions among clinicians, scientists, patients, and other members of the lay public.

BASIC BIOLOGY OF THE SPIROCHETAL BACTERIA

Spirochetes are a highly specialized group of motile gram-negative spiral-shaped bacteria (Table 1), usually having a slender and tightly helically coiled structure. They range from 0.1 to 0.5 μm in width and from 10 to 50 μm in length. One of the unique features of spirochetes is their motility by a rapidly drifting rotation, often associated with a flexing or undulating movement along the helical path. Such locomotion is due to the presence of axial fibrils, also known as flagella, that are wound around the main body (protoplasmic cylinder) and enclosed by the outer cell wall or sheath of these organisms. These bacteria belong to the order Spirochaetales, which includes two families: Spirochaetaceae and Leptospiraceae. Important members of these groups include the genera *Borrelia, Leptospira*, and *Treponema*.

The spirochetes are generally a fastidious group of bacteria, i.e., they can be difficult to grow (and therefore to study) in the laboratory, often requiring highly specialized media and culture conditions (such as low oxygen tension) in order to optimize their replicating capabilities. Some, such as *T. pallidum*, can only be maintained consistently in a replicating state by *in vivo* passage in rabbits. The spirochetes live primarily as extracellular pathogens, rarely, if ever, growing within a host cell. Unlike most bacteria, spirochetes do not stain well with aniline dyes such as those used in the Gram stain procedure. Their cell walls do, however, resemble structurally and biochemically those of other Gram-negative bacteria and are thus classified within this very large group of bacteria. The best way to visualize spirochetes is through the use of dark-field or phase-contrast microscopy or after staining with a fluorochrome dye, such as acridine orange (1), and then viewing under a microscope equipped for fluorescence microscopy (Figure 1). When present, their appearance in tissue specimens can often be revealed by the silver-staining technique.

The infections caused by the spirochetes are important public health problems throughout the world leading to such diseases as Lyme and the relapsing fever borrelioses, syphilis and the other treponematoses, and leptospirosis (Table 2). A better understanding of the molecular biology, pathogenesis, and immunobiology of the disease-causing spirochetes has become crucial in efforts to develop effective vaccines, because there has been no significant modification in excessive sexual activity, personal hygiene practices, or vector control. Further knowledge of immune responses to spirochetes is essential for their eventual control by immunization, and studies of the host-spirochete relationship have led to important new insights related to the immunobiology of these pathogens. Serologic techniques have now become indispensable diagnostic tools for detection of many of the spirochetal diseases, especially Lyme disease and syphilis. Unfortunately, as may occur in other infectious processes, the host response to the spirochetes, as part of the normal protective mechanisms, may paradoxically cause an immunologically induced disease in the affected individual, leading to the complications of arthritis

TABLE 1
Unusual Features of the Pathogenic Spirochetes

Large bacteria: up to 50 microns in length, but very thin in diameter; compared with other bacteria (e.g., cocci and rods are 1 to 3 μ in length), red blood cell diameter is 6 to 8 μ; they also exhibit a unique spiral, helical shape.
Most of them require special staining techniques and microscopy for visualization, such as silver stain, fluorescence, or dark-field microscopy.
They exhibit a slow rate of growth: 24- to 33-hour division time *in vivo*; compare with *E. coli*: 20 minutes.
They are extremely sensitive to elevated temperatures (≥38°C).
Pathogenic treponemes *cannot* be cultivated on artificial media; other spirochetes can be grown with some difficulty or with special media.
They cause chronic, stage-related and sometimes extremely debilitating or crippling disease in the untreated host.
They do *not* seem to produce toxins.
The interplay or interrelationship between the invading spirochetes and the subsequent host response as factors in the disease process have yet to be clearly or fully defined.
They have endoflagella intertwined between the cell wall and protoplasmic cylinder—also called axial fibrils. Most bacterial flagella are extracellular.
Most pathogenic spirochetes (*Borrelia, Treponema*) are microaerophilic (once thought to be anaerobes).
B. burgdorferi are the most unique organisms in that they have linear plasmids that code for outer-surface proteins.

and the neuropathies of Lyme disease, as well as aortitis, immune-complex glomerulonephritis, and the gummatous lesions of syphilis.

LYME DISEASE: GENERAL FEATURES

In the mid-1970s, a geographic clustering of an unusual rheumatoid arthritis-like condition involving mostly children and young adults occurred in northeastern Connecticut. This condition proved to be a newly discovered disease, named Lyme disease after the town of its origin (2). The arthritis is characterized by intermittent attacks of asymmetric pain and swelling primarily in the large joints (especially the knees) over a period of a few years. Epidemiologic and clinical research showed that the onset of symptoms was preceded by an insect bite and unique skin rash probably identical to that of an illness following a tick bite, first described in Europe at the turn of the century (3). The beneficial effects of penicillin or tetracycline in early cases suggested a microbial origin (likely, bacterial) for what was initially called Lyme arthritis.

Lyme disease is now the most common tick-transmitted illness, and it has been reported in at least 43 states. However, it occurs primarily in three geographic regions: the coastal areas of the Northeast from Maine to Maryland, the Midwest in Wisconsin and Minnesota, and the far West in parts of California and Oregon. These geographic areas parallel the location of the primary tick vector of Lyme disease in the United States—*Ixodes scapularis* (formerly *dammini*) in the East and Midwest and *Ixodes pacificus* in the far West. Lyme disease has been reported in many other countries, especially in western Europe, corresponding to the distribution of *Ixodes ricinus* ticks. The greatest concentration of cases is in the northeastern United States, particularly in New York state, where the disease is endemic on Long Island and just north of New York City in neighboring Westchester County.

In the early 1980s, spirochetal organisms were isolated and cultured from the midguts of *Ixodes* ticks taken from Shelter Island, NY (an endemic focus), and shortly thereafter they were cultured from the skin rash site, blood, and cerebrospinal fluid of patients with Lyme disease. This newly discovered spirochete, called *B. burgdorferi*, is microaerophilic, resembles other spirochetes morphologically, and is slightly larger than the treponemes. Unlike the pathogenic treponemes, *B. burgdorferi* can be readily cultivated *in vitro* in a highly fortified growth media (4).

Protection to *B. burgdorferi* may develop slowly, and it is unclear whether resistance to reinfection occurs. Experimental animal studies have shown that immune sera can transfer protection to normal recipients challenged with *B. burgdorferi* (5). Monoclonal antibodies to borrelial outer surface proteins are also protective (6) and have thus become the major target antigens for a vaccine.

CLINICAL ASPECTS

Lyme disease is an illness having protean manifestations with symptoms that include the following: (1) an erythematous-expanding red annular rash with central clearing; (2) fever, headache, stiff neck, nausea, and vomiting; (3) neurologic complications such as facial nerve (Bell's) palsy

TABLE 2
Epidemiology of Spirochetal Infections

Pathogenic Spirochete	Human Disease	Vector or Source of Infection
Borrelia		
B. burgdorferi	Lyme disease	Ixodid ticks
B. recurrentis	Epidemic relapsing fever	Body louse, ped. humanus
B. hermsii		
B. turicatae	Endemic relapsing fever	Ornithodoros ticks
B. parkeri		
Leptospira		
Leptospira interrogens	Leptospirosis (Weil's Disease)	Exposure to contaminated animal urine
Treponema		
T. pallidum subspecies (ssp) pallidum	Syphilis	Sexual contact, transplacental
T. pallidum ssp endemicum	Bejel (endemic syphilis)	Direct contact with contaminated eating utensils
T. pallidum ssp pertenue	Yaws	Direct contact with infected
T. carateum	Pinta	skin lesions

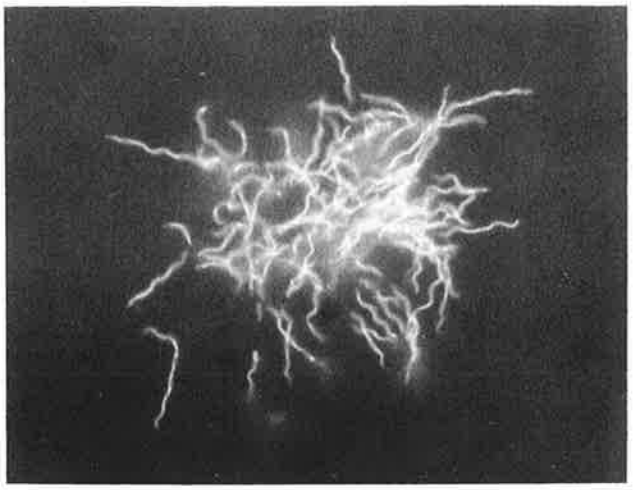


FIG. 1. Photomicrograph of *B. burgdorferi* (strain B31 in BSK media) after staining with the fluorochrome dye, acridine orange. Magnification times 500. Note the colony formation or clumping phenomenon of this low-passage *in vitro* culture.

and meningitis; and (4) arthritis in about 50% of untreated patients (7). These symptoms occur most frequently from May to November, when ticks are active and numerous and people are engaged in many outdoor activities. The most characteristic feature of early Lyme disease is a skin rash, often referred to as erythema migrans (EM), which appears shortly (3 to 32 days) after a bite from an infected tick. The lesion typically expands almost uniformly from the center of the bite and is usually flat or slightly indurated with central clearing and reddening at the periphery. It is noteworthy, however, that many Lyme disease victims do not recall being bitten by a tick or do not apparently develop classic EM. On the other hand, at various intervals after the initial rash, some patients develop similar but smaller multiple secondary annular skin lesions that last for several weeks to months. Biopsy of these skin lesions reveals a lymphocytic and plasmacytic infiltrate. Various flulike symptoms such as malaise, fever, headache, stiff neck, and arthralgias are often associated with EM. The extracutaneous manifestations of Lyme disease may include migratory and polyarticular arthritis, neurologic and cardiac involvement with cranial nerve palsies and radiculopathy, myocarditis, and arrhythmias. Lyme arthritis typically involves a knee or other large joint. It may enter a chronic phase, leading to destruction of bone and joints if left untreated. Interestingly, Lyme arthritis is less common in Europe than the United States, but neurologic complications are more prevalent in Europe. Unique strain variations expressing antigenic subtypes between European and North American isolates of *B. burgdorferi* probably explain these dissimilarities (8).

In most cases, humoral and cell-mediated immune responses are activated during borrelial infection (7). Antibody, mostly of the IgM class, can be detected shortly after the appearance of EM; thereafter, there is gradual increase in overall titer and a switch to predominant IgG antibody response for the duration of an untreated infection. Most notably, very high levels of antibody have been found in serum and joint fluid taken from patients with moderate to severe arthritis. Although the presence of such high antibody titers against *B. burgdorferi* may reduce the spirochete load somewhat, they appear not to ameliorate the disease process completely and, indeed, may actually contribute to some of the pathologic changes. These serologic responses form the basis of laboratory tests designed to aid in the diagnosis of Lyme borreliosis. On the basis of lymphocyte transformation assays, peripheral blood T cells from Lyme disease patients respond to borrelial antigens primarily after early infection and following successful treatment (9, 10). Also, addition of antigens to synovial cells *in vitro* from infected patients triggers the production of interleukin-1, which could account for many of the harmful inflammatory reactions associated with this disease (11). Human mononuclear and polymorphonuclear phagocytes can both ingest and presumably destroy *Borrelia* (12, 13). Thus, borrelial antigen-stimulated T cells or their products may activate macrophages, limiting dissemination and resulting in enhanced phagocytic activity and the eventual clearance of spirochetes from the primary lesion.

DIAGNOSIS

Clinically, Lyme disease mimics other disorders, many of which are not infectious and therefore would ordinarily not be responsive to antibiotic therapy. Because *B. burgdorferi* is the causative agent, demonstration of the organism in suspected cases is the most definitive diagnosis. However, in the vast majority of cases, the Lyme spirochete cannot be isolated or identified, and immune responses (antibody production) specific for *B. burgdorferi* must be used to confirm the diagnosis (14). Unfortunately, the antibody response is often not detectable in the early treatable stage and the clinical impression cannot always be confirmed.

Isolation of the spirochete unambiguously confirms the diagnosis of Lyme borreliosis. Recovery of *B. burgdorferi* is possible, but the frequency of isolation from the blood or other body fluids of acutely ill patients is very low (1, 15, 16). Better success rates in isolating *Borrelia* have been achieved after culturing skin-biopsy specimens of clear-cut EM rashes (17). Despite such success, borrelial cultivation can usually only be done in a few laboratories or institutions because the medium is expensive, and cultures require up to 8 to 12 weeks of incubation for detection of the spirochetes (1).

Visualization of the spirochetes in tissue or body fluids has also been used to diagnose Lyme borreliosis (18). In the early stage of the disease, when erythema migrans is present, the Warthin-Starry or modified Dieterle silver stain can identify spirochetes in one-half or more of skin biopsies obtained from the outer portion of the lesion (19). However, few microorganisms are present, and they can be confused with normal skin structures by inexperienced laboratory personnel. Immunohistologic examination of tissue has rarely been successful in determining the presence of *Borrelia*, and in chronic Lyme disease, spirochetes are rarely detectable by any microscopic technique (20).

Serologic tests are, for all practical purposes, the only detection systems routinely available for the confirmation of Lyme borreliosis. One of the standard serological tests, either an enzyme-linked immunosorbent assay (ELISA) or an indirect immunofluorescence assay (IFA) (14), is available in many public and private laboratories. Blood samples obtained within 3 weeks of the onset of erythema migrans are frequently serologically negative in both assays (21). In addition, these assays have not been standardized, with laboratories using different antigen preparations and "cut-off" values. Workers, using the same set of sera, have reported interlaboratory variation in results and interpretations (22, 23). There is also considerable variability in the

serologic response pattern of patients with Lyme disease. Finally, if antibiotics are administered during early illness, antibody production can be aborted or severely curtailed (24, 25).

The existence of antigenically different strains of *B. burgdorferi* throughout the world (8) may account for some of the variability in antibody response. In addition, assays currently either use the whole spirochete or a crude bacterial sonicate, as antigen. With these assays, cross-reactions have been observed with other spirochetes, in particular *T. pallidum* and the relapsing fever *Borrelia* species (25).

Attempts to improve antibody detection have used Western (immunoblot) analysis for the detection of IgM and IgG antibodies and have used purified flagellin antigen in the ELISA. Immunoblots are more sensitive and more specific than ELISAs (26). Although not standardized, commercial Western immunoblot test kits are now being offered to further verify a routine serologic test result, especially in troublesome cases. Some studies have shown, however, that immunoblotting could not overcome the inability to detect antibody during the first 3 weeks of infection (27). The performance of the ELISA has been improved by the use of purified flagellin protein as antigen (28). Antibodies to the 41-kDa flagellum-associated component peak at 6 to 8 weeks. Unfortunately, epitopes on this antigen are shared by many other spirochetes, and neither IgM nor IgG antibodies to this antigen are specific for *B. burgdorferi* (27).

The prevailing sentiment within (as well as outside) the Lyme disease research and diagnostic community is that serological verification of this disorder is fraught with difficulties. False negative results are likely to occur if serum is obtained within 4 weeks of initial infection or if the patient has been treated with antibiotics. False positives occur if large numbers of patients with a low *a priori* probability of having Lyme borreliosis are examined. Interlaboratory agreement on what constitutes a positive varies in part, because methods for the preparation of antigen and for the absorbance of cross-reacting antibodies varies among the test systems developed by individual laboratories who wish to establish their own "in-house" testing. Clearly, serologic testing for the early diagnosis of Lyme disease is at an inadequate juncture (14). Compounding this problem, diagnosis of initial infection can be difficult clinically since only 60 to 75% of patients with Lyme borreliosis present with or recall erythema migrans, or have a clear and consistent epidemiologic history (29).

Recently, attempts meant to address these apparent shortcomings in serologic testing have led to the development of nonserologic diagnostic procedures such as the polymerase chain reaction (PCR) and the lymphocyte proliferation assay. By using the PCR and selective probes, it is possible to detect a single organism in a serum or tissue sample, and such gene amplification procedures show great promise for the early detection of *B. burgdorferi* (30, 31). In this regard, using primers directed at the rRNA genes of *B. burgdorferi*, our research group has identified the Lyme disease spirochete directly from skin biopsy material (32) as well as from short-term cultures of tissue extracts (33). From a realistic standpoint, however, and because it may be technically demanding and not cost-effective for most diagnostic labs handling just a few specimens, PCR may continue to be primarily a research tool rather than a routine diagnostic procedure.

While PCR-based procedures seem promising as an exquisite and novel diagnostic tool for selective stages of Lyme disease, serological testing, for a variety of reasons, will continue to be the mainstay for the laboratory detection of the vast majority of Lyme disease cases, as it currently is for syphilis (caused by a related spirochete) and for certain other infectious and non-infectious disorders. Nonetheless, continued refinements along these lines will be needed and should be geared toward developing as economical a system as possible combined with one having optimal sensitivity and specificity.

Finally, attention has focused recently on an assay system (9, 10) designed to measure past or current exposure to *B. burgdorferi* by virtue of the patient's lymphocytes to respond *in vitro* to undergo DNA synthesis in the presence of specific borrelial antigens. This laboratory procedure is generally considered to be a good *in vitro* correlate of the classic DTH reaction (34) commonly used to measure *in vivo* tuberculin sensitivity. For purposes related to Lyme disease, it has been reported (10) that these lymphocyte proliferation assays have been helpful in identifying patients with active disease in the absence of detectable antibody (serologic) responses. Like serologic tests, however, this assay has yet to be standardized, and there is growing concern over the evidence (35) for elevated responses occurring in some healthy controls, thereby possibly limiting the usefulness of this technique.

PROPHYLACTIC MEASURES

Avoiding *Borrelia*-infected ticks or tick-infested areas will guarantee protection against Lyme infection. For those living in endemic areas, a few simple precautions will help minimize possible exposure. These include wearing clothing that fully protects the body and using repellents that contain DEET (diethyltoluamide). If a tick does attach to the skin, careful removal with tweezers shortly after it attaches, followed by application of alcohol or another suitable disinfectant will make borrelial transmission unlikely.

Considerable attention has now turned toward the development of a vaccine for Lyme disease. A canine vaccine consisting of whole inactivated organisms (Bacterin) has existed for a few years (36), whereas those being developed for humans consist of recombinant outer surface proteins of *B. burgdorferi*. Early human clinical trials of a recombinant-derived vaccine have now begun involving a few selected research centers throughout the United States including here at New York Medical College and Westchester Medical Center. It is not known, however, how successful these human trials will be, nor will this information be available for at least several years. It is important to realize, nevertheless, that the development of such vaccines still requires answers to many questions, such as those that follow:

1. What type of vaccine will induce maximal antibody responses?
2. What type of vaccine will induce maximal cell-mediated immunity?
3. Is long-lasting protection against the disease achievable, and does it depend on antibodies or cellular immune responses?
4. Is this protection limited to only a few of the target organ sites or is it complete?
5. Will a vaccine provide cross-protection against all tick-transmitted *B. burgdorferi* infections, or will it affect only some?
6. Are adjuvants useful or necessary? Which ones should be used?
7. Can vaccine formulations be prepared in such a way to

avoid the development, or minimize the risk, of undesirable side effects?

The possibility of developing vaccines that prevent *Borrelia* infections has gained major impetus by recent reports describing considerable success in protecting animals from experimental *B. burgdorferi* infection by immunizing them with inactivated spirochetes (5) or with recombinant borrelial protein [outer surface protein A (OspA)] (37, 38). It has also been shown (6) that monoclonal antibodies directed against OspA could protect mice against *B. burgdorferi* infection and the development of disease.

Although these advancements make the production of protective immunogens much more credible, problems exist in the conceptualization, design, and implementation of practical vaccination regimens or formulations. Except for one recent report (38) describing an immunoprotective lipoprotein, most other experimental vaccine studies have relied on the use of recombinant proteins incorporated with toxic adjuvants that would be unacceptable for human use. Another important limiting factor to active immunization is our lack of full understanding of how host defense mechanisms interact in controlling the spread of *Borrelia* from the primary lesion site. For despite the accumulation of a relatively large amount of clinical and experimental data (7), it is still unclear what the related roles of humoral and cell-mediated immunity are in the pathogenesis of, and protection against, acute and chronic disease. Current evidence (5, 6, 9, 10, 12, 13) suggests the involvement of both forms of immunity, although to what extent each contributes to the elimination of this spirochete, or restricts its growth *in vivo*, or confers long-term protection, is just now beginning to emerge. This information is crucial for the purpose of developing meaningful immunization strategies likely to be effective in preventing Lyme disease.

RELAPSING FEVER BORRELIOSIS

Relapsing fever is an acute febrile disease of worldwide distribution and is caused by arthropod-borne spirochetes belonging to the genus *Borrelia*. Two major forms of this illness are louse-borne relapsing fever (for which humans are the reservoir, and the body louse, *Pediculus humanus*, is the vector) and tick-borne relapsing fever (for which rodents and other small animals are the major reservoirs, and ticks of the genus *Ornithodoros* are the vectors). *Borrelia recurrentis* causes louse-borne relapsing fever and is transmitted from human to human, following the ingestion of infected human blood by the louse and subsequent transmission of spirochetes onto the skin or mucous membranes of a new host when the body louse is crushed. The disease is endemic in parts of Central and East Africa and South America. The causative organisms of tick-borne relapsing fever are numerous and include *B. hermsii, B. turicatae*, and *B. parkeri* in North America; *B. hispanica* in Spain; *B. duttonii* in East Africa; and *B. persica* in Asia. Ticks become infected by biting and sucking blood from a spirochetemic animal. The infection is transmitted to humans or animals when saliva is released by a feeding tick through bites or penetration of intact skin.

After an individual has been exposed to an infected louse or tick, *Borrelia* penetrate the skin and enter the bloodstream and lymphatic system. After a 1- to 3-week incubation period, spirochetes replicate in the blood, and there is an acute onset of shaking chills, fever, headache, and fatigue. Concentrations of *Borrelia* can reach as high as 10^8 spirochetes/mL of blood, and these are clearly visible after staining blood smears with Giemsa or Wright's stain. During the febrile disease, *Borrelia* are present in the patient's blood but disappear prior to afebrile episodes and subsequently return to the bloodstream during the next febrile period. Jaundice can develop in some severely ill patients as a result of intrahepatic obstruction of bile flow and hepatocellular inflammation; if left untreated, patients can die from damage to the liver, spleen, or brain. The majority of untreated patients, however, recover spontaneously. They produce borrelial antibodies that have agglutinating, complement-fixing, borreliacidal, and immobilizing capabilities and that render patients immune to reinfection with the same *Borrelia* serotype. Serologic tests designed to measure these antibodies are of limited diagnostic value because of antigenic variation among strains and the coexistence of mixed populations of *Borrelia* within a given host during the course of a single infection. Diagnosis in the majority of cases requires demonstration of spirochetemia in febrile patients.

LEPTOSPIROSIS

Leptospirosis is an acute, febrile disease caused by various serotypes of *Leptospira*. Often referred to as Weil's disease, infection with *Leptospira interrogans* causes diseases that are extremely varied in their clinical presentations and that are also found in a variety of wild and domestic animals. Transmission to humans occurs primarily after contact with contaminated urine from leptospiruric animals. In the United States, dogs are the major reservoir for exposure of humans to this disease. The routine vaccination of dogs against *Leptospira* is probably an important preventive measure. After entering the body through the mucosal surface or breaks in the skin, leptospiral bacteria cause an acute illness characterized by fever, chills, myalgias, severe headaches, conjunctival suffuseness, and gastrointestinal problems. Most human infections are mild and anicteric, although in a small proportion of victims, severe icteric disease can occur and be fatal, primarily as a result of renal failure and damage to small blood vessels. After infection of the kidneys, leptospiras are excreted in the urine. Liver dysfunction with hepatocellular damage and jaundice is common. Antibiotic treatment is curative if begun during early disease, but its value thereafter is questionable.

Diagnosis of leptospirosis depends upon either seroconversion or the demonstration of spirochetes in clinical specimens. The macroscopic slide agglutination test, which uses formalized antigen, offers safe and rapid antibody screening. Measurement of antibody for a specific serotype, however, is performed with the very sensitive microscopic agglutination test involving live organisms. This method provides the most specific reaction with the highest titer and fewer cross-reactions. Agglutinating IgM-class-specific antibodies are produced during early infection and persist in high titers for many months. Protective and agglutinating antibodies often persist in sera of convalescent patients and may be associated with resistance to future infections.

SYPHILIS: GENERAL FEATURES

The origin and history of syphilis are filled with many mysteries and hypotheses. Biblical references suggest its presence in early civilization. Other evidence points to its prevalence primarily after Columbus and his crew returned

to Europe from the New World in 1493. From that point on, syphilis spread throughout Europe affecting all levels of society including political and religious leaders. Indeed, the dementia and insanity associated with late-stage syphilis that may have occurred in certain afflicted rulers or monarchs probably changed the course of history during the 16th and 17th centuries. In the preantibiotic era, many toxic drugs were used for treatment, and as early as 1905, a blood test was developed for the diagnosis of syphilis; the so-called Wasserman test—the prototype for the current nonspecific serologic tests designed to measure antibodies to cardiolipin.

Syphilis is still a significant worldwide problem and, after gonorrhea and chlamydial infections, it is the third most common sexually transmitted disease in the United States. The most recent rise in heterosexual infection (Fig. 2) has paralleled an alarming increase in congenital syphilis in many urban areas where drug abuse and the frequent exchange of sexual services for drugs are common practices among those who use illicit drugs.

Treponema pallidum is the spirochetal bacterium that causes syphilis that, if left untreated, can have severe pathologic effects leading to irreversible damage to the cardiovascular, central nervous, and musculoskeletal systems. The organism cannot be grown on artificial media; it is highly motile, infectious, and it replicates extracellularly and very slowly *in vivo*. Limited growth in tissue culture has been achieved, but this pathogen must still be passaged *in vivo* using rabbits. Because of this problem, it has taken many years of research in order to acquire our current understanding of the *treponemes* and has probably delayed efforts toward any possible vaccine development. *Treponema pallidum*, like the other treponemes, is shorter and more tightly coiled than the *Borrelia*.

With the institution of antibiotic therapy in the mid-1940s, the incidence of syphilis fell sharply from a high of 72 cases per 100,000 in 1943 to about 4 per 100,000 in 1956. During the 1970s and early 1980s, syphilis increased rapidly within the homosexual community and, for the past several years, the Centers for Disease Control and Prevention has periodically reported significant increases (Fig. 2) in primary and secondary cases. Such findings can be attributed, in part, to changing lifestyles, sexual practices, and other factors such as an unusually high prevalence and reduced efficacy of antibiotics in patients with acquired immunodeficiency syndrome (AIDS). Syphilis continues to rank annually as the third or fourth most frequently reported communicable disease in the United States.

The course of syphilis in humans is marked by several interesting phenomena. Without treatment, the disease will usually progress through several well-defined stages (somewhat resembling Lyme disease). This is unlike most other infectious diseases, which are ultimately eliminated by the host's immune system or, in severe cases, result in death. The relatively slow generation time of treponemes, which is estimated at 30 to 33 hours, contributes to this unique course. During the first two stages (primary and secondary syphilis), there is almost unimpeded rapid growth of *T. pallidum*, leading to an early infectious spirochetemic phase of the disease. The third stage (tertiary syphilis) occurs much later, following a prolonged latency period. Alterations in this stage are due primarily to tissue-damaging immune responses elicited by small numbers of previously deposited or disseminated spirochetes.

Syphilis activates both humoral and cell-mediated immunity, but this protection is only partial. The relative importance of each type of immune response is not fully known. Protective immunity against re-exposure is incomplete, especially during early stages, when it develops relatively slowly.

CLINICAL ASPECTS

The severe late manifestations or complications of syphilis occur in the blood vessels and perivascular areas. However, sexual contact is the common mode of transmission, with inoculation on the mucous membranes of genital organs.

The first clinically apparent manifestation of syphilis (primary syphilis) is an indurated, circumscribed, relatively avascular and painless ulcer (chancre) at the site of treponemal inoculation. Spirochetemia with secondary metastatic distribution of microorganisms occurs within a few days after onset of local infection, but clinically apparent secondary lesions may not be observed for 2 to 4 weeks. The chancre lasts 10 to 14 days before healing spontaneously.

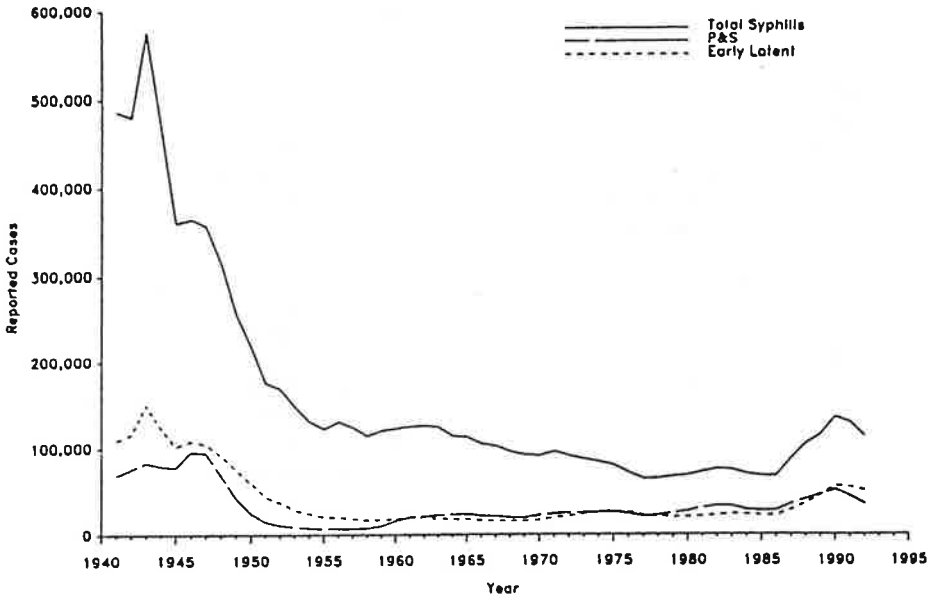
The presence of metastatic infection (secondary syphilis) is manifested by highly infectious mucocutaneous lesions of extra-ordinarily diverse description as well as headache, low-grade fever, diffuse lymphadenopathy, and a variety of more sporadic phenomena. The lesions of secondary syphilis ordinarily go on to apparent spontaneous resolution in the absence of treatment. However, until solid immunity develops (a matter of about 4 years), 25% of untreated syphilitic patients may be susceptible to repeated episodes of spirochetemia and metastatic infection.

Following the resolution of secondary syphilis, the disease enters a period of latency, with only abnormal serologic tests to indicate the presence of infection. During this time, persistent or progressive focal infection is presumably taking place, but the precise site remains unknown in the absence of specific symptoms and signs. One site of potential latency, the central nervous system, can be evaluated by examining the cerebrospinal fluid, in which pleocytosis, elevated protein levels, and a positive serologic test for syphilis are indicative of asymptomatic neurosyphilis.

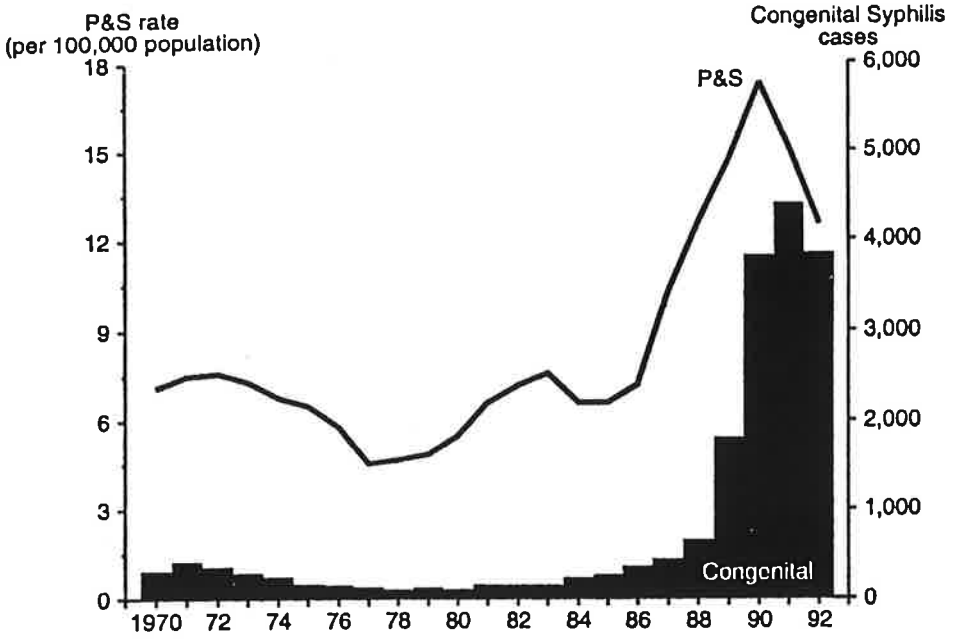
Only about 15% of patients with untreated latent syphilis go on to develop symptomatic tertiary syphilis. Serious or fatal tertiary syphilis in adults is virtually limited to disease of the aorta (aortitis with aneurysm formation and secondary aortic valve insufficiency), the central nervous system (tabes dorsalis, general paresis), the eyes (interstitial keratitis), or the ears (nerve deafness). Less frequently, the disease becomes apparent as localized single or multiple granulomas known as "gummas." These lesions are typically found in the skin, bones, liver, testes, or larynx. The histopathologic features of the gumma resemble those of earlier syphilitic lesions, except that the vasculitis is associated with increased tissue necrosis and often frank caseation. With its myriad of organ system involvement and symptomotology, syphilis, not surprisingly, has long been called "The Great Imitator."

Congenital syphilis is the direct result of treponemes crossing the placenta and fetal membranes, especially during mid-pregnancy, leading to spirochetemia and widespread dissemination after entering the fetal circulation. Fetal death and abortion can occur. Surviving babies with the disease have prominent early symptoms of hepatosplenomegaly, multiple long bone involvement, mouth and facial anomalies (saddle nose), and skin lesions. Treponemal antibodies (especially IgM) found in the newborn's blood is highly diagnostic.

SYPHILIS — By year, United States, 1941–1992



CONGENITAL SYPHILIS — Reported cases in infants <1 year of age and rate of primary and secondary syphilis among women: United States, 1970–1992



NOTE: The surveillance case definition for congenital syphilis changed in 1989.

FIG. 2. Reported incidence of syphilis (all cases from Centers for Disease Control, MMWR 1992, vol. 41, No. 55): (a) by year and (b) congenital syphilis in infants <1 year, and syphilis among women.

DIAGNOSIS

In its primary and secondary stages, syphilis can be diagnosed by dark-field microscopic examination of material from suspected lesions. Diagnostic serologic changes do not begin to occur until 14 to 21 days following acquisition of infection. Serologic tests provide important confirmatory evidence for secondary syphilis but are the only means of diagnosing latent infection. Many forms of tertiary syphilis can be suspected on clinical grounds, but serologic tests are important in confirming the diagnosis. Spirochetes are notoriously difficult to demonstrate in the late stages of syphilis.

Two main categories of serologic tests for syphilis (STS)

are available; tests for reaginic antibody and tests for treponemal antibody.

*A. Tests for Reaginic Antibody*This is an unfortunate and confusing designation; there is no relationship between this antibody and IgE reaginic antibody. Patients with syphilis develop an antibody response to a tissue-derived substance (from beef heart) that is thought to be a component of mitochondrial membranes and is called "cardiolipin." Antibody to cardiolipin antigen is known as Wassermann, or reaginic, antibody. Numerous variations (and names) are associated with tests for this antigen. The simplest and most practical of these are the VDRL test (Venereal Disease Research Laboratory of the U.S. Public Health Service), which involves a slide microflocculation technique and can provide qualitative and quantitative data, and the rapid plasma reagin (RPR) circle card test. Positive tests are considered to be diagnostic of syphilis when there is a high or increasing titer or when the medical history is compatible with primary or secondary syphilis. The tests may also be of prognostic aid in following response to therapy, because the antibody titer will revert to negative within 1 year of treatment for seropositive primary syphilis or within 2 years of that for secondary syphilis. Because cardiolipin antigen is found in the mitochondrial membranes of many mammalian tissues as well as in diverse microorganisms, it is not surprising that antibody to this antigen should appear during other diseases. A positive VDRL test may be encountered, for example, in patients with infectious mononucleosis, leprosy, hepatitis, and systemic lupus erythematosus. Although the VDRL test lacks specificity for syphilis, its great sensitivity makes it extremely useful.

*B. Tests for Treponemal Antibody*The first test used for detecting specific antitreponemal antibody was the *T. pallidum* immunobilization (TPI) test. Although highly reliable, it proved to be too cumbersome for routine use. A major test used until recently was the fluorescent *T. pallidum* antibody (FTA) test. If virulent *T. pallidum* from an infected rabbit testicle is placed on a slide and overlaid with serum from a patient with antibody to treponemes, an antigen-antibody reaction will occur. The bound antibody can then be detected by means of a fluoresceinated antihuman immunoglobulin antibody. The specificity of the test for *T. pallidum* is enhanced by first absorbing the serum with nonpathogenic treponemal strains. This modification is referred to as the FTA-ABS test. (If specific anti-IgM antibody to human gamma globulin is used, the acuteness of the infection or the occurrence of congenital syphilis can be assessed. However, this test may sometimes be falsely positive or negative in babies born of mothers with syphilis.)

The FTA-ABS test is reactive in approximately 80% of patients with primary syphilis (versus 50% for the VDRL test). Both tests are positive in virtually 100% of patients with secondary syphilis. Whereas the VDRL test shows a tendency to decline in titer after successful treatment, the FTA-ABS test may remain positive for years. It is especially useful in confirming or ruling out a diagnosis of syphilis in patients with suspected biologic false-positive reactions to the VDRL test. However, even the FTA-ABS test may be susceptible to false-positive reactions, especially in the presence of lupus erythematosus.

The microhemagglutination-*T. pallidum* (MHATP) test, a simple passive hemagglutination test, is a satisfactory substitute for the FTA-ABS test. Its principal advantages are economy of technician time and money. Its results correlate closely with those of the FTA-ABS test, except during primary and early secondary syphilis, when both the VDRL and FTA-ABS are more likely to show reactivity. The VDRL test is the only one that can be used with reliability in the evaluation of cerebrospinal fluid.

The interpretation of serologic data from patients with syphilis may be extremely complex in some cases. For example, a prozone phenomenon may be encountered in secondary syphilis; serofastness may characterize late syphilis; and the VDRL test may be negative in up to one-third of patients with late latent syphilis.

PROPHYLACTIC MEASURES

Prevention of syphilis requires the practice of safe sex techniques such as the use of condoms. These, if used properly, can be an effective barrier against the sexual transmission of *T. pallidum*. Early treatment with antibiotics is the only way known to prevent the later ravages of syphilis. Experimental vaccines have proven to be impractical or fail to afford complete protection.

TREATMENT

Penicillin is the drug of choice for syphilis in all its stages. Because the lesions of tertiary syphilis may be irreversible, it is crucial to identify and treat the disease before tertiary lesions begin. The AIDS patients with syphilis must be treated more intensively with penicillin (39). This reinforces the notion that curing syphilis depends on interactions between an intact immune system and the treponemicidal effects of antibiotics.

NONVENEREAL TREPONEMATOSES

The causes of yaws (*T. pallidum subsp pertenue*), pinta (*T. carateum*), and bejel (*T. pallidum* and *endemicum*) are human pathogens responsible for this group of contagious diseases, which are endemic among rural populations in tropical and subtropical countries. Unlike syphilis, these diseases are not transmitted by sexual activity but arise when treponemes are transmitted primarily by direct contact, mostly among children living under poor hygienic conditions. These three treponemal species are morphologically and antigenically similar to *T. pallidum* but give rise to slightly different disease manifestations. Pinta causes skin lesions only; yaws causes skin and bone lesions; and bejel (so-called endemic syphilis) affects the mucous membranes, skin, and bones. They do resemble venereal syphilis by virtue of the self-limiting primary and secondary lesions, a latency period with clinically dormant disease, and late lesions that are frequently highly destructive. The serologic responses for all three diseases are indistinguishable from one another and from that of venereal syphilis, and there is the same degree of slow development of protective immunity associated with prolonged untreated infection.

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Missouri Lyme Disease: 1989 through 1992

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The existence and characteristics of Lyme disease in Missouri are being discussed. Centers for Disease Control (CDC) surveillance criteria are being used and symptom patterns of cases reported nationally and in Missouri are compared. The results show that Missouri Lyme disease is consistent with a true borreliosis.

Key words: Lyme disease, Signs and Symptoms, Surveillance Definition

BACKGROUND

With increasing frequency, physicians in Missouri have diagnosed and reported cases of Lyme disease that have met the rigorous Centers for Disease Control (CDC) surveillance criteria. Missouri had previously been considered a nonendemic state, and there has been some controversy over whether or not these cases represent a true borreliosis.

METHODS

To address this issue, we analyzed data from all cases of Lyme disease reported to the Missouri Department of Health from 1989 through 1992 that were confirmed as meeting the CDC surveillance case definition. These data were then compared to the 1990 national Lyme disease statistics reported by the CDC at the Fifth International Conference on Lyme Borreliosis in 1992 (1).

All physician-diagnosed cases of Lyme disease in Missouri from 1989 through 1992 that met CDC's surveillance criteria were analyzed according to signs and symptoms and compared with national reporting data. Additionally, photographs of physician-diagnosed erythema migrans in Missouri were collected and presented for comparison.

RESULTS

The confirmed Lyme cases from 1989 through 1992 were separated according to the presence of the following signs or symptoms: erythema migrans, arthritis, Bell's palsy, radiculoneuropathy, encephalitis or meningitis, and second or third degree heart block.

Missouri cases were compared to national cases with regard to the distribution of signs and symptoms in the two groups (Table 1).

In a total of 672 Missouri Lyme disease cases from 1989 through 1992 that met CDC's surveillance criteria, signs and symptoms occurred in the following percentages: erythema migrans 50.9%; arthritis 64.7%; Bell's palsy 4.9%; encephalitis or meningitis 1.49%; second or third degree heart block 1.33%; and radiculoneuropathy 4.46%.

DISCUSSION

The 672 Missouri Lyme disease cases were broken down into signs and symptoms and compared by percentage to the 4966 national cases in 1990. Both the Missouri and the national groups met the CDC surveillance case definition. In four of the five categories, i.e., arthritis, Bell's palsy, encephalitis or meningitis, and second or third degree heart block, the Missouri experience as a percent of total cases exceeded the 1990 national percent data. In all but the arthritis category, the results were strikingly similar.

There is speculation that many Missouri physicians do not report Lyme disease unless there is overt arthritis. Although more than the 30.5% 1990 national figure. Missouri arthritis percentages are strikingly similar to the 60% arthritis figure given by Steere et al. (2, 3).

In effect, reports made in a single year are an accumulation of cases that have developed over several years. The true ratio of acute versus chronic cases may change in a specific year if previous to that year the diagnosis and treatment of that disease did not exist. This phenomenon might skew the relative percentages of signs, making a chronic sign like arthritis, for instance, appear far more common and an acute sign like erythema migrans appear less common. It could be argued that because physicians in Missouri were unaware of Lyme disease until recently, present reporting does not accurately reflect the typical distribution of acute and chronic cases.

CONCLUSION

We conclude that Lyme disease reported in Missouri is similar in terms of signs and symptoms to Lyme disease reported nationally and is consistent with a borreliosis. The geographic distribution of Lyme disease in the United States has been both changing and controversial. Oliver et al. (4) challenges previous geographic restrictions based upon the presence of the *Ixodes (I.) dammini* deer tick species by demonstrating that *I. dammini* is not a separate tick species but is the same as *I. scapularis*, which is prevalent in Missouri. *Borrelia (B.) burgdorferi* has been isolated in Oklahoma (5,6), Georgia (7), Florida (7), and Texas (8). Spirochetes morphologically consistent with *B. burgdorferi* and which stain variably with monoclonal antibodies for *B. burgdorferi* have been observed in Missouri ticks by one of the authors and other researchers (9–12). Additionally, pictures of Missouri erythema migrans cases have been published (13).

We conclude that Lyme disease reported in Missouri is similar in terms of signs and symptoms to Lyme disease reported nationally.

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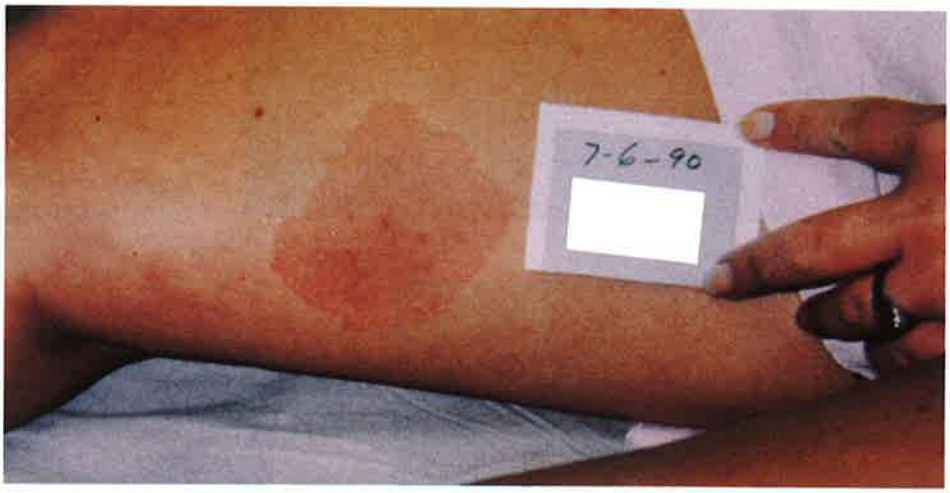
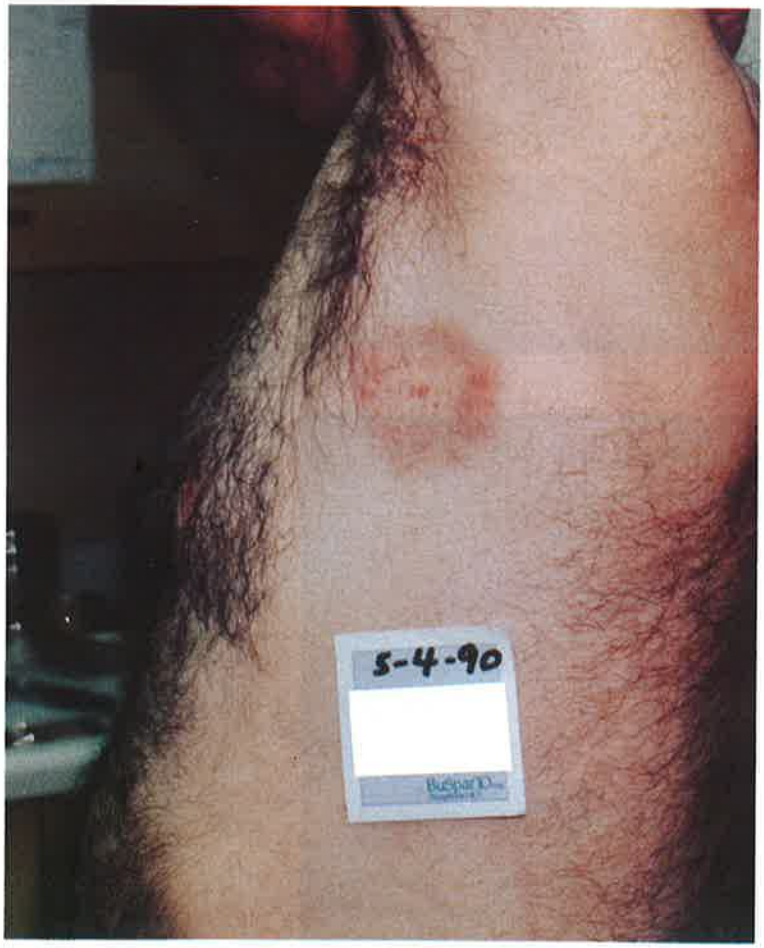
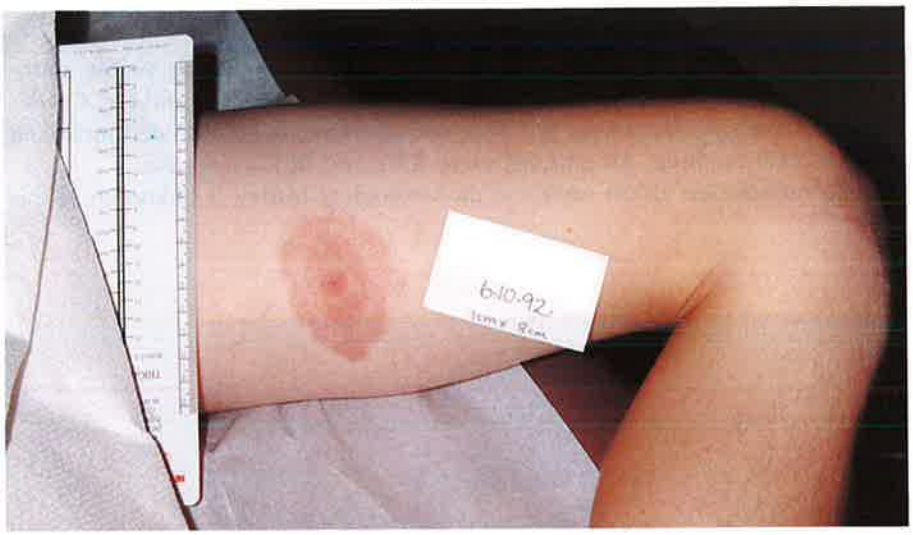
Erythema Migrans in Missouri

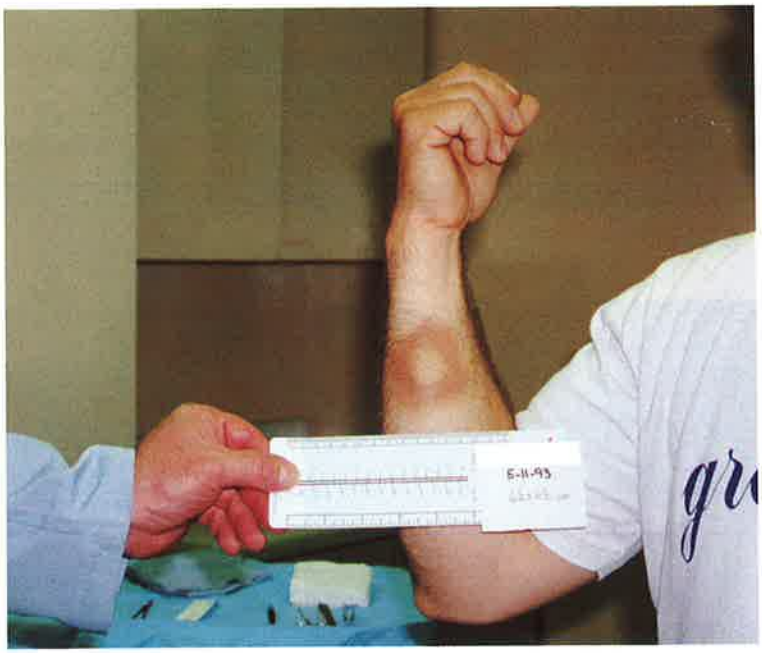
Erythema migrans patients in Missouri have had positive skin biopsies for Lyme disease, including visible spirochetes. Biopsies were performed by Dr. Duray of Boston, MA, Dr. DeKoning of the Netherlands, and Dr. Cordes of Cape Girardeau, MO. In addition, positive serologies from over ten different labs have been obtained including ELISA and Western blot. Some patients were PCR positive. All patients were RA's and RPR's negative.

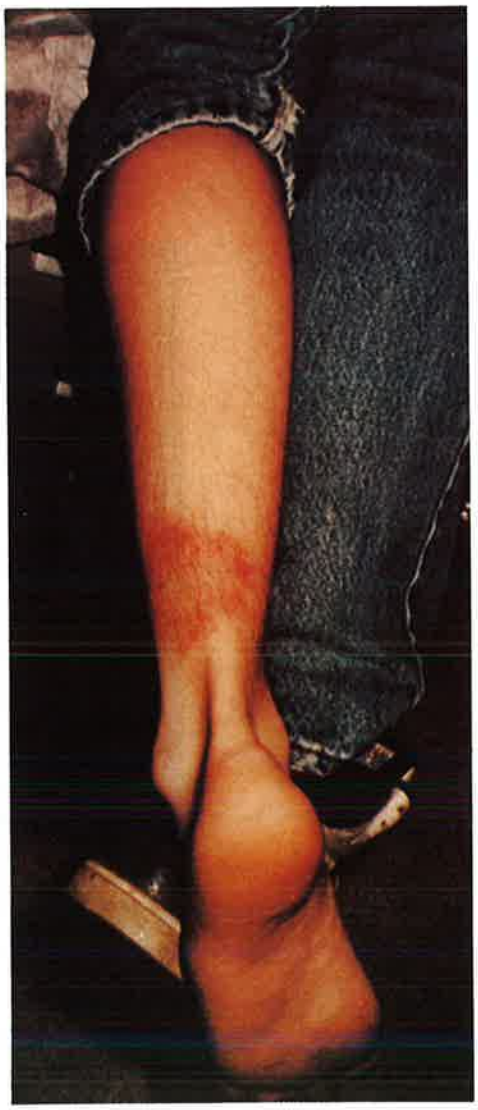
The potential impact of probable *Borrelia burgdorferi* strain variation on serological testing is unknown at this time.

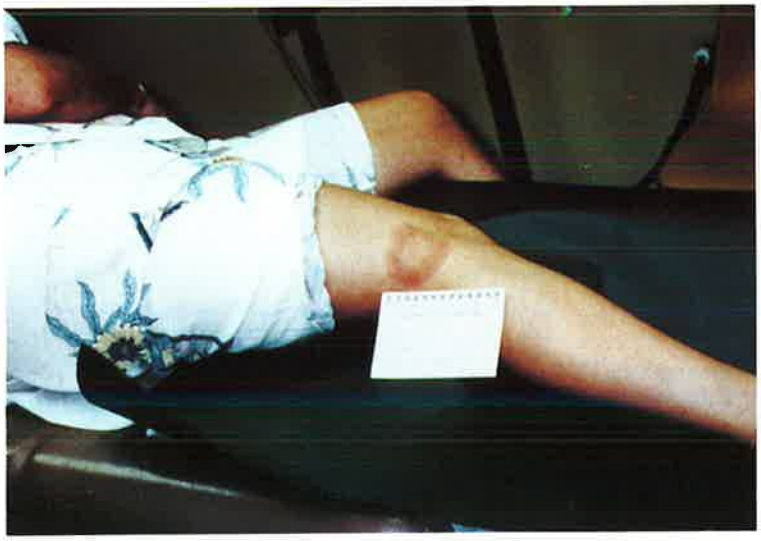


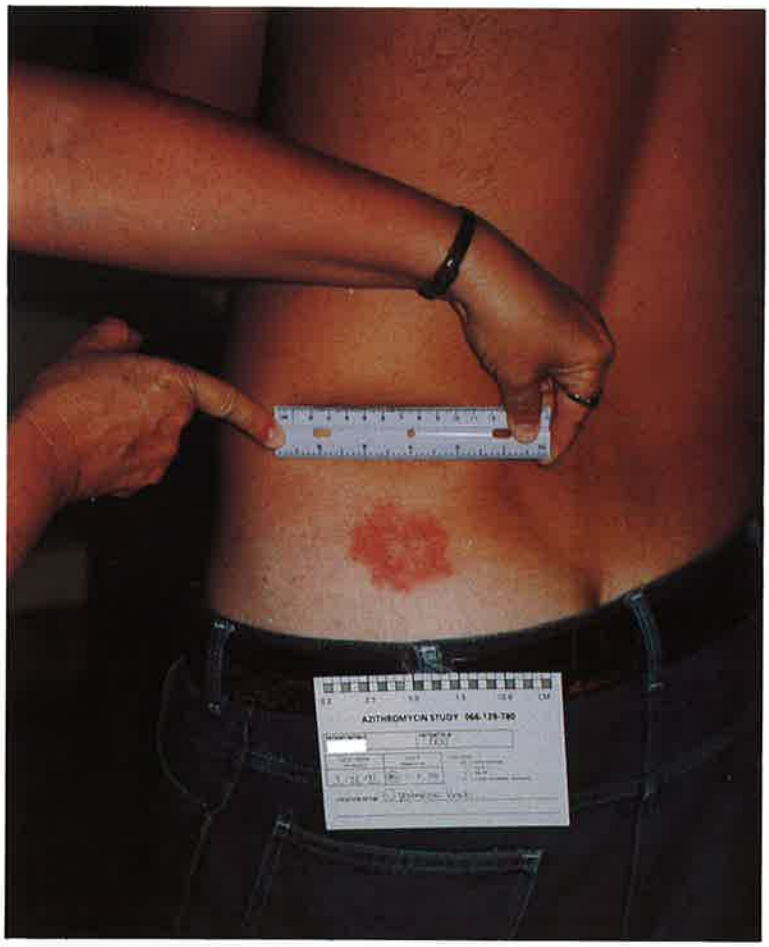








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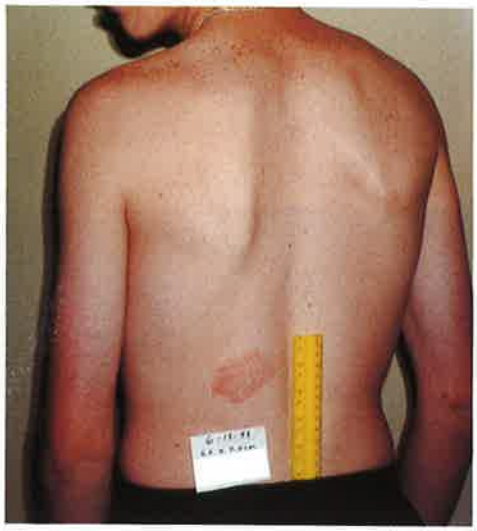

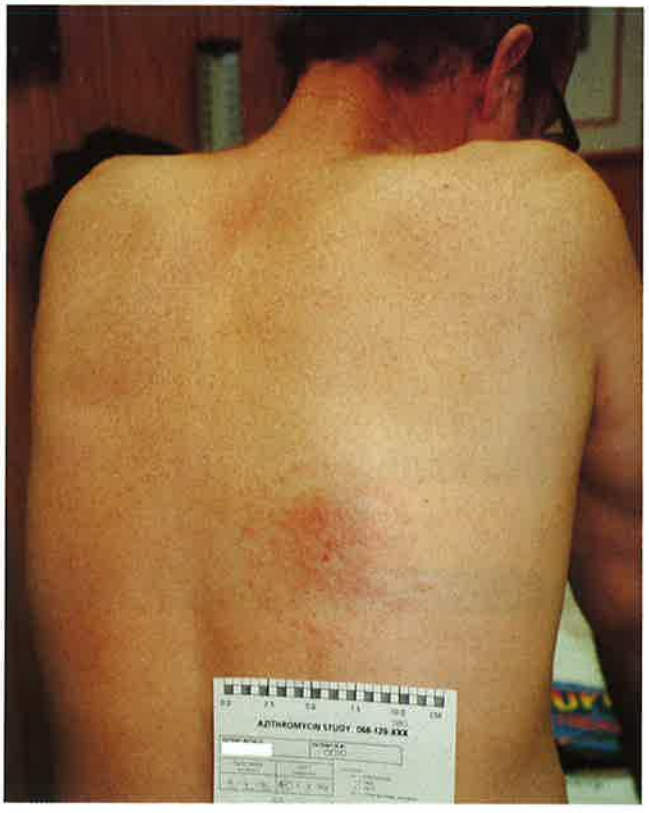
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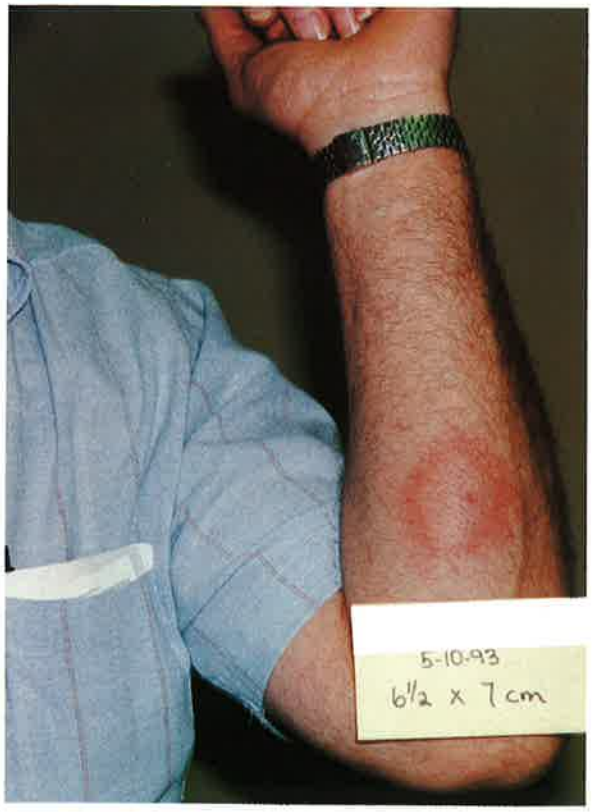
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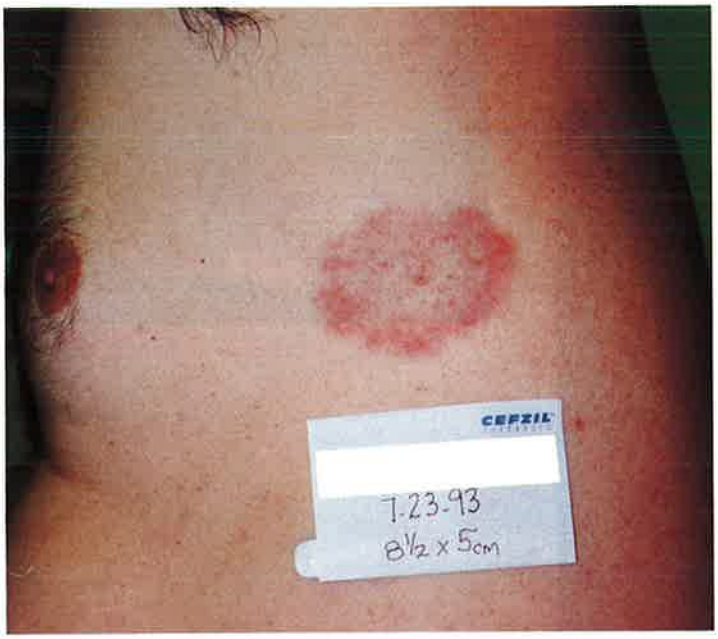
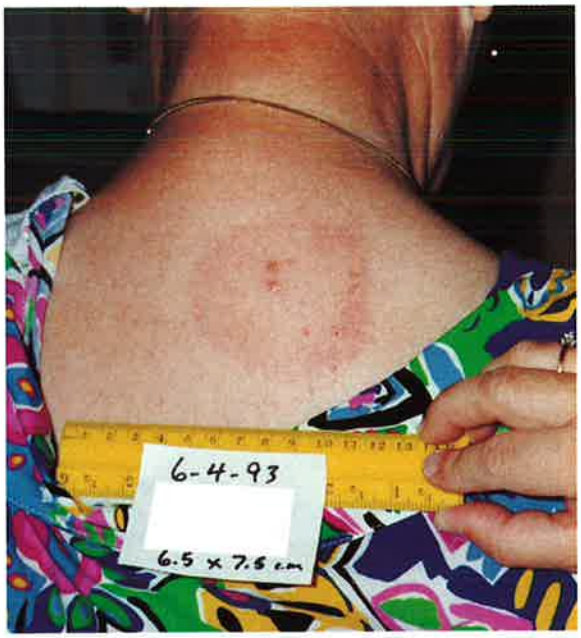

CEFZIL
7-23-93
8½ x 5cm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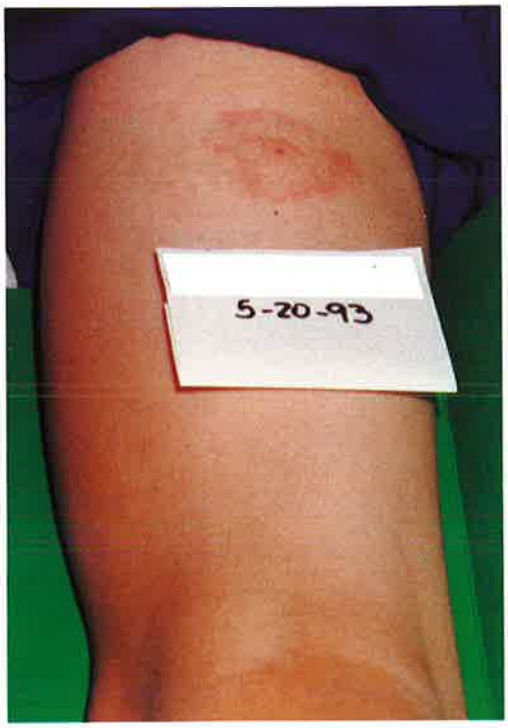
6.5 x 7.5 cm


5-20-93

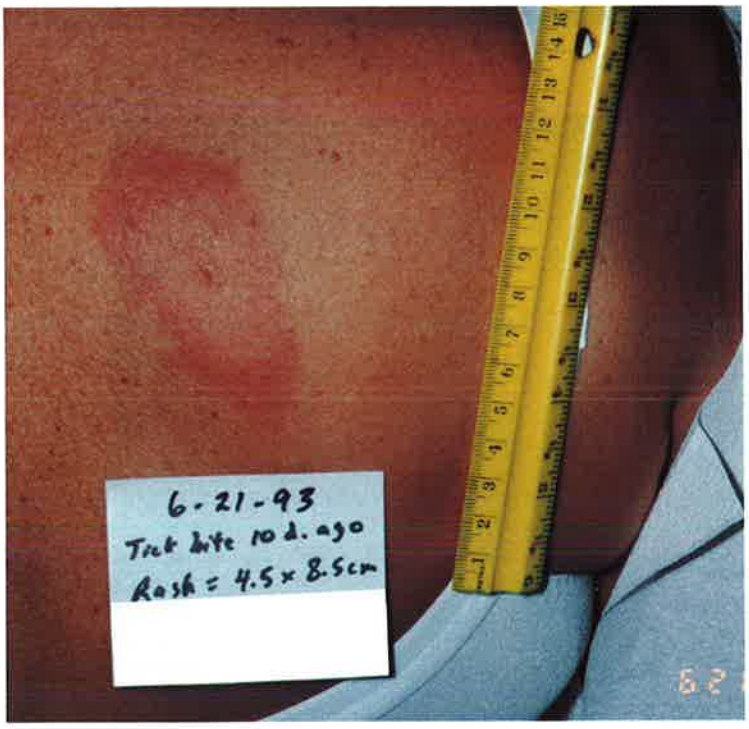

6-21-93
Tick bite 10 d. ago
Rash = 4.5 x 8.5cm

TABLE 1

Missouri and National Lyme Disease Compared by Signs and Symptoms (Cases Confirmed as Meeting CDC Surveillance Criteria) 1989 through 1992

	Missouri 1989 through 1992		1990 National		1990 National Excluding Missouri Cases	
	Total Cases	% of Total	Total Cases	% of Total	Total Cases	% of Total
Total cases confirmed CSTE/CDC	672	100	5171	100	4966	100
Erythema migrans	342	50.9	3736	72.2	3632	73.1
Arthritis	435	64.7	1657	32	1515	30.5
Bell's palsy	33	4.91	200	3.9	193	3.9
Encephalitis or meningitis	10	1.49	50	0.97	47	0.95
Second or third degree heart block	9	1.33	25	0.48	25	0.50
Radiculoneuropathy	30	4.46	No data		No data	

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Laboratory Aids for the Diagnosis of *Borrelia burgdorferi* Infection

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B. burgdorferi is the causative agent of Lyme disease, laboratory aids for the diagnosis of Lyme disease include culture of the etiologic agent, amplification of *B. burgdorferi* DNA, detection of antigenic components in body fluids such as urine, and monitoring of the immune response. Specific antibody may be detected by indirect immunofluorescence (IFA), ELISA, Western immunoblotting, and a borreliacidal antibody test. The failure to detect the antibody response in a patient with Lyme disease may be a function of inadequate tests, limited understanding of the patients immune dynamics, or antigen-antibody complexes.

Key words: Lyme disease, *Borrelia burgdorferi*, ELISA, antibody test, Western blot, PCR (polymerase chain reaction)

Lyme disease is the most prevalent tick-borne infection in the United States. Some counties of California, Connecticut, Massachusetts, Wisconsin, and New York have reported incidences of up to 200 cases per 100,000 population. Cases of Lyme disease have been reported from at least 47 states in the United States. Lyme disease is primarily a clinical diagnosis. Like other infectious diseases, however, laboratory assistance is essential for diagnosis and management of the patient as well as for a clear understanding of the epidemiology of the disease.

Laboratory tests for confirming or establishing a diagnosis of Lyme disease have been affected by inadequate commercial kits for *Borrelia (B.) burgdorferi* antibody detection, lack of a well standardized antigen detection test, difficulty in growing or visualizing *B. burgdorferi* from clinical material, and ignorance of the growth dynamics and localization of this microorganism in the human host. Clinicians and laboratorians also fail to recognize that the use of any diagnostic test in a population where the disease prevalence is low leads to problems of both sensitivity and specificity. This critical review presents selected information on laboratory aids for diagnosis of Lyme disease and is not exhaustive. Rather, it will focus on a few essential issues in the laboratory detection of *B. burgdorferi* infection.

THE TICK VECTOR

The deer tick, *Ixodes* (*I.*) *dammini*, has been the principal tick vector in the United States. A recent report (1), however, has indicated that there is no taxonomic difference between *I. dammini* and *I. scapularis*. *Ixodes dammini* and *I. scapularis* are synonymous and both should be called *I. scapularis* or "black-legged" ticks. The implication of this report by Oliver on *Ixodes* taxonomy is that failure to find *I. dammini* (*I. scapularis*) in a region does not rule out the possibility of Lyme disease acquisition in that area.

One report (2) documents the transmission of *B. burgdorferi* to humans by *Amblyomma americanum*, the Lone Star tick, but not by species of *Dermacentor*, the common dog tick or wood tick. The isolation of *B. burgdorferi* from or the detection of DNA in a possible vector such as *Dermacentor* does not necessarily indicate that dog ticks transmit Lyme disease. The apparent transmission of Lyme disease by a horsefly bite (3) should not implicate this class of insect as an important vector until sufficient epidemiologic evidence has accumulated.

New ticks hosting *B. burgdorferi* have been identified. They include *I. spinapalpis* and *I. angustus* (4), both in California or the Pacific northwest. Transmission of *B. burgdorferi* by *I. angustus*, (5) but not *I. spinapalpis*, to humans has been documented.

THE MICROORGANISM

There is extensive literature on the biology of *B. burgdorferi*. *B. burgdorferi* has been well-characterized as to its molecular and immunological fingerprints (6). Less has been published on the applicability of these organisms as "reagents" for use in diagnostic tests.

Two questions are relevant:

1. Does the *B. burgdorferi* strain that is used as a capture antigen for enzyme-linked immunosorbent assay (ELISA) or indirect fluorescence antibody (IFA) based serological tests possess sufficient epitopes to recognize human Lyme disease from diverse geographic areas?
2. Does the strain used possess all of the antigens that will recognize specific antibodies by Western immunoblotting?

The most commonly used strain of *B. burgdorferi* for serological testing is B31 (ATCC 35210). In the author's laboratory, strain 2591 (a Connecticut isolate) is used. Others (7) have used *B. burgdorferi* 639/40, a strain isolated from ticks in Connecticut, as well as a variety of other North American and European isolates. Until recently, most assumed that strain B31 detected the major antibodies produced in response to infection by *B. burgdorferi*. Ryan (personal communications) has indicated that high-passage *B. burgdorferi* B31 failed to produce a major 22–25 Kda determinant and that low-passage strains produced insignificant quantities. Antibodies to the 22–25 Kda protein (probably a lipoprotein) are among the first to arise in early Lyme disease (8). Kurashige et al. (9) reported the isolation of an unusual strain of *B. burgdorferi* from *I. pacificus*. This strain had abundant protein with a molecular weight of 25 Kda, but no apparent outer surface protein A (OspA) (31 Kda) or outer surface protein B (OspB) (34 Kda) proteins. Kit manufacturers must ensure that the strain used to coat the solid phase possesses immunodeterminants suffi-

cient to detect the major antibodies produced. Poor kit results may reflect an inappropriate choice of capture antigen.

There is now clear evidence of phylogenetic diversity among the spirochetes initially classified as *B. burgdorferi*. Baranton et al. (10) proposed three groups: *B. burgdorferi*, *B. garinii*, and "Group VS461." Group VS461 has now been named *B. afzelii*. While this report has not changed the approach to antibody detection in the United States, geographic or regional strain diversity may well alter the effectiveness of testing. However, U.S. isolates and some Western European isolates are still included in the original species designation, *B. burgdorferi*.

There are similar concerns for strains used in Western immunoblotting assays. With certain exceptions, we now recognize those proteins that are either genus or species specific as well as those antigenic determinants that are highly cross-reactive. It follows that if specific immunodeterminants are critical in the interpretation of a Western blot, then such determinants should be present in the *B. burgdorferi* strain that is electrophoresed and subsequently transblotted.

TESTS FOR ANTIBODY

Indirect fluorescent antibody (IFA)

The IFA microscopy detection of antibody to *B. burgdorferi* was one of the first tests available (11). Whole cells of *B. burgdorferi* are fixed to a glass slide, and then dilutions of patient sera are added. Staining with fluorescent-labeled antihuman antisera detects those patients who have antibody to *B. burgdorferi*. Some laboratories (12) find that IFA is as reproducible as ELISA while others (13) report lack of specificity. It is clear that the interpretation of an IFA test is more subjective than ELISA and much more difficult to automate.

Enzyme immunoassay (EIA) and ELISA

The ELISAs for specific antibody have been the mainstay of laboratory diagnosis of Lyme disease. These tests fall into two categories: "in-house" assays and "kits."

Fister et al. (14) reported on the three commercial kits available in 1989 and indicated that only one was both sensitive and specific compared with immunoblot confirmatory tests and clinical diagnosis. This report included the FIAX (Bio-Whittaker), which is an automated, quantitative fluoroimmunoassay. FIAX proved to be the least sensitive of the products tested. In contrast, the in-house assay in this study was 92% sensitive and 96% specific. The number of commercial products increased rapidly, and over 20 different Lyme disease antibody kits are now marketed. Their performance has not improved. Bakken et al. (12) demonstrated both intra- and interlaboratory variations in Lyme disease proficiency testing representing users of 10 different kits. Thus, 4 to 21% of laboratories failed to identify positive serum samples with an IFA titer of $\geq$1:512 using polyvalent antisera or IgG tests only. Reactive samples with less antibody ($\leq$1:512), when tested revealed that 55% of participating laboratories failed to identify a case-defined serum. False-positive rates increased to 27% using an IgG conjugate. Some laboratories had great difficulty reproducing their own results.

Data reported by the College of American Pathologists in their 1993 "Lyme Proficiency Surveys" (Set Ly-A) confirm that some problems exist. Nearly 475 laboratories used a wide variety of kits in different formats as well as in-house assays. While most laboratories reproduced their kit results on two duplicate specimens, "normal" donors were reported positive by a number of laboratories as was a patient with syphilis. One sample with elevated IgG antibody and borderline IgM antibody revealed variable results using IgM-specific conjugates. High IgG levels tend to interfere with IgM antibody detection unless an IgM capture method is used.

Other reasons for variable antibody test results include lack of technical expertise, problems with the accuracy of instrumentation, lack of reliable and reproducible controls, and lot-to-lot variation of test kits.

A recent report (15) described an in-house assay for antibody to *B. burgdorferi* that has been used by at least two laboratories for the past 6 years. This test is similar to that reported by Magnarelli et al. (13) and is similar to many of the noncommercial antibody tests used in Lyme disease reference centers. This ELISA is semikinetic, and results are reported as separate IgG and IgM titers.

Titers <1:160 are not considered significant; 1:160 titers are "equivocal," and titers of $\geq$1:320 are "reactive."

This ELISA is technically demanding and time-consuming (4 hours). Some of the advantages include a "nonspecificity" control for each patient and an automatic retesting of each sample that shows reactivity at a titer of $\geq$1:160, either IgG or IgM. The enzymatic reaction is not stopped as a function of incubation time, but at an arbitrary optical density. Improved standardization of assay conditions as they affect test variability result from such a procedure.

An unpublished study of over 600 clinically characterized Lyme disease patients indicated that this ELISA was 79% sensitive and 98.5% specific with reproducibility of 99%.

A recent report on serodiagnosis of early Lyme disease (15a) indicated that Western blotting was more sensitive than ELISA (25% vs. 13%) for patients with EM less than 7 days duration. Sera of all 14 patients with EM greater than 14 days duration were positive both by Western blotting and ELISA. Of interest was the fact that seroconversion was observed in nearly 75% of evaluable patients despite antibiotic therapy.

IgM capture ELISA

Two groups (16, 7) have shown that a capture IgM assay is more sensitive and specific than the conventional sandwich ELISA. By definition, a capture ELISA "captures" all IgM, specific and nonspecific. *B. burgdorferi*-specific IgM is then determined as a proportion of total IgM. This procedure results in less interference from IgG antibody and rheumatoid factor.

IgM capture assays developed in the author's laboratory approximate the same sensitivity as an IgM Western blot in early Lyme disease. There appears to be little advantage in using an IgG antibody capture method, as the relative proportion of *B. burgdorferi*-specific IgG to total IgG is relatively low compared to the high ratio of specific IgM to total IgM.

Borreliacidal antibody

Callister et al. (17) have indicated that a borreliacidal antibody test termed the "Gundersen test" is useful for diagnosis of Lyme disease. This test was initially described by Pavia et al. (18) and is based on similar methodology to the *Treponema (T.) pallidum* immobilization test (TPI). Sadzene et al. (19) also indicated that borreliacidal antibodies inhibited growth of the bacterium and that this in-

hibition was more strain specific than the ELISA. Correlation between ELISA and the Gundersen test has been poor, although some (17) indicated better correlation with disease. This lack of correlation is not surprising, as functional antibodies such as those detected by the Gundersen test and ELISA antibodies may be entirely different. We believe that these bactericidal assays should be reserved for special situations such as response to experimental vaccines.

Peptide ELISA antibody tests

Two single-peptide EIAs are available for detection of specific antibody to *B. burgdorferi*. They include tests based on the detection of antibody to the 41 Kda antigen and to the 39 Kda antigen. The 41 Kda-based tests may be very sensitive but relatively nonspecific, whereas tests for the 39 Kda antibody have been reported to be specific (20) but lacking in sensitivity. Some new information (21) indicates that a test based on a subunit of the 41 Kda flagellin protein may be more specific than detecting antibody to the entire protein.

Dias et al. (8) recently evaluated a single-epitope 25 Kda ELISA and reported that it was both sensitive and specific, particularly for early Lyme disease. Comparison of the 25 Kda EIA with the IgM antibody capture assay on 30 patients with early Lyme disease shows similar sensitivity for both tests in early Lyme disease (unpublished results, Tilton RC and Ryan RW).

Western immunoblot for detection of antibody to B. burgdorferi

Ideally, all positive ELISA tests should be confirmed by Western blotting as there is significant cross-reactivity observed in patient sera submitted for Lyme disease testing. Magnarelli et al. (22) reported significant EIA antibody test cross-reactivity in patients with periodontal disease, because one of the etiologic agents is a spirochete with a cross-reacting 41 Kda antigen.

Tilton and Ryan (15) recently reviewed Western blots. The following is excerpted from this report:

> "Until recently, a Western blot was considered positive if a certain number of bands was detected (two to five or more) (23). Cross-reactive antibodies present in Lyme disease patients and normal serum controls make such a nonselective interpretation less useful. Tilton and Ryan (24) and Zoller et al. (25) proposed that for a Western blot to be reactive, antibodies (or bands) had to be present that were specific for *B. burgdorferi*. The following proteins have been shown to stimulate antibody response in patients with Lyme disease:
>
> 1. 83 Kda. LeFebvre et al. (26) suggested that this chromosomally expressed protein is genus-species specific. Dorward (27) confirmed the species specificity of this antigen.
> 2. 39 Kda. A major protein determinant in *B. burgdorferi* (21).
> 3. 34 Kda protein. Outer surface protein B.
> 4. 31 Kda protein. Outer surface protein A.
> 5. 25/22 Kda protein. Outer surface protein C (28).
> 6. 12 Kda protein. Genus-species specific but function unknown (29).
> 7. 41 Kda. This protein is characteristic of the flagellum of *B. burgdorferi*, but also of other treponemes such as oral treponemes that are involved in periodontal disease, other borrelias, and occasionally *Leptospira*. IgM antibody to 41 Kda is usually the first antibody to appear after infection with *B. burgdorferi*.

It is our experience and that of others that patients with late-stage or recurrent Lyme disease may develop a variety of antibodies to other proteins (9, 20, 37, 38, 45, 50, 55, 57, 60, 66, 75, 100 Kda) but similar patterns may occasionally be seen in control subjects. The significance of these proteins is unknown.

The interpretation of immunoblots for Lyme disease should be based on both the number and type of bands present. We do not feel that interpretation based solely on the number of bands without regard to the nature of the proteins is justified. For example, more emphasis is placed on antibodies to proteins such as 22, 25 Kda, OspA (31 Kda), OspB (34 Kda), 39 Kda, and 83 Kda than the cross-reactive proteins such as 41 Kda or those with undefined significance. While cross-reactive, antibody to the 41 Kda protein is still important for the detection of early Lyme disease.

There are three categories of antibodies observed in Western blots:

Category 1- known cross-reactive and other undefined bands (9, 20, 37, 38, 45, 50, 55, 57, 60, 66 Kda);
Category 2- genus-family specific (41 Kda);
Category 3- genus-species specific (12, 22, 25, 31, 34, 39, 83 Kda).

Depending on the number of bands present in Category 1, 2, or 3, blots are reported as nonreactive, indeterminate, or reactive. Any bands present in Category 3 (genus-species specific) will result in either an equivocal interpretation (1 band) or a reactive result ($\geq$2 bands). If bands (representing antibody) are present only form Categories 1 and 2, the results are either nonreactive or equivocal, depending on the number of bands present.

The "equivocal" interpretation is used to indicate that while antibodies are present, they may reflect a cross-reacting antibody. The "equivocal" interpretation should be used with the knowledge of the patient's history, clinical signs and symptoms of Lyme disease, as well as evidence of syphilis or other cross-reacting spirochetal diseases. Patient sera with "equivocal" results should be repeated in 3–4 weeks.

We have downgraded the importance of antibody to the 41 Kda flagellin antigen (Category 2, genus/family specific). A recent evaluation of IgG Western blots indicated that approximately 65% of those tested had antibody to the 41 Kda antigen. In the majority of these patients, the 41 Kda antibody was the only band present. We recognize that the 41 Kda IgM antibody is one of the first antibodies observed in early Lyme disease. However, Dias et al. (8) reported that the early antibody response is almost always to either both the 25 Kda antigen and the 41 Kda antigen or to the 25 Kda antigen alone.

Recently, one other immunogenic outer surface protein has been described as OspD (30).

Antibody in Cerebrospinal Fluid

Lyme disease was first described in the United States as an "arthritis," but neurologic defects have been quite common. In Europe, serious late neurologic abnormalities are much more prevalent. In some parts of the United States, the majority of Lyme disease patients present with erythema chronicum migrans (ECM) and subsequent neurologic defects such as facial palsy.

Some investigators have suggested that the neurotropism of the Lyme spirochete may be manifested much earlier in

the disease process than once thought. Garcia-Monco et al. (31) presented evidence in rats of early central nervous system (CNS) invasion by *B. burgdorferi*, resulting in permeability changes in the blood-brain barrier. In three out of five human patients with early Lyme disease, specific antigen was detected in cerebrospinal fluid (CSF). Coyle (32) also provided evidence for early CNS invasion by the Lyme spirochete.

Detection of *Borrelia* in CSF by the polymerase chain reaction (PCR) or antigen detection prior to specific antibody formation may prove to be a sensitive and specific indicator of early CNS invasion. However, once antibody synthesis is initiated, the laboratory diagnosis of CNS Lyme disease is best made by evaluation of the intrathecal antibody response.

A quantitative total IgG determination is performed on both CSF and serum. The fluids are diluted so that both CSF and serum have equivalent protein concentrations. A Lyme-specific ELISA (IgG) is performed on both specimens, and the titer is recorded. Intrathecal antibody production is present when the titer in CSF is greater than the companion serum titer (index ≥ 1.0).

AMPLIFICATION OF *B. BURGDORFERI* DNA

The detection of small quantities of *B. burgdorferi* DNA by PCR is potentially a useful laboratory tool. Direct detection of a microorganism or its components is superior to reliance on the immune response, particularly when the immunologic dynamics of Lyme disease are not well-understood. The traditional methods such as culture are insensitive because of smaller numbers of organisms present and possible sequestration in tissues not available for routine culture.

Four types of body fluids are potential candidates for PCR: blood, urine, CSF, and synovial fluid. Liebling et al. (33) tested all of these body fluids by nested PCR. They were able to detect the equivalent of <10 organisms per milliliter of fluid. Overall, the specificity of PCR was 96.4%, but the sensitivity was only 76.7%. Sensitivity ranged from 100% in CSF and urine to 80 and 59%, respectively, for synovial fluid and urine when results were compared to antibody response and clinical presentation. Apart from the disappointing sensitivity, there were several important aspects of this study. The detection of DNA in 13 CSF samples from patients with neurological manifestations and in the serum of eight patients with late-stage Lyme disease suggests that intact bacteria are present in the late stages of the disease. The presence of *B. burgdorferi* DNA in the urine of three patients with active infection may reflect bacterial proliferation in either the kidney or the bladder since the target DNA was too large to be filtered intact through a normal kidney.

There are a number of ongoing studies that are assessing the value of PCR for Lyme disease diagnosis. The obvious questions that must be answered relate to which body fluid is best and at what stage of the disease. Collection and transport procedures and pretreatment methods of specimens for PCR remain unresolved. Our experience with PCR for Lyme disease in conjunction with the University of Connecticut School of Medicine suggests a number of caveats:

a. Specimens should be frozen. *Borrelia* DNA was degraded at 4°C. The PCR reactive specimens left at 4°C for 7 days became nonreactive.
b. Whole blood and serum from patients with late-stage Lyme disease lacked sensitivity. Most PCR positive serum/whole blood was on patients with early-stage Lyme disease in which a spirochetemic phase has been documented.
c. The specimen with the greatest yield of reactive results was CSF. Preliminary results with a limited number of synovial fluid aspirates indicate that this, too, is a productive specimen.

Schwartz et al (33a) reported on the use of PCR for detection of *B. burgdorferi* DNA in skin biopsies. Twenty-one of 37 (57%) biopsies from untreated patients were positive by culture. Twenty-two of 37 specimens (59%) were positive by PCR. Although culture is time consuming, it appears to have the approximate sensitivity of PCR for skin biopsies.

CULTURE OF *B. BURGDORFERI*

The culture of *B. burgdorferi* from a skin lesion or body fluid is definitive evidence of disease. This "gold standard" test is fraught with difficulty owing to:

- low numbers of organisms in skin and body fluids;
- difficulty in initiating growth of *B. burgdorferi* from clinical specimens;
- apparent disappearance of cultivatable organisms from body fluids or reduction in numbers to a level where cultural methods are not effective; and
- nonstandardized media.

The availability of BSK-A medium (Sigma Chemical Co.) and a pretested rabbit serum additive now ensures that the growth medium is optimal for recovery of the organism from skin and body fluids. It is our experience that for nonsterile specimens, such as skin biopsies and gastrointestinal specimens, rifampin and kanamycin should be added to the medium. The BSK-A medium must be used as a transport medium if a shipping delay is anticipated.

The optimal growth temperature for *B. burgdorferi* is between 30 and 37°C. Our experience indicates that the best yields of organisms occur at 33°C. The generation time is 8 to 24 hours. *Borrelia* will grow in stationary culture. Shaking (aeration) does not appear to increase the yield, but the individual spirochetes clump as if microcolony formation is being initiated.

Notwithstanding the availability of a standardized growth medium, culture with the possible exception of erythema migrans (EM) lesions is a low-yield procedure. However, a positive culture is diagnostic. The frequency of recovery of *B. burgdorferi* was reported to range from 6 to 45% (6).

ANTIGEN DETECTION

The studies on direct detection of *B. burgdorferi* antigen have focused on detection of *B. burgdorferi* antigen from urine, blood, and tissue specimens. Benach et al. (34) found evidence of an outer surface protein in infected hamster urine. Hyde et al. (35) detected antigen in 10 patients with Lyme disease, eight of whom had EM. This diagnostic kit used monoclonal antibodies to detect Lyme antigen in human urine samples. The test was marginally sensitive.

Dorward et al. (36) have shown that OspA and several other proteins are exported from *B. burgdorferi* cells in membrane vesicles. These vesicles are captured from body fluid, immobilized on an electron microscope grid, and stained with a gold preparation. These vesicles can also be detected using an ELISA format. It is possible that the "gold"

test for *B. burgdorferi* antigen will be available for routine clinical use during 1994.

SERONEGATIVE LYME DISEASE

The concept of seronegative Lyme disease is controversial. Some patients with clinical signs and symptoms consistent with or similar to Lyme disease have no demonstrable antibody to *B. burgdorferi*. There are several possible explanations:

a. Immune complexes. Schutzer et al. (37) demonstrated immune complexes in seronegative patients with Lyme disease. Attempts in our laboratory to reproduce this work using polyethylene glycol disruption of immune complexes, acid dissociation, and sonification have not been successful. While the idea of immune complexes in blood is appealing, the low numbers of bacteria present in blood would not appear to be sufficient to complex large amounts of specific antibody.
b. Altered antibody synthesis. There is evidence (38) to suggest that antibiotic therapy abrogates antibody formation. Berardi et al. (7) speculated that treatment could impair the transition between IgM and IgG antibody synthesis.
c. Other tick-borne diseases. Ticks do not harbor a single species of microorganism. In addition to *Borrelia*, rickettsiae, including *Ehrlichia*, *Rickettsia*, and *Spiroplasma* may be found in a variety of tick species. More than one microorganism may be transferred by a tick bite, and patients may develop one or more diseases simultaneously.
d. Strain diversity could attribute to variations in antibody test efficacy on various geographic regions. Testing European patients with North American kits may cause inaccurate results because of the differences in European and North American strains of *B. burgdorferi*. Tilton (39) reported that the ELISA antibody test did not accurately detect antibody in patients from Missouri who had Lyme disease or a Lyme-like disease. In this series of patients, the Western blot was a more sensitive test. These results are difficult to explain as the same strain of *B. burgdorferi* (2591) was used for both ELISA and Western blot.
e. The patients with Lyme or Lyme-like symptoms do not have Lyme disease, but some other unrelated illness.

LYMPHOCYTE STIMULATION TEST

Dattwyler et al (40) reported that 17 seronegative Lyme disease patients showed a highly reactive T lymphocyte response when exposed to the *B. burgdorferi* antigen. This early report was enthusiastically received. However, like other potentially useful diagnostic tests for Lyme disease, results have been difficult to consistently reproduce. Dressler et al (41) adjusted the cutoff values of the T-cell assay and improved the specificity (95%) probably at the expense of sensitivity (45%). There may be small groups of seronegative patients with persistent symptoms who potentially might benefit from this test, particularly if T-cell stimulation was observed. However, a negative test would not necessarily rule out Lyme disease.

CONCLUSION

There is a large variety of laboratory tests available to the clinician to supplement his/her clinical judgment. These include serological, direct antigen detection, cultural, and molecular methods. Because of the clinical and epidemiological complexity of Lyme disease, no one test is definitive for diagnosis. Optimal diagnosis and management continues to rely on a combination of laboratory results and clinical acumen.

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Lyme Borreliosis in Domestic Animals

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Lyme borreliosis has been reported in dogs, cats, horses, sheep, and cattle. A large percentage of domestic animals exposed to the causative agent, *Borrelia* (*B.*) *burgdorferi*, are asymptomatically infected. Lameness and joint swelling are the predominant clinical signs in domestic animals with clinical disease. A variety of other clinical signs have been reported less frequently, including myocarditis, encephalitis, renal disease, abortion, lethargy, and behavior changes. Presumptive diagnosis depends on clinical signs, elimination of other causes of lameness and joint swelling, supportive serology, and response to antibiotic therapy. Definitive diagnosis relies on recovery of the organism from affected tissues, which is difficult and costly. Improved diagnostic tests are needed. Treatment regimens have been largely extrapolated from laboratory animal studies and human patient experience. Antibiotics from the penicillin and tetracycline families are typically employed. Exact drugs used vary by species. Experimental infection, evidenced by seroconversion, has been accomplished in several domestic animal species; however, animals in most of these studies remained clinically normal. A recently developed canine model using infected tick feeding to induce infection seems to mimic naturally occurring canine Lyme borreliosis. This model may be instrumental in improving understanding of the natural disease in dogs and other species.

Key words: Lyme disease, Borrelia burgdorferi, veterinary medicine, domestic animals, cattle, horse, sheep, dog, cat

Lyme borreliosis has been reported in dogs, cats, horses, cattle, and sheep (1–36). The causative agent, *Borrelia* (*B.*) *burgdorferi*, is usually transmitted to the host via the bite of infected *Ixodes* species (spp) ticks (37). Both nymphal and adult ticks can parasitize and infect domestic animals, although adult ticks may prefer the larger animal hosts (23, 25, 37). Other species of ticks, fleas, and biting insects have been reported to harbor *B. burgdorferi* less frequently and may be involved in transmission of the disease to a lesser degree (38, 39). Contact transmission has been reported in experimentally infected dogs and mice (40, 41), and urine shedding of *B. burgdorferi* has been demonstrated in naturally infected cattle (5, 9, 32) as well as the reservoir host, *Peromyscus leucopus* (white-footed mouse) (42).

Many domestic animals infected with *B. burgdorferi* seroconvert but do not show clinical signs (4, 5, 15, 27). Those animals that do develop clinical signs exhibit primarily single or shifting limb lameness and swollen joints, with or without fever (1, 5, 11, 19, 23, 29). Less commonly observed clinical signs reported include behavioral changes (43), seizure activity (43), encephalitis (8), renal dysfunction (19, 44), cardiac arrythmia (45), and reproductive disorders (5, 46). With the possible exception of cattle (32), domestic animals do not commonly demonstrate an erythematous skin lesion at the site of tick bite, although *B. burgdorferi* has been cultured from normal-appearing skin in this region (47, 48).

Presumptive diagnosis of Lyme borreliosis in domestic animals relies primarily on clinical signs, supportive serology [indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA), or Western blot] (49–56), and response to therapy (31); other causes of lameness and joint swelling must be ruled out. Because of the large number of asymptomatically infected animals in endemic regions, serological evidence alone is insufficient to support a diagnosis of Lyme disease in domestic species. Definitive diagnosis relies on demonstration of the organism in affected body fluids or tissues (57).

Immunostaining, silver staining (57, 58), culturing (59–62), polymerase chain reaction (61, 63), and other techniques have been used to demonstrate the organism. The sparseness of *B. burgdorferi* in affected tissues, difficulty in culturing as a result of slow generation time, fastidiousness of the organism, and the relative lack of inflammatory reaction surrounding organisms in tissue have made definitive diagnosis difficult, time-consuming and costly (57–60).

Treatment of Lyme borreliosis in domestic animals has been largely extrapolated from laboratory animal studies and experience in human therapy. Clinical trials of therapeutic agents for this disease have been delayed by difficulty in experimental reproduction of disease in domestic species. Drugs in the penicillin and tetracycline families are typically employed (19, 31). Dosages and particular drugs used vary with species. Duration of therapy ranges from 2 to 4 weeks initially. Prolonged antibiotic treatment is instituted in animals that experience clinical relapse following cessation of therapy (19, 21, 49). The percentage of animals that are cured by antibiotic treatment versus those that remain infected but no longer show clinical signs is unknown. Recrudescence of clinical illness may indicate survival of the organism (presumably in privileged sites) or re-exposure to the agent. The clinical decision of whether to treat clinically normal seropositive animals to prevent subsequent clinical manifestations of disease is controversial.

Experimental infection of several domestic animal species (dogs, cats, horses, cattle, sheep) has been accomplished (14, 17, 40, 47, 48, 51, 64–67), but only rarely has clinical disease been reproduced during experimental infection of these species.

Risk of zoonotic transmission of *B. burgdorferi* from domestic species appears to be low (19, 68, 69), although further research in this area is warranted. Shedding of the organism in urine (5, 9, 32), colostrum (5), and milk (9) has been demonstrated in infected cattle.

Serosurvey of domestic animals (particularly dogs) has been a useful epidemiological tool in determining emergence of this disease in new regions (71).

Lyme borreliosis in dogs

Lyme borreliosis has been investigated more thoroughly in dogs than in other domestic species. Several recent reviews address this topic in depth (1, 18–20). Dogs commonly serve as hosts for nymphal and adult *Ixodes spp* ticks, as well as a variety of other tick species. Most canine Lyme borreliosis is thought to be a result of tick-borne infection (19, 25, 26, 37). Transplacental (65) and contact transmission (40) have been demonstrated in experimentally infected dogs. The importance of these modes of transmission in the natural disease epidemiology is unknown. In regions endemic for Lyme disease, serological estimates of exposure to *B. burgdorferi* range from approximately 40 to 89% (4, 10, 21, 25, 26). The majority of infected dogs are clinically normal. Clinical signs develop in approximately 5% (10, 21) of dogs exposed to *B. burgdorferi*. Dogs with clinical illness most commonly present with acute onset of single or shifting limb lameness, swollen joints, fever, and depressed attitude (1, 19, 22). A less common presentation is that of a reoccurring single-joint lameness with only low-grade or no fever, possibly representing a more chronic infection (19). The joints most commonly affected include carpus, elbow, and tarsus (19); radiographic findings are unremarkable. Other less frequently reported manifestations of Lyme borreliosis in dogs include myocarditis (22, 45), renal failure (19, 44), seizure activity (43), and behavior changes (43). The true incidence of these less common manifestations is unknown, as definitive diagnosis is difficult. Additionally, the infection may precipitate disease indirectly, through immune mediated mechanisms or molecular mimicry (72, 73).

A presumptive diagnosis of canine Lyme borreliosis is based on clinical signs, ruling out other causes of lameness, positive serology, and response to antibiotic therapy. The possibility of false positive serological tests should be considered in regions where dogs may become infected with other borrelial species, such as *B. hermsi* (25). Most dogs have a positive antibody titer (IFA or ELISA) to *B. burgdorferi* when clinical signs are first noted, indicating that infection may be present for a variable time prior to development of clinical disease (26). Antibody titers in naturally infected dogs tend to remain elevated for ≥6 months (74), even if treated appropriately with antibiotics. Persistence of antibody titer >1 year may indicate subclinical infection or re-exposure to the organism (19, 74). Western blot analyses of naturally infected dogs show development of antibody against the outer surface protein A (OspA), OspB, and flagellar proteins of *B. burgdorferi*, as well as a multitude of other proteins (21, 49, 51, 56). Production of antibody against OspA may be depressed in naturally infected dogs compared to dogs experimentally infected with *B. burgdorferi* culture. Western blot analysis is useful in the interpretation of a low or equivocal ELISA titer (52, 56). The banding patterns seen on Western blot can distinguish antibodies produced against *Leptospira spp* from *B. burgdorferi* (56) and are also useful in distinguishing *B. burgdorferi* vaccine response from natural infection (19). Detection of *B. burgdorferi* by dark-field microscopy, immunostaining, culture in BSK medium, and polymerase chain reaction (PCR) analysis of body fluids and tissues has been reported in both naturally and experimentally infected dogs (25, 39, 44, 47, 48, 65, 67). The organisms are difficult to recover from clinical specimens, as discussed previously, and these techniques are therefore infrequently used in practice. One possible exception may be examination of skin biopsy by culture or PCR analysis. Two groups have used this technique to detect *B. burgdorferi* in dogs experimentally infected by ticks (47, 48). The clinical usefulness of the technique remains to be seen.

Treatment of canine Lyme borreliosis has been largely extrapolated from laboratory animal data (60) and human clinical experience (75). Acutely ill dogs are commonly treated with either oral amoxicillin or oral doxycycline for 2 to 4 weeks (19). Clinical response is usually rapid (24 to 48 hours) (19) especially in acutely infected dogs. Even in rapidly responding dogs, the full treatment length should be observed. A small percentage of patients relapse following cessation of treatment, usually experiencing intermittent lameness that is again responsive to antibiotic therapy. These individuals may require extended or repeated antibiotic treatment (19). The recent development of canine models of clinical Lyme disease (48, 67) (see below) may enable therapeutic trials to determine optimum antibiotic treatment for complete clearance of the organism. A whole cell-killed bacterin of *B. burgdorferi* has been commercially produced for dogs and used in endemic regions (76). Vaccine use has been controversial, particularly in endemic regions, where many dogs presented for vaccination may be subclinically infected (77). As Lyme disease pathogenesis may have immune-mediated components (72, 73), the possibility that vaccination of infected animals may precipitate immune-mediated disease has been raised (78), although solid evidence to support this theory has not been presented. A recently published study involving a large number of dogs in an endemic region showed no evidence of immune mediated disease in vaccinated dogs; the study also showed that the vaccine was more likely to prevent development of clinical disease in dogs that were vaccinated prior to natural exposure to *B. burgdorferi* (77). The possibility of vaccine-precipitated immune-mediated disease should lessen as more of the canine population becomes vaccinated prior to natural exposure. Examination of incidence of clinical disease in this population will also give a better indication of vaccine efficacy under field conditions.

Experimental infection of dogs with *B. burgdorferi*, as evidenced by seroconversion or recovery of the organism, has been accomplished by several laboratories (17, 40, 47, 48, 51, 65, 67). Transplacental infection of pups has also been demonstrated experimentally (65). The dogs in most of these studies did not develop clinical signs. Clinical signs were produced in one laboratory using frequent inoculation of *B. burgdorferi* in dexamethasone treated dogs (67). More recently, a model of canine Lyme disease was developed using *B. burgdorferi* infected ticks as the source of infection (48). In this model, which more closely mimics natural infection, dogs seroconverted 4 to 6 weeks following infection developed intermittent lameness 2 to 4 months after infection and had persistent antibody titers for more than 1 year post infection. The production of OspA and OspB antibodies was greatly decreased in tick-exposed dogs compared to dogs infected with *B. burgdorferi* culture in the same study. Histopathology of joints of infected dogs with acute lameness showed a fibrinopurulent arthritis and synovitis, while joints of clinically normal infected dogs showed a mild nonsuppurative plasma cell and lymphocytic infiltrate of synovial membrane and joint capsule.

These findings should be of great value in interpretation of the natural disease in dogs.

Lyme borreliosis in cats

Cats in endemic regions are frequent hosts of nymphal *Ixodes spp* ticks (23). Serological evidence indicates that

B. burgdorferi infection commonly occurs in cats. Cats may be more resistant to development of clinical signs of Lyme borreliosis than dogs (1). Cats that do develop clinical illness tend to exhibit mild lameness that responds well to amoxicillin therapy. Definitive diagnosis of *B. burgdorferi* infection is typically difficult (1, 23). Experimental infection of cats (without development of clinical signs) has been accomplished by inoculation of *B. burgdorferi* by intravenous, oral, and conjunctival routes (64). The white-footed mouse, *Peromyscus lecopus*, is an important host of immature *Ixodes spp* ticks and *B. burgdorferi* (37). This raises questions concerning infection of cats during natural rodent hunting behavior (64). Preliminary results from another laboratory demonstrated induction of lameness and hematological changes in cats infected intradermally with *B. burgdorferi* (14). The pathogenesis of Lyme disease in cats warrants further investigation.

Lyme borreliosis in horses

Equine Lyme borreliosis has been the subject of recent reviews (11, 24, 29). Serosurveys indicate that approximately 14 to 24% (27, 54, 70) of horses from northeastern U.S. regions endemic for Lyme disease have measurable antibody titers to *B. burgdorferi*, while only approximately 10% (27, 54) of the seropositive animals develop clinical signs typical of Lyme disease. Seroprevalence in Texas horses approached zero (50). Horses may serve as hosts for adult *Ixodes spp* ticks and less commonly for nymphal stages (11). Pastured horses are at increased risk for tick exposure compared to confined horses. The most common clinical sign of Lyme disease reported in horses is lameness with or without joint swelling, sometimes accompanied by fever (11, 24, 29, 30). Less frequently reported clinical signs include laminitis (30), uveitis (7), encephalitis (8), abortion (46), and lethargy (30). Lyme disease is particularly difficult to diagnose in the equine species as the anatomy and athletic use of this species predisposes them to a wide variety of musculoskeletal disorders with resulting lameness (24). Presumptive diagnosis of Lyme disease therefore requires elimination of other causes of lameness (which may involve radiography, nerve blocking, and extensive neurological and musculoskeletal examinations), as well as supportive serological tests indicating exposure to *B. burgdorferi*. The IFA or ELISA titer is generally used in serodiagnosis; Western blot analysis can be used as a more specific serological test (5, 49, 50, 70) if the preliminary IFA or ELISA test result is equivocal. Diagnosis of less specific clinical signs can be extremely challenging (11, 24, 29). When antibiotics are given as the sole therapeutic agent, response to therapy can be a useful diagnostic tool. However, febrile Lyme disease suspect horses are generally treated with antibiotics plus anti-inflammatory agents to prevent laminitis (30). Anti-inflammatory agents will provide temporary improvement in lameness of many different etiologies. Anti-inflammatory drugs are also necessary components of therapy in laminitis, encephalitis, and other disease entities that may or may not be related to Lyme borreliosis.

Treatment regimens vary. Intramuscular procaine penicillin, benzathine penicillin, and intravenous oxytetracycline have been used. Oral tetracycline use in horses has been associated (rarely) with intractable colitis, so caution with its use in this species is advised (79). Oral trimethoprim-sulfa drugs have been used to treat Lyme disease in horses, but *in vitro* studies have shown *B. burgdorferi* to be resistant to this agent (80). Recently, ceftiofur, an injectable newer generation cephalosporin licensed for use in cattle, has been used (off-label) to treat horses with reoccurring Lyme disease. Treatment length is generally 2 to 4 weeks. Controlled clinical trials are not available to access effectiveness of the various antibiotic regimens. A Jarrish-Herxheimer reaction has been observed in a seropositive asymptomatic horse treated with antibiotics (J Post, personal communication).

Experimental infection of a pony with *B. burgdorferi*-infected tick material produced seroconversion without clinical signs of illness (70).

Much remains to be learned about the natural course of Lyme disease in the equine species.

Lyme borreliosis in cattle

Although *B. burgdorferi* infection in cattle is probably widespread in endemic regions, less is known about Lyme borreliosis in this species than in dogs or horses. Cattle may serve as hosts for adult *Ixodes spp* ticks (SL Bushmich, unpublished data). Parasitism by ticks infected with *B. burgdorferi* is presumably an important route of infection in cattle (31). Infected cattle have been shown to shed *B. burgdorferi* in the urine; urine shedding may persist in some animals (5, 9, 32). A urine-oral mode of transmission has been postulated (5). As *B. burgdorferi* is quickly killed by dehydration, direct splashing of urine onto mucous membranes or abraded skin would presumably be needed for contact transmission. Although the relative importance of the two potential modes of transmission in natural bovine disease is unknown, urine transmission could enhance widespread exposure in group housing (e.g., free stall) situations. *Borrelia burgdorferi* has also been demonstrated (rarely) in blood, colostrum, milk, synovial fluid, and aborted fetal tissues of infected dairy cattle (5, 9, 81).

Asymptomatic infection appears to be common in cattle as well as in other domestic animal species. When present, clinical disease usually occurs as a herd problem, with first calf heifers most severely affected (29). The predominant clinical signs include lameness and joint swelling (5, 9, 29, 32). Less frequently reported clinical signs include erythematous skin rash, laminitis, fever, weight loss, depressed milk production, and abortion (5, 9, 29, 32). Presumptive diagnosis (based on clinical signs, elimination of other causes of lameness, serology, and response to therapy) is particularly difficult in cattle. Recent evidence suggests that serological testing may be less reliable in cattle compared to other species (9). Cross-reacting antibodies to other *Borrelia* that infect cattle (*B. coriaceae, B. theileri*) (82, 83), or possibly rumen spirochetes, may cause positive serology by IFA and Western blot analysis (9). Cows with confirmed *B. burgdorferi* infection (e.g., in which the organism has been demonstrated in fluids/tissues) may produce only low antibody titers (5, 9). Response to therapy is hindered by the reluctance of dairy producers to use antibiotics for any extended period of time, owing to the economic loss incurred when milk cannot be sold. Clinically ill infected cattle that are not treated seem to recover slowly, over weeks to months, compared to more rapid recovery observed in infected cattle that are treated (32). Penicillin, oxytetracycline, and ceftiofur (all off-label usages) have been employed to treat dairy cattle for Lyme disease (29, 32). Controlled therapeutic trials have not been reported. Detection of *B. burgdorferi* in urine of suspect cows, using direct fluorescent antibody staining or PCR, may be a useful diagnostic tool (9). Preliminary studies indicate that dairy cows with clinical Lyme borreliosis may be more likely to

shed spirochetes in the urine than asymptomatically infected cows (9).

Potential zoonotic spread of the disease via splashing of bovine urine containing *B. burgdorferi* into mucous membranes has been postulated (29). One controlled study addressing this possibility found no difference in the rate of Lyme disease seropositivity between dairy farmers and crop farmers working in the same endemic area (68). Food safety questions have also been raised. Culture innoculums of *B. burgdorferi* have been shown to survive in refrigerated milk for extended periods of time; however, pasteurization kills the organism, so minimal potential for zoonotic spread exists by this route (69). The ability of *B. burgdorferi* to survive gastric acid in humans is unknown, but oral inoculation of mice and cats using large numbers of cultured organisms has been accomplished experimentally (41, 64). As a general rule of food safety, consumers are advised to cook meat thoroughly and drink only pasteurized milk.

Lyme borreliosis in sheep

A small number of cases of suspected Lyme borreliosis in lambs have been reported. The predominant clinical signs of infection included lameness, anorexia, and poor body condition (13). Experimental infection of lambs with cultured *B. burgdorferi* produced seroconversion without clinical signs (66). The prevalence and pathogenesis of the natural disease in sheep is largely unknown.

CONCLUSION

Most domestic animal species are susceptible to *B. burgdorferi* infection. Many infected domestic animals appear clinically normal. The susceptibility to clinical illness seems to vary by species and individual. Clinical signs most frequently include lameness and joint swelling, but other less common manifestations may occur. Improved diagnostic tests are needed to enable veterinarians to definitively diagnose this disease and to enable appropriate controlled therapeutic trials to ensue.

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Place: Sheraton Hotel, Stamford, Connecticut, U.S.A.
Date: April 22 & 23, 1994

Conference Chair:	Martina Ziska, M.D.	Scientific Director
Conference Co-Chairs:	Andrew D. McBride, M.D.	Stamford Department of Health
	Julie Rawlings, M.P.H.	Texas Department of Health
	Steven Schutzer, M.D.	Univ. of Medicine & Dentistry of N.J.

Topics to include:

- Diagnosis (including differential diagnosis)
- Clinical Manifestations
- Neuroborreliosis
- Treatment Options
- Immunopathogenesis
- New Research Discoveries
- Other Tick-Borne Diseases
- Other Spirochetal Diseases
- Public Health Issues
- Writing & Submitting Articles to Peer-Reviewed Journals
- Community Education Training
- Poster Session

Organized by: Lyme Disease Foundation, Inc.
1 Financial Plaza, Hartford, CT 06103

For more information contact: Martina Ziska, M.D. Phone 203-525-2000
Fax 203-525-TICK

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Accreditation requested: ☐ MD ☐ RN ☐ RPH ☐ AAFP ☐ DO ☐ DVM

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Affiliation _____

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City _____ State _____ Zip _____

Work Telephone _____ Home Telephone _____ FAX _____

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