

# 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 axis 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  $\mu\text{m}$  in width and from 10 to 50  $\mu\text{m}$  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 labora-

tory, 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

**TABLE I**  
*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  $\mu$  in length), red blood cell diameter is 6 to 8  $\mu$  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 (greater than or equal to 38 degrees 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.

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 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 rheu-

matoid 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.

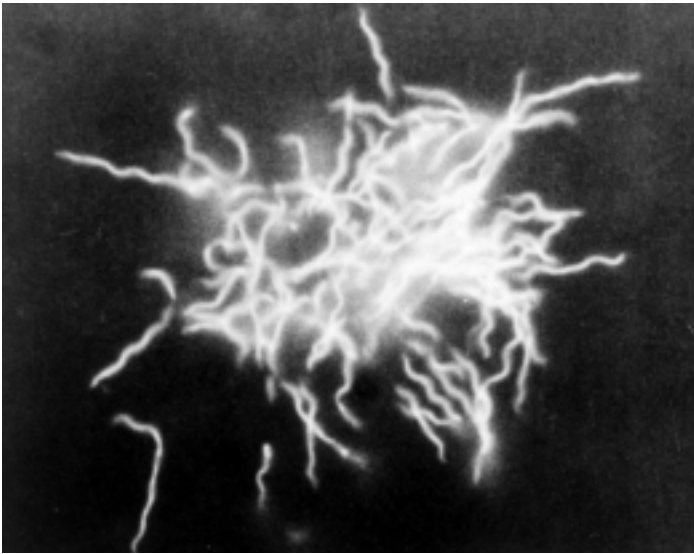
Lyme disease is now the most common tick-transmitted illness, and it has been reported in at least 43 states. However, it occurs primarily in three geographic regions: the coastal areas of the Northeast from Maine to Maryland, the Midwest in Wisconsin and Minnesota, and the far West in parts of California 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.

**TABLE 2**  
*Epidemiology of Spirochetal Infections*

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

## 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 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 on 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 or 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 blood stream 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, borrelicidal, 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 aggluti

nating 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 unim-

ped 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.

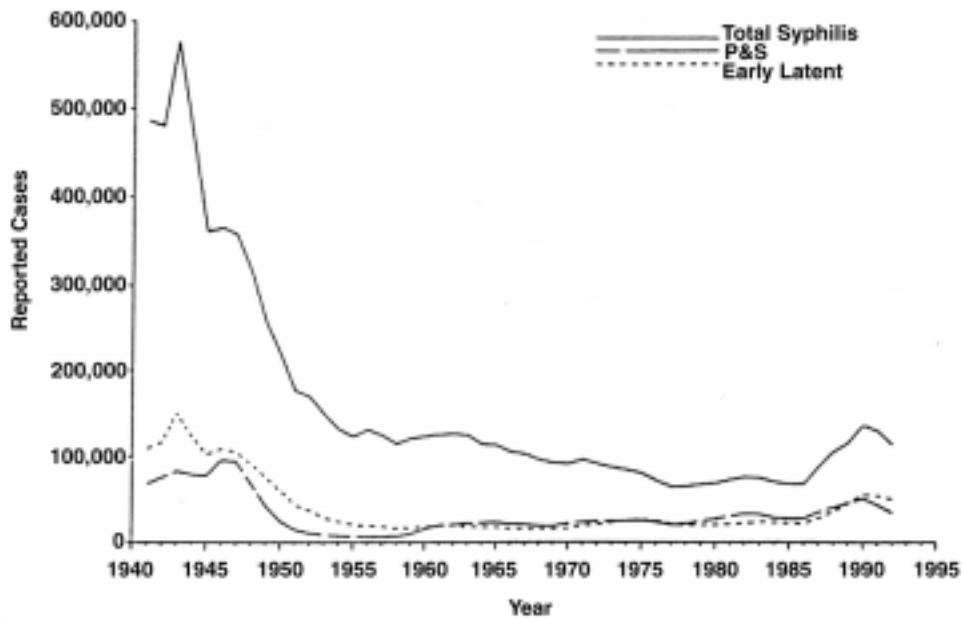
The presence of metastatic infection (secondary syphilis) is manifested by highly infectious mucocutaneous lesions of extra-ordinarily diverse description as well as headache, low-grade fever, diffuse lymphadenopathy, and a variety of more sporadic phenomena. The lesions of secondary syphilis ordinarily 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.

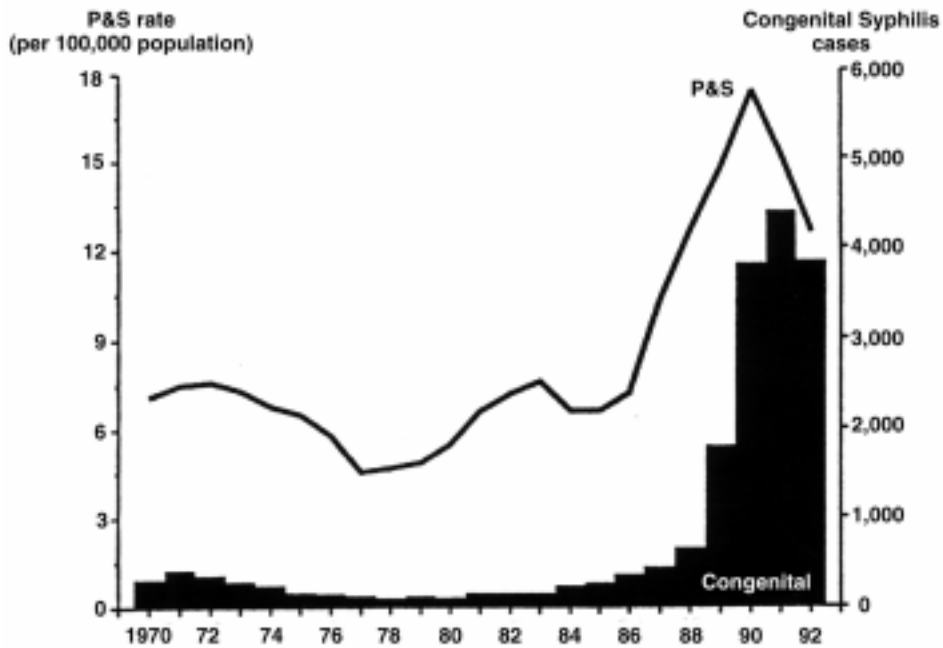
Only about 15% of patients with untreated latent syphilis go on to develop symptomatic tertiary syphilis. Serious or fatal tertiary syphilis in adults is virtually limited to disease of the aorta (aortitis with aneurysm formation and secondary aortic valve insufficiency), the central nervous system (tabes 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 symptomatology, 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,

SYPHILIS - By year, United States, 1941 - 1992



CONGENITAL SYPHILIS - Reported cases in infants <1 year of ages 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.**

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.

**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 anti

body 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 in- primary and early secondary syphilis, when both the VDRL and FFA-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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