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PREVALENCE OF *BORRELIA BURGDORFERI* AMONG TICKS COLLECTED FROM MICHIGAN

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ABSTRACT

PREVALENCE OF BORRELIA BURGDORFERI AMONG TICKS

COLLECTED FROM MICHIGAN

BY

ABEDALHAI MUHNNA

An indirect immunofluorescence test was used to determine the prevalence of Borrelia burgdorferi, the etiologic agent of Lyme disease, in 1307 ticks collected from different sites in Michigan during the period of October 1988 - December 1989. Eleven(22.9%) of 48 Ixodes dammini ticks and seven (1.3%) of 553 Dermacentor variabilis ticks collected from forty-one study sites in nineteen counties in the Lower Peninsula and twenty-two sites in three counties in the Upper Peninsula of Michigan were found to contain spirochetes. B.burgdorferi was not found in other species of ticks . A specific monoclonal antibody [H5332] directed to the outer surface protein A (OSP A) was used for the detection of B.burgdorferi in the ticks midgut tissues. I.dammini is the primary vector of B.burgdorferi in Michigan.

This is dedicated to my wife and my parents for their continuing support.

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Introduction

Lyme disease is a systemic, immune-mediated inflammatory disorder. It is characterized by an unusual skin rash called Erythema Chronicum Migrans (ECM) which may be followed by involvement of the central nervous system, heart and joints. The early stages of the illness are almost always seen in summertime. The disease is caused by a spirochete, Borrelia burgdorferi , and is transmitted to humans by the bite of ixodid ticks and, perhaps, other arthropods as well [122]. In many patients the disease follows a chronic, relapsing course and, during the latter stages, arthritis is the principal clinical manifestation. Endemic foci of the disease are known to exist in at least 43 states in three main regions in the country, as well as parts of Europe and Australia. All ages are affected . The disease tends to occur in individuals living or visiting rural, wooded neighborhoods or recreational areas [122]. Lyme disease is now the most common tick-borne illness recognized in the United States [46]. In short, were it not for the Acquired Immune Deficiency Syndrome (AIDS), Lyme disease would be the number one disease facing us today [98].

Forty-one cases of Lyme disease were reported in Michigan in 1988. Prior to 1988, Lyme disease in Michigan was thought to be acquired only by travel in endemic areas. Although this disease is now known to occur in Michigan, the scope of the problem is unknown.

In Michigan, the first official reported human case of Lyme disease was in 1985. From 1985 through 1987, there was a total of 8 cases reported, all from the western Upper Peninsula. In 1988, cases were also reported from 17 counties in mid and lower Michigan. More than 30 cases were identified during 1988 and it is anticipated that the number of reported cases will continue to increase.

The threat of Lyme disease has generated increasing alarm because it can cause such serious complications as arthritis, cardiac dysfunction and neurological disorders if not treated promptly with antibiotics.

This study describes examination of ticks collected from various sites throughout Michigan concentrating on those areas where there is a high deer population, and where Lyme disease patients are suspected to have contracted the disease. This study is not limited to examining Ixodes dammini. Various species of ticks common to Michigan will be examined since it has been demonstrated that more than one species of tick may harbor the causative agent, B.burgdorferi [29,7,32,114,5,12,64]. The laboratory portion of this study required dissecting the tick, smearing and fixing the midgut on a slide for examination by an indirect fluorescent antibody test using B.burgdorferi-specific monoclonal antibody H5332 (provided by Dr. Alan Barbour, University of Texas, San Antonio) which is reactive to a 31,000 dalton molecular surface protein [17].

This study will attempt to demonstrate the prevalence of the spirochete associated with Lyme disease in Michigan. This study will provide data that will not only increase the level of awareness among clinicians and other health care personnel, but will provide information that will permit eradication measures in endemic areas.

History

The story of Lyme disease is a fascinating account of modern medical science. The discovery of this disease, from its recognition as a clinical entity to the identification of its causative agent, is a triumph of modern medical research and attributable to the collaborative efforts of a great many scientists. It begins in 1975 when two mothers in the town of Old Lyme, Connecticut, became concerned over the large number of juvenile rheumatoid arthritis diagnoses in their small community [46]. One called the Connecticut State Health Department to express her concern , and the other called Yale Rheumatology Clinic and the Connecticut State Health Department. Dr. Allen Steere, a postdoctoral fellow in rheumatology found a very high incidence of what appeared to be juvenile rheumatoid arthritis in the three contiguous communities of Old Lyme, Lyme, and East Haddam [46]. Adults were affected as well as children; the disease occurred only in summer and early fall; and about a quarter of the patients recalled a peculiar skin rash which had preceeded joint symptoms. This rash started as a small bump and then an area of redness with a bright red outer border resembling a bull's-eye. This bump expand to a median diameter of 15 cm with a range of 6 to 52 cm and last about three weeks [116]. Steere concluded on the basis of these findings that he was dealing with a previously unrecognized disease. He named it Lyme arthritis for the town in which it was first described [117].

A review of the literature disclosed a report in 1909 by Afzelius in Sweden who noted a similar rash called Erythema Chronicum Migrans [ECM]. That occured following bites by the tick Ixodes ricinus. However, arthritis was not noted

[46]. European physicians had successfully treated ECM with penicillin, indicating that the most likely agent of infection was not a virus but a bacterium. Yet when fluid was removed from the joints of Lyme disease patients affected with arthritis and cultured, no microorganisms could be found. Meanwhile, the number of cases of Lyme disease continued to increase [46].

In 1977 nine patients affected by the ECM rash remembered they had been bitten by a tick at the site of the rash [46]. One of them had removed the tick and saved it, and was able to give it to Steere for identification. The tick was identified by Andrew Spielman of the Harvard school of Public Health as I.dammini, a species closely related to I.ricinus, the tick responsible for European ECM. Now that I.dammini had been identified, investigators working on Lyme disease hoped to isolate the actual agent of infection. First they had to be certain that the tick was indeed the vector for Lyme disease. The distribution of the dog tick, Dermacentor variabilis, was equally common on both sides of the Connecticut River, but I.dammini was more abundant on the east side- near Lyme, Old Lyme and East Haddam - where Lyme disease was by that time known to be endemic. The workers were convinced that I.dammini must be the primary vector in the transmission of Lyme disease.

The discovery of the etiological agent was almost accidental. All cultures made from clinical material were negative. Fortunately, because of a fatal case of Rocky Mountain Spotted Fever in the fall of 1981 on Shelter island, the New York State Department of Public Health sent a team of biologists to collect live ticks. Their objective was D.variabilis, the vector of Rocky Mountain Spotted Fever in that region. This species was not found that late in the fall, but I.dammini were found and sent to Rocky Mountain Laboratories in Hamilton, Montana. There, these ticks were dissected by Willy Burgdorfer and found to

contain a spirochete which could be grown in artificial media [46]. When ticks containing these forms fed on rabbits, lesions appeared at the bite site 10 to 12 weeks after feeding and these lesions resembled those lesions seen in humans. Sera from patients with Lyme disease were tested by indirect immunofluorescence and positive reactions were obtained at titers ranging from 1:80 to 1:1280 [29].

Fortunately, Alan G. Barbour, then at the Rocky Mountain Laboratories, was able to grow the spirochete in pure culture and obtain them in sufficient quantities for experimentation. It was interesting that organisms presenting the morphological characteristics of spirochetes were said to be associated with ECM as early as 1948 [73]. From this point investigations proceeded rapidly. By the summer of 1982 spirochetes had been isolated from the blood, skin and CSF of Lyme disease patients.

Russell C. Johnson and his colleagues at the University of Minnesota Medical School studied the Lyme disease spirochete and determined on the basis of DNA homology , that it was a new species in the genus Borrelia [46]. In 1984, to honor its discoverer, Burgdorfer, they named it Borrelia burgdorferi.

Since the outbreak in Old Lyme, Connecticut, additional foci of the disease have become evident. As noted earlier, the cutaneous disease (ECM) had been described and named as early as 1909. Although in the United States the first reported cases of ECM occurred in Wisconsin in 1970 [95], and south eastern Connecticut [87], Lyme disease as a new form of inflammatory arthritis was first recognized in 1975 in Lyme, Connecticut [116]. Since 1982, Lyme disease has been reported with increasing frequency. The majority of reported cases have occured along the east coast from Delaware to Massachusetts,in the Mid-West from Wisconsin to Minnesota, and in California. However, cases have been reported from 43 states, including Michigan (Fig.1).

In 1984, isolated cases had been reported from 10 states outside the range of either I.dammini or I.pacificus [37]. The finding of the spirochete in Amblyoma americanum ticks collected in New Jersey [96] may explain some of these isolated cases.

The Lyme disease vector

Lyme disease is transmitted by ticks. In north-central and north-eastern states, the vector is the northern deer tick,I.dammini [29,26] (Fig.2). Not all I.dammini carry the organism; however, in endemic areas, 20% to 60% of the ticks may be infected.

The life cycle of I.dammini normally spans two years (Fig.3). Eggs are deposited in the spring and hatch into free living larvae a month later. During the first summer the larva feeds once for a period of 2 days on the blood of a host (usually a rodent such as the white-footed mouse, Peromyscus leucopus, which serves as one of the primary reserviors for B.burgdorferi [6,7,72]). Then the larvae enter a resting stage coincident with the onset of cold weather in the fall. The following spring, the larva molts, enters a second immature stage called the nymphal stage and again attaches itself to an animal host, this time to feed for 3-4 days. Although the larvae and nymphs attach to a variety of vertebrates, the majority of the ticks in these age cohorts are found on the white-footed mouse, P.leucopus. It is at this stage that ticks are most likely to attach themselves to humans [46].

At the end of the summer, nymphs molt into the adult stage. They can be found in brush about one meter above the ground, where they can easily attach themselves to larger mammals. Like the immature ticks, the adults feed on a

variety of mammalian hosts; but in the northeastern U.S they are found predominately on the white-tailed deer, Odocoileus virginians. The adult ticks mate on the host soon after the female attaches herself to it. Only the females overwinter; the male dies soon after mating. It is not known where the eggs are deposited, but they hatch in the spring and the entire cycle is repeated [46].

Newly hatched larvae are usually not infected because transovarial transmission of the spirochete appears to be minimal. The larvae become infected primarily by feeding on infected rodents, and the spirochetes are maintained through each subsequent development stage. Although both male and female adult ticks may contain spirochetes, it is only the female that has been reported to transmit the disease [98].

Transmission of the spirochete does not occur immediately at the time the tick attaches to its host. Approximately 7% of rodents exposed to spirochete-carrying ticks were infected after 24 hours, 36% after 48 hours, and 93% after 72 hours [93].

Although all developmental stages of the deer tick will feed on humans, most human cases of Lyme disease are acquired from ticks in the nymphal stage because the nymphs are active in the summer (a time when people wear comparatively little protective clothing and are likely to be camping or hiking in the woods). By contrast, adult ticks have less opportunity to transmit the disease because they are active in the early spring and late fall when people wear more protective clothing and are less likely to be in wooded areas [98].

Although B.burgdorferi has been isolated from the dog tick, D.variabilis [7], there is no evidence that the dog tick is capable of transmitting the spirochete (D.variabilis does transmit Rickettsia rickettsie, the etiologic agent of Rocky Mountain Spotted Fever). The adult deer tick is approximately

one-third the size of the dog tick (Fig.4), and the nymphal stage is no longer than the head of a pin (Fig.5). Borrelia burgdorferi has also been isolated from mosquitoes, deer flies, and horse flies, but their ability to transmit the spirochete has not been established [82].

In the western states, B.burgdorferi is carried by the California black-legged tick, Ixodes pacificus. Amblyoma americanum-the lone star tick- has been identified as a vector of Lyme disease in New Jersey [93]. The major vector of the disease in Europe is I.ricinus, and in Russia and Asia is I.persulcatus [98].

What seems clear is that Lyme disease is spreading in the U.S.A. Infected ticks are being disseminated to new areas locally by birds and mammals and to distant areas by birds. In addition to transporting infected ticks, birds can themselves be infected [4].

One approach to counter the spread of Lyme disease is to nab the immature ticks that carry it. According to Dr.Thomas Mather of the Harvard University School of Public Health, these ticks are thought to acquire Lyme disease spirochete by sucking blood from infected field mice. Because field mice often use cotton to build their nests, Mather has successfully used pesticide-tainted cotton balls placed in cardboard tubes and scattered in mouse habitats to stem the Lyme disease-infected tick population in a tested area. The Massachusetts and New York State Parks Departments are currently evaluating Mather's system.

The etiologic agent of Lyme disease

Discovery of the Lyme disease spirochete

In the late 1940's and early 1950's, two Swedish investigators, Lennhoff and Hollstrom, discovered spirochetal structures in the lesions of ECM and

demonstrated the efficacy of penicillin in its treatment [73,55]. Early speculation that Lyme disease might be caused by a virus diminished with the report of rapid resolution of ECM and other symptoms with early antibiotic treatment [107].

In 1982 Burgdorfer et al [29] isolated a treponema-like spirochete from the midgut of the tick Ixodes dammini collected on Shelter Island, New York. The spirochete had irregular coils ranging from 10 to 30 micrometer long and 0.18 to 0.25 micrometer in diameter. This size allowed it to pass through many filters designed to retain bacteria [46]. When these infected I.dammini ticks were allowed to feed on New Zealand white rabbits, there was no immediate reaction. However, after 10 to 12 weeks small skin lesions developed and progressed into typical (ECM). Samples of serum from all exposed rabbits showed high titers of antibody to the spirochete by indirect immunofluorescence, as did sera from 9 patients with typical Lyme disease [117]. A year later, in 1984, Berger and colleagues reported the presence of spirochetes accompanied by a lymphoplasmacytic infiltrate in ECM [25].

Cultivation of the spirochetes by Dr. Alan G. Barbour [10] paved the way for detailed studies of the infectious agent and a definitive identification of B.burgdorferi as the etiologic agent of Lyme disease. The spirochete was first isolated from blood, skin, and spinal fluid of patients by Dr. Steere and coworkers [114] and Dr. Jorge L. Benach and colleagues [22]. Subsequently B.burgdorferi was identified as the causative agent of European Lyme disease [8,30,92,123], where it is referred to under a number of names, including lymphocytic meningoradiculitis (Bannwarth's syndrome), ECM, acrodermatitis chronicum atrophicans, and erythema migrans disease.

General Bacteriology: Taxonomy and Ultrastructure

Borreliae are spirochetes and as such have in common with other spirochetes the following characteristics [54,66] :

i) The cells are helically shaped and motile with three modes of movement.

ii) An outer cell membrane surrounds the protoplasmic cylinder complex, consisting of the cytoplasm, the inner cell membrane, and the peptidoglycan.

iii) Flagella, which are equivalent to other bacterial flagella in architecture, are located not at the cell's surface but in the periplasmic space between the outer cell membrane and the protoplasmic cylinder.

Ecological and biochemical characteristics that serve to identify the genus Borrelia are :

i) All species in this genus are transmitted to vertebrates by hematophagous arthropods; there often is transovarial transmission of the borreliae in arthropods.

ii) The gaunine -cytosine content of the genomic DNA is between 2% and 32% [59,61,62].

B.burgdorferi is about 200 mm wide and 10 to 30 micrometer long. There are seven to eleven periplasmic flagella, the longitudinally transversing filaments that characterize the spirochetes. Borrelia have both an inner(cytoplasmic) and an outer membrane. The outer membrane of B.burgdorferi, like those of other Borrelial species, is easily disrupted [17,38]. The rigidity which can be imparted by components such as the lipopolysaccharides of gram-negative bacteria, is not seen in the outer membrane of B.burgdorferi [17].

B.burgdorferi is a typical spirochete. It is a unicellular loosely coiled, left-handed helix,that is; it coils in a counterclockwise direction. Like most

spirochetes, it is small and difficult to detect (Fig.6).

Lyme disease spirochetes lack cytoplasmic tubules and divide by binary fision. They are microaerophilic and they lack the catalase enzyme. Their major carbon and energy source is glucose, and lactic acid is their principal metabolic end product. In vitro, Lyme disease spirochetes can be cultivated successfully in modified Kelly's medium [122]. By either phase-contrast or dark-field microscopy of live organisms, or standard light microscopy of stained, fixed organisms, B.burgdorferi can usually be distinguished from other Borreliae by its looser and more irregular coiling [18].

Typing strains

As the number of isolates of B.burgdorferi from different human and animal sources and from different parts of the world increases, greater attention is being paid to strain distinctions. Several options are available, including poly-acrylamide gel electrophoresis profiles of cellular proteins, reactivities of monoclonal antibodies, and plasmid analysis [18].

The initial isolates of B.burgdorferi in the U.S.A were almost identical in their polyacrylamide gel electrophoresis protien profiles [11,16,17,32]. They all had major proteins of 31(OpsA) and 41(flagellin)KDa. A large majority had an abundant 34-KDa surface protein, OpsB, but some isolates either lacked this protein or had an OpsB with a slightly different electrophoretic migration [11,16].

Although polyclonal antibodies raised against an isolate of B.burgdorferi will react with other isolates, differences between strains are being realized. Through the use of monoclonal antibodies, variations in the outer membrane proteins have been observed [124]. This antigenic heterogeneity may be plasmid-

mediated. Although many bacteria have plasmids, this characteristic is limited among the spirochetes.

Plasmids were first identified in Borrelia in 1984 by Hyde and Johnson [59]. Barbour and Garon recently examined B.burgdorferi to ascertain whether the OpsA and OpsB genes were located on a plasmid [13]. They found that these genes were located on a unique 49 Kilobase double-stranded plasmid that existed in a linear form with covalently closed ends.

DNA hybridization of whole chromosomal DNA has shown that B.burgdorferi is a distinctive species in the genus Borrelia and that strains within the species differ in the amount of DNA relatedness [58,61,62,103]. These differences may not be great enough, however, to use genomic DNA hybridization as a routine typing procedure for B.burgdorferi [18].

Clinical manifestations of Lyme disease

Lyme disease is a multisystem inflammatory disorder which,in its classical form, affects in turn the skin, the nervous system and/or the heart, and finally the joints [116]. Transmission of B.burgdorferi from vertebrate to vertebrate depends on blood-feeding arthropods. Infected vertebrate hosts are lightly spirochetemic for days to weeks and during this time the infection may spread to other organs [20,28,31,63,69,105]. During the spirochetemic phase of illness, humans commonly have fever and constitutional symptoms. Some of these systemic effects may be the consequence of Interleukin-1 production by leukocytes exposed to whole cells or released components of the Borreliae [36,45]. Following spirochetemia, the organisms are to be found in various organs [42,63].

In many patients, the inflammation that follows may be due in part to the persistence of viable Borreliae and in part to the host's immune response to the bacteria [18].

Lyme disease is rarely fatal; thus, the knowledge of the pathology of human infection is not extensive and depends on the rare autopsy case, biopsies, and animal infections [18]. The predominant finding in biopsy specimens is a lymphocytic and plasmacytic infiltrate, usually greatest in perivascular areas [25,41,42,43,65]. Neither granulomas nor necrosis is found, but marked fibrin deposition and obliterative microvascular lesions have been noted [18].

Early B.burgdorferi infection may be either asymptomatic or of such a nonspecific nature that it cannot be distinguished by respondents from an influenza like illness. Marked variations in the expressions of the disease have been observed.

The manifestations of Lyme disease can be roughly placed in one of three stages according to when they occur during the course of the infection [18,122] (Fig.7). Erythema chronicum migrans (ECM) is the hallmark of the first stage and the best clinical and epidemiological marker of Lyme disease [108,110] (Fig.8). ECM is currently designated simply as EM (erythema migrans), due to the fact that the lesions are no longer considered chronic. ECM is analogous to the primary chancre of syphilis. Typically, this lesion appears at the site of a tick bite sustained 3 to 14 days previously. ECM is characterized by an advancing, slightly elevated, annular erythema which leaves a central clear area without scaling. The outer edge is usually more distinct than the inner edge of the ring. The primary skin lesions may not always take this

classical form and may appear instead as an erythromatous plaque which extends its margin. During early infection the patient may complain of low or moderate fever, headache, easy fatigue-ability, arthralgias, stiff neck, and myalgias. Approximately half of the patients with untreated ECM develop one or more metastatic annular lesions at sites distant from the original rash [18].

The rash begins as a small macule or papule which expands over days to form a large, annular, red lesion. ECM occurs most commonly on the proximal extremities (thigh,buttock,and axilla) and trunk, a distribution consistent with the behavior of a crawling, rather than a flying vector. In most patients, the rash fades over a period of 3 days to 8 weeks [122].

The leukocyte count and hepatic transaminases may be elevated in the blood during acute disease [108,119]. Low to moderate levels of circulating immune complexes have been found in ECM patients [48-50]. Patients with elevated serum total IgM concentrations and cryoglobulins are more likely to have a complicated disease course [88,115]. Concentrations of total serum IgM correlate with the degree of disease activity [24].

In the second and third stages of Lyme disease there may be skin, joint,nervous system, or cardiac involvement [9,91,94,110]. The second stage manifestations usually start a few months after the initial ECM. Third stage manifestations occur months or years after onset of infection [18].

The heart disorder in Lyme disease is a diffuse myocarditis and is self-limited in almost all cases [86,90]. Nonetheless, Lyme carditis is the most potentially serious complication. Cardiomegaly and heart failure

are rare, but there may be evidence of mild ventricular dysfunction and electrocardiographic changes consistent with acute myopericarditis [18].

Approximately 10% of Lyme disease patients develop cardiac disease within several weeks to months after onset of illness [116,110]. The most common abnormality observed in one such study of 20 patients, mostly young men, was atrioventricular block (AV), which fluctuated in degree [122].

The second stage neurologic disorders may appear suddenly a few weeks after appearance of ECM or advance insidiously over months [51,53,89,94,99,118]. Approximately 30% to 40% of patients with disease progressing beyond ECM have neurologic complaints.

The clinical complex of neurologic abnormalities accompanying or following ECM had been known in Europe for a half century and were called Tick-borne meningopolyneuritis or Bannwarth's syndrome [2,100]. The illness was characterized by chronic radicular pain, cranial or peripheral neuropathy and chronic lymphocytic meningitis. Patients were treated symptomatically and the illness often lasted for weeks to months.

The arthritic manifestations of Lyme disease differ depending on when, in relation to the onset of illness, joint involvement begins [116,109]. In patients who develop arthritic symptoms soon after the first stage of disease, the manifestations tend to be transient, less well defined, and characterized by migratory pain in joints, bones, muscles, or bursae. In contrast, frank arthritis usually does not begin for months after the initial symptoms.

The frank arthritis typically involves a knee or other large joints [52,60,112,116]. Some of these patients, if untreated, continue to have a chronic, destructive arthritis of one or more large joints. There may

be erosion of the cartilage and bone and a proliferative synovium. Chronic Lyme arthritis may last for years and is then considered part of the third stage of infection.

An added clinical problem is the maternal-fetal transmission of B.burgdorferi. The organism, like other pathogenic spirochetes, is probably transmissible via the placenta to the fetus [14]. B.burgdorferi infection of fetuses has been documented [76,102].

Diagnosis

The diagnosis of Lyme disease is relatively straightforward during stage 1 if a typical ECM is present and the physician is aware of the possibility of this illness. Unfortunately, approximately 30% of adults and 50% of children do not develop ECM. The clinical picture is less clear in late disease as manifestations mimic a variety of neurologic, arthritic, and dermatologic disorders. Thus if ECM is absent or unrecognized and a history of tick bite is lacking, diagnosis becomes more dependent on laboratory findings, particularly in the late stages of the illness [98].

The rash can be confused with: (1) cellulitis, particularly streptococcal or staphylococcal; (2) erythema multiforme, although its lesions are usually smaller and more urticarial; or (3) erythema marginatum. If there is a necrotic or vesicular center in the ECM lesions, it may resemble the lesion of Tularemia, but the latter is not expansive and not associated with similar complications. It is particularly important to distinguish Lyme disease from acute rheumatic fever

especially with the resurgence of the latter [125,120].

Other forms of arthritis that might be confused with Lyme disease include: (1) pauciarticular juvenile rheumatoid arthritis; (2) reiter syndrome; (3) psoriatic arthritis; (4) gonococcal arthritis; (5) reactive arthritis associated with Salmonella, Shigella or Yersinia infections; (6) postinfectious or infectious arthritis; and (7) temporomandibular joint syndrome [111]. There are usually several distinctive features that allows prompt differentiation from Lyme disease. A definitive diagnosis of Lyme disease can be made based on isolation of B.burgdorferi from patients. Presently this is a low-yield procedure, and 3 weeks or more are required before cultures become positive. Direct examination of specimens is not productive because of the paucity of spirochetes in tissues and body fluids.

Serologic tests for antibodies to B.burgdorferi are currently the most useful diagnostic tools available [40,81,97]. Both Indirect immunofluorescence (IFA) and ELISA are used to detect total Immunoglobulins or class-specific IgM and IgG. While the tests are of similar sensitivity and specificity, the ELISA is often preferred because the tests can be automated and the results statistically analyzed [98].

Sensitivity of both tests (IFA,ELISA) varied with the stage of disease but was 100% for both tests during complicated Lyme disease [97]. Investigators found that both tests are highly specific and sensitive for complicated Lyme disease but relatively insensitive for patients with ECM alone [97].

The IFA was the first test to become established. The test is, however, subjective and difficult to automate. The IFA is being gradually

superseded in the USA by the ELISA as it is more sensitive and specific [40,81,97].

Either an IFA or ELISA for total immunoglobulins is adequate for routine confirmation of Lyme disease. However, the antibody response to B.burgdorferi is relatively slow, with IgM titers peaking between the third and the sixth weeks of illness and IgG titers peaking months later. The IgM response is not necessarily limited to early Lyme disease, it may persist in patients with prolonged illness, and a new IgM response may appear late in the disease [39].

Serologic testing therefore is likely to be most helpful in evaluation of patients from or visitors to endemic areas who develop systemic illness in whom ECM is absent or who present primarily with neurologic, cardiac or rheumatologic disorders. False positive tests are most often seen with other spirochetal diseases. Patients with early disease and\or early treatment are least likely to have a positive response [19].

Because cross reactivity is largely associated with IgM antibodies, an assay for IgG antibodies may help differentiate later stages of Lyme disease from other neurologic, arthritic, and dermatologic disorders. These serologic assays will not, however, discriminate between Lyme disease and relapsing fever. Although cross reactivity does occur between B.burgdorferi and pathogenic Treponema, Rapid Plasma Reagin (RPR) and microhemagglutination tests can be used to differentiate Lyme disease from those of syphilis and yaws because B.burgdorferi antibodies are nonreactive in these tests [77].

Despite the usefulness of serologic tests, interpretation of results is not straightforward. Results vary from laboratory to laboratory for

lack of standardized tests of patient antibody response to B.burgdorferi. Depending on the laboratory, IFA titers ranging from 1:64 to > 1:256 are considered evidence of Lyme disease in patients with compatible clinical symptoms. Interpretation is further complicated by reports of seronegative Lyme disease and the fact that approximately 50% of patients are serologically negative during early stage 1 disease [98].

Currently, diagnosis of Lyme disease cannot be based upon laboratory findings. However, because of nonspecific symptoms, a greater reliance on serology is often necessary in late disease. The observation that patients with Lyme disease may excrete antigens of B.burgdorferi in the urine, provides the possibility of an additional diagnostic test.

Routine laboratory testing is usually nonspecific and not helpful. The sedimentation rate is often elevated. Leukocyte counts are commonly normal. Aspartate aminotransferase and alanine aminotransferase concentrations can be mildly elevated in early disease. Complement studies are variable. Serum IgG and IgA concentrations are usually normal. However, IgM and cryoglobulin M are often elevated, particularly in patients with severe disease who later develop neurologic complications or arthritis [115]. Immune complexes can be found in patients with Lyme disease and may be involved in its pathogenesis [48]. In some children with arthritis the antinuclear antibody is elevated [101].

Synovial fluid in patients with arthritis can have leukocyte counts from 500 to 98,000 cells/cubic millimeter with a predominance of polymorphonuclear leukocytes. Total protein is usually 3 to 8 g/ml. Joint fluid antinuclear antibody, rheumatoid factor and complement determinations are normal, but cryoglobulin is almost always present [19].

Direct detection

Stanek and colleagues were able to detect as few as 10,000 Borreliae per ml of mouse blood by microscopic examination of a wet mount of blood [105]. Other investigators detected spirochetes in the urine of about half of all field mice examined in an endemic area [27]. In one report, a spirochete was seen by electron microscopy in the skin biopsy of a patient with ECM [121]. In exceptional cases, spirochetes have also been detected in synovial tissue biopsies with either the standard or modified Dieterle Silver Stain [43,65]. When seen, the numbers of spirochetes present were very low.

Polyclonal antibodies have been used successfully in immunohistologic studies to demonstrate spirochetes in tissues [27,68]. However, with monoclonal antibodies not only are spirochete structures demonstrated, but also the particular type of spirochete can be determined [15-17].

Direct and indirect immunofluorescence assays with antiborrelial antibodies have been used to determine the prevalence of infected ticks in different geographic areas [5,32]. Although this approach has proved successful in field studies, laboratory experiments with ticks have shown some borreliae in the ticks may either not react at all with certain monoclonal antibodies or react more weakly than they usually do with polyclonal antisera [32,71]. This phenomenon suggests that antigenic variation occurs. Another strategy for direct detection of organisms is with DNA probes, using cloned B.burgdorferi genes [47,56,57].

The presence of B.burgdorferi may also be suggested by the detection of borrelial antigens in body fluids. Using an immunoassay, Benach et al found evidence of an outer membrane in the urine of infected hamsters

[23]. Endotoxin is an indicator of Gram-negative infection, but endotoxin-like activities have not been found in the blood of patients with Lyme disease [104].

In Vitro Cultivation

The culture medium for in vitro cultivation is complex and expensive and has a short shelf life. Only a minority of cultures from definite cases of Lyme disease yield spirochetes. Under these circumstances, B.burgdorferi cultivation can hardly be considered the diagnostic method of choice, but this approach remains the only way to confirm a diagnosis. Recovery of B.burgdorferi from a patient indicates an active or latent disease state and not simply an inconsequential colonization [18].

Burgdorfer, et al were the first to recover a spirochete from Ixodes dammini ticks by using Stoenner's version of Kelly's medium [29]. By additional modifications of Stoenner-Kelly medium to improve the buffering capacity and make preparation easier (BSK medium), Burgdorfer, was also able to isolate borrelia from Ixodes ricinus ticks of Europe and to grow B.burgdorferi from a single organism [12].

B.burgdorferi is grown at temperatures between 30C and 37C in the laboratory. At temperatures above 38C, Borrelial growth slows substantially [26]. The cap or lid of the culture vessel is usually tight or sealed to prevent loss of carbon dioxide from the medium. The generation time is 8 to 24 hours, and culture-adapted strains achieve cell densities of about 100,000,000 spirochetes per ml [10]. The microaerophilic character of Borrelia is indicated by its preference for the bottom portion of the culture medium during initial growth [10,61]. All human lymph node aspirates cultures have been negative to date [114].

Lyme disease spirochetes have been recovered from several types of feral and domestic animals. These include field mice(Peromyscus leucopus), raccoons, voles (Microtus pennsylvanicus), dogs, horses, cows and birds [3,4,5,26,35,44,75].

B.burgdorferi has been isolated from Ixodes spp. ticks [5,29,32,114,1,12,64]. Although organisms have been isolated from whole ticks ground up and inoculated into culture medium, the midgut is the site most likely to contain cultivable spirochetes [29,30,32].

In Vivo Cultivation

Neubert et al. implanted skin biopsies from patients with ECM in nude mice and observed spirochetes in the blood of these animals a few days later [24]. Borreliae may also be present in the kidney's and urine of animals but usually not in humans. Little overt renal disease has ever been documented in human with Lyme disease, and the human urine specimens that have been cultured have been negative [113].

Lyme disease Pathogenesis

It is interesting that Lyme disease patients experience an extensive array of symptoms in spite of the presence of only a small number of spirochetes. Two theories of Lyme disease pathogenesis have been advanced to explain this fact; both involve the immune system and both appear to be operative.

The first theory holds that immune complexes, which consist of antigens from the spirochete and antibodies and complement from the human host, accumulate in a patient's joints. This build up in turns attracts

neutrophils, which release a variety of enzymes that attack the antigen-antibody complexes. According to this hypothesis, it is the enzymes released by the neutrophils that attack the joint and erode bone cartilage tissue to cause arthritis-like symptoms.

Work done by Gail S. Habicht et al [46] at the State University of New York at Stony Brook suggests a second hypothesis. They believe the pathological effects of spirochetes are amplified not only by neutrophil-secreted enzymes but also by the immune system mediator called Interleukin-1(IL-1).

IL-1 is a protein with a molecular weight of 17000 daltons that is synthesized primarily by the phagocytic white blood cells ,macrophages. It is a regulator of the body's immune response and acts as the molecular orchestrator of nonspecific defense mechanisms against a variety of environmental insults.

One of the most powerful stimuli for the release of IL-1 is a lipopolysaccharide (LPS), a complex of sugar and lipid molecules, that is found in the outer envelope of the cell wall of all Gram-negative bacteria. It is speculated that B.burgdorferi might contain LPS that could trigger the release of IL-1, which in turn would exert powerful local and systemic effects on the human body (Fig.9). The Jarisch-Herxheimer reaction (J-H) experienced by some Lyme disease patients, is consistent with Habicht et als theory mentioned above: antibiotic treatment kills large numbers of the spirochetes at the same time, releasing large quantities of LPS into the blood stream and triggering the production of IL-1.

Prevention and control

Prevention of Lyme disease occurs only by avoidance of contact with the tick vector. It is important to realize that not all ticks carry the Lyme disease spirochete. Even in endemic areas where a large percent of the ticks are infected, the chance of acquiring disease depends in part on duration of attachment of the tick [93,21].

Some of the important preventive methods include: (1) avoidance of high risk areas, particularly wooded, grassy areas; (2) if walking in such areas, wearing long pants, long-sleeved shirts, high socks and sneakers; (3) use of insect repellents such as DEET (for skin) and Permethrins (for clothing); (4) most important by conducting careful "tick patrols" everyday or after every potential exposure to look carefully for the ticks; and (5) removal of ticks by pulling straight out with tweezers or protected fingers [18]. Finally, although there is no vaccine available for Lyme disease, the finding that hamsters can be protected from experimental B.burgdorferi infection suggests the potential for a vaccine in the near future [67].

Treatment

Fortunately, Lyme disease can be treated successfully at any stage with broad-spectrum antibiotics administered orally, including penicillin, tetracycline, and erythromycin. Treatment during the first stage greatly reduces the likelihood of developing neurologic, cardiac or arthritic complications. Even if it is left untreated until the third stage, Lyme

disease can still be eradicated in most patients by antibiotic therapy, although hospitalization and intravenous administration of the antibiotics may be necessary at this stage [46].

Currently, there is no vaccine for Lyme disease, although researchers are pursuing this possibility. Development of an effective vaccine may be difficult because the Lyme disease bacterium belongs to a group of bacteria that genitically often change their protein coats, thereby deceiving the immune system defenses that vaccines prompt.

Physicians who treat patients with Lyme disease have observed an unusual phenomenon. Immediately following antibiotic therapy there is a temporary exacerbation of symptoms. This phenomenon known as J-H reaction was also observed following treatment of other spirochetal infections, such as syphilis and relapsing fever [46]. The reaction gave investigators a major clue for elucidating the pathogenesis of Lyme disease.

Figure 2. Adult female Ixodes dammini. Unengorged (left),and blood-engorged state (right) (about seven times actual size).

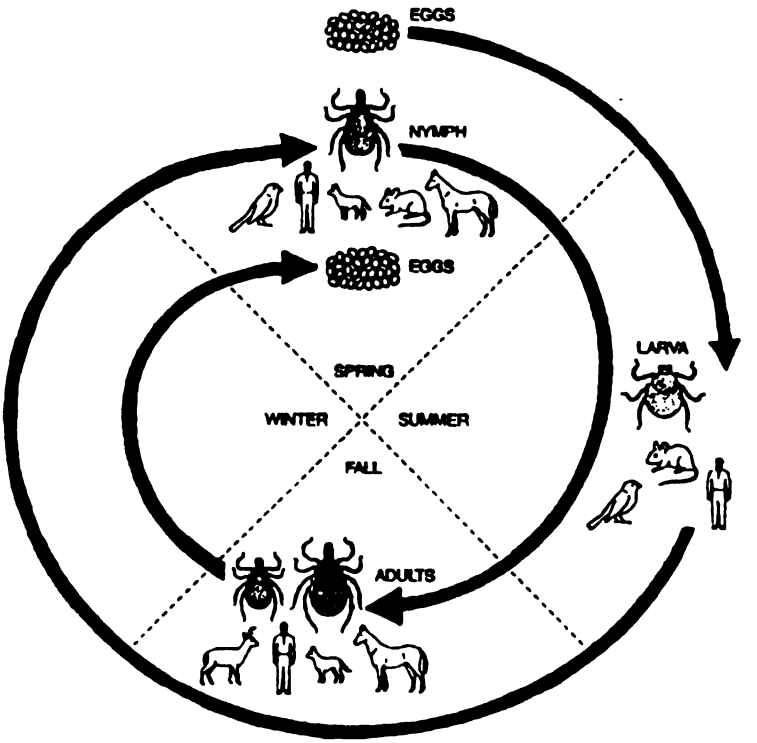

Figure 3. The life cycle of the tick I. dammini (Scientific American, July 1987)

Figure 4. Adult Dermacentor variabilis, the american dog tick, and I.dammini (smaller tick).

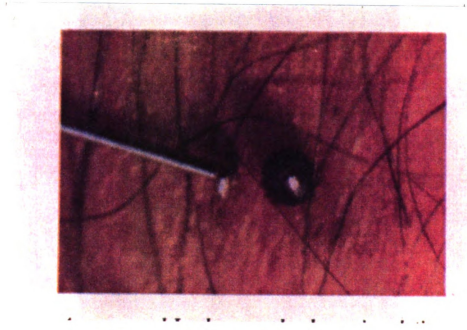

Figure 5. Engorged I.dammini nymph in the act of drawing blood from its human host, shown in relation to the size of a common pin.

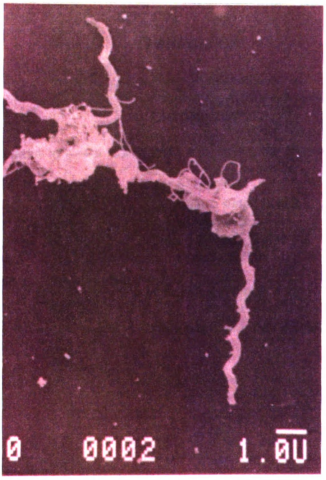

Figure 6. B.burgdorferi, shown under the scanning electron microscope (4400X).

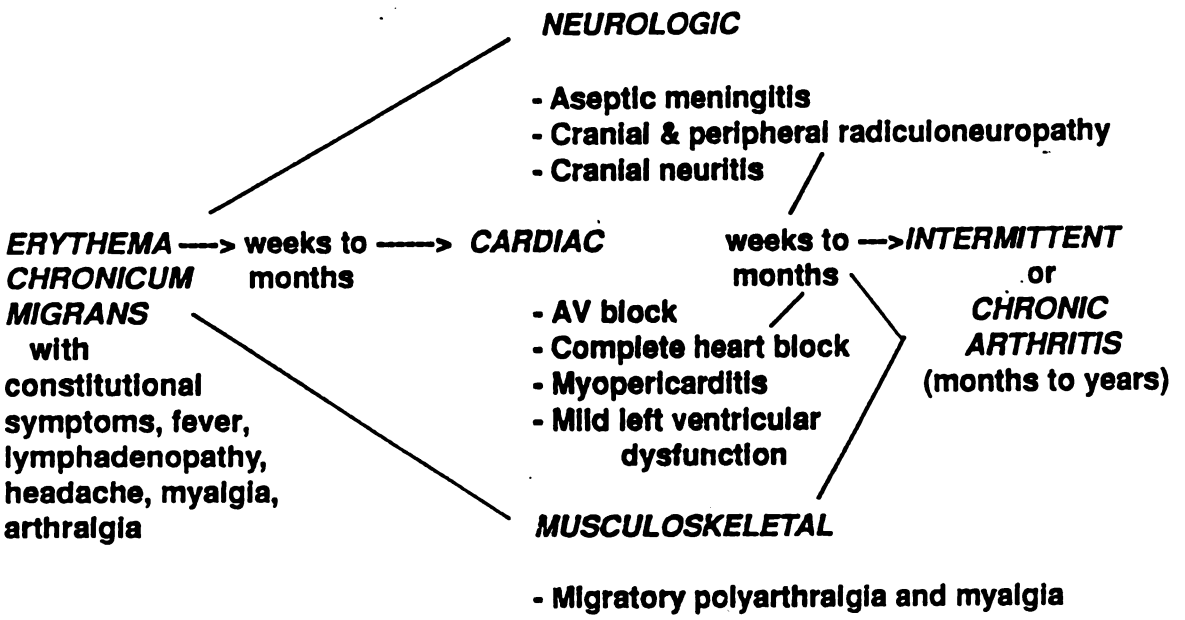

Figure 7. The three stages of Lyme disease

Figure 8. Erythema chronicum migrans (photo by Dr.Melski)

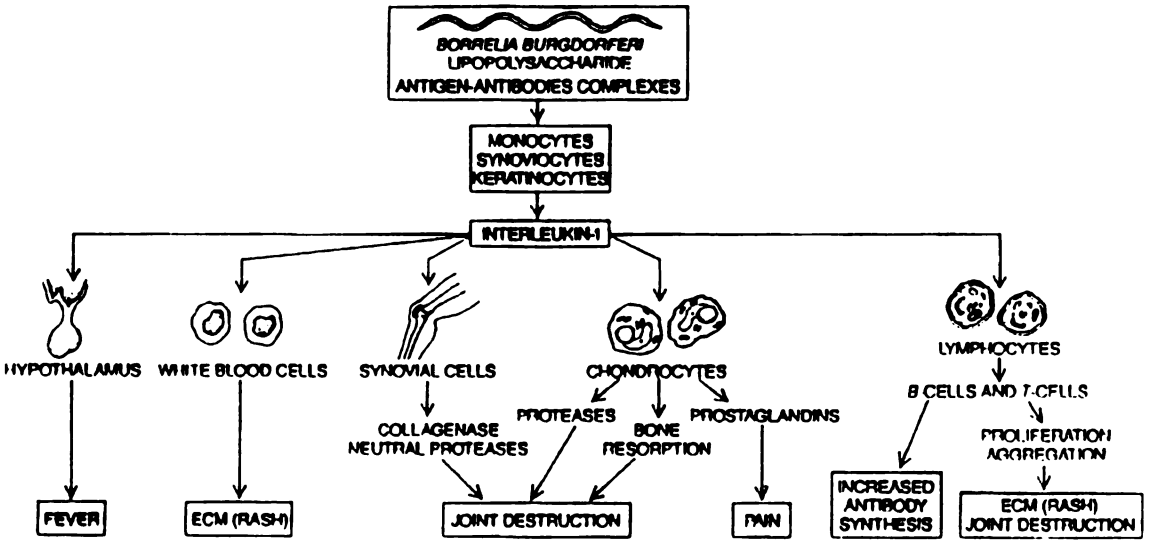

Figure 9. The major role of IL-1 in Lyme disease pathogenesis (Scientific American, July 1987)

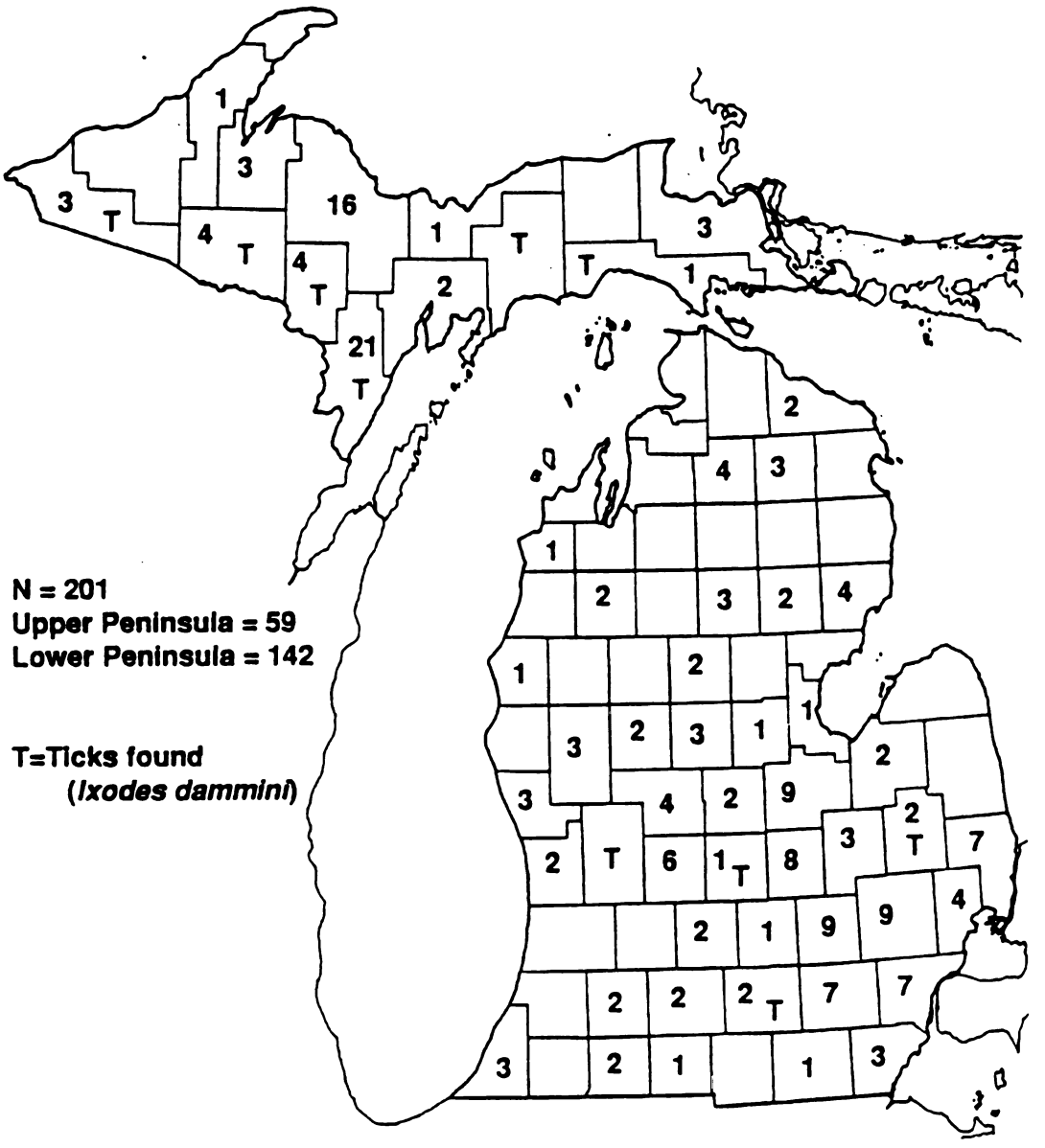

Figure 10. Reported cases of Lyme disease by county of likely exposure.

MATERIALS AND METHODS

Tick collection

Ticks were collected by the staff of the Insect and Rodent Control Section at Michigan Department of Pulic Health, from forty-one study sites in 19 counties in the Lower Peninsula and 22 sites in three counties in the Upper Peninsula of Michigan. Study sites were determined from review of the human Lyme disease case report forms [Fig.10].

A case definition for Lyme disease varies from one part of the country to another. The latest case definition from the Center for Disease Control (CDC) in August, 1988 states that only ECM will be required for endemic counties, and ECM plus a positive serology will be required in nonendemic counties. The Michigan Department of Public Health, however uses a more complicated case definition that includes ECM with exposure occuring no more than 30 days prior to onset of ECM. If ECM is absent, organ involvement and either a positive serology or isolation of B.burgdorferi from a clinical specimen could be called Lyme disease [Michigan Department of Public Health,Lyme disease Diagnostic Criteria for Surveillance,1989]. This case definition holds true only in endemic areas, with non-endemic counties having different criteria.

A white flannel cloth was used to drag the area to collect questing ticks. Animals were trapped alive, placed in a wide mouth anesthetizing chamber filled with ether soaked cotton balls, and examined for ticks. Pets

and road killed animals were also examined for ticks. Local Health Departments, Department of Natural Resources, and the public were invited to submit ticks. Collection site, sex, and species were noted for each tick and submitted to the laboratory for examination for B.burgdorferi. Ticks midgut tissues were dissected and smeared on glass-microscope slides as described previously by Anderson, et al and Burgdorfer, et al [5,29] . Slides were fixed for 10 minutes in acetone, air dried and stored at -70C for later examination using IFA.

Chemicals and reagents

- Antibody (a murine monoclonal [H5332]) was donated by Dr. Alan Barbour at the University of Texas Health Science Center at San Antonio.

- Phosphate buffer saline (PBS) pH 7.2 (Formula per liter: NaCl 7.65 g, Na2HPo4 0.724 g, KH2Po4 0.21 g).

- Fluorescein-labeled rabbit anti-mouse (IgG) antibody [Cappel Research Reagents, Division of Organon Teknika Corp, West Chester,PA 19380].

Ticks Testing Procedure

Ticks were tested for the presence of B.burgdorferi by using an indirect fluorescent monoclonal antibody technique with standard methods. The slides fixed earlier were overlaid with murine monoclonal antibody (H5332) diluted 1:16 in phosphate buffer saline (PBS) solution. This antiserum was directed to outer membrane surface protein A (OSP A), a polypeptide of approximately 31 KD that is common to all North American isolates of

B.burgdorferi [17]. The slides were then incubated in a moist chamber for 30 minutes at 37C, washed 3 times for 5 minutes each in PBS and air dried. The fixed slides were overlaid with fluorescein-labeled rabbit anti-mouse (IgG) antibody diluted 1:80 in PBS. They were then incubated in a moist chamber for 30 minutes at 37C, washed 3 times for 5 minutes each in PBS and air dried. Slides were mounted with buffered glycerol and evaluated using a fluorescent microscope at 40X. The specificity of this monoclonal antibody, dilution of reagents, and other procedures used in the indirect fluorescent-antibody (IFA) staining procedures have been reported [5,17,82].

Positive and negative controls for B.burgdorferi were included in the tick testing procedure. Treponema pallidum was also included as a control to rule out crossreactivity with B.burgdorferi.

Results

During October 1988 to December 1989, 1307 ticks were collected from Michigan. Eleven (22.9%) of 48 Ixodes dammini ticks were found to harbor spirochetes that reacted with fluorescein-labelled rabbit antisera to B.burgdorferi. A total of 553 Dermacentor variabilis ticks were collected. Of those only 7 (1.3%) were found positive for B.burgdorferi. All other tick species were IFA negative for B.burgdorferi [Table.I].

Two of the 11 positive I.dammini were adult female, two were in the nymph stage, and seven were in the larva stage. One out of the seven positive D.variabilis was nymph, and six were larvae.

Table 1. Tick species submitted for determination of presence of *B.burgdorferi* by IFA.

Tick species	Total examined	No.IFA Positive(%)
Ixodes dammini	48	11 (22.9)
Dermacentor variabilis	553	7 (1.3)
I.cookeii	32	0 (0)
I.marxi	15	0 (0)
I.texanus	9	0 (0)
I.kingi	5	0 (0)
I.muris	1	0 (0)
D.alpobictus	586	0 (0)
Amblyoma americanum	4	0 (0)
Rhipicephalus sanguiheus	2	0 (0)
Heamaphysalis leporisphuris	17	0 (0)
D.nigrolineator	4	0 (0)
Flea	1	0 (0)
Mite	10	0 (0)
Black flies	20	0 (0)
Total	1307	18 (1.4%)

DISCUSSION

In the present study, we report the first survey to determine the prevalence of B.burgdorferi of tick species in Michigan. Ticks were collected from 22 counties, and B.burgdorferi was found in Menominee county. Spirochetes were detected in 11 (22.9%) of 48 Ixodes dammini and 7 (1.3%) of 553 Dermacentor variabilis. The degree of infection varied; some ticks contained only a few spirochetes, others contained large numbers. Our results support previous studies [5,29], which established I.dammini as a primary vector of B.burgdorferi. Our finding infected specimens of D.variabilis is consistent with observations made by Anderson et al [89]. In Connecticut, I.dammini is the chief vector of B.burgdorferi [5,10], and the proportion of the infected I.dammini differed from 11% to 54% depending on the site, season, and sampling method. At Shelter Island, N.Y., an infection rate of 61% has been reported [29].

Of the eleven infected I.dammini, two of seven were adult females (28%), two of eight were nymphs (25%), and seven of thirty-three were larvae (21.2%). Since transovarial transmission of B.burgdorferi is low in I.dammini [83], larvae mainly acquire these spirochetes by feeding on infected hosts. Of the seven infected D.variabilis, one of 82 nymphs (1.2%) were infected and six of 78 larvae were infected (7.7%). Based on the lower percentage of infected D.variabilis nymphs and the absence of B.burgdorferi in questing adults of this species, transstadial transmission in D.variabilis is probably inefficient.

B.burgdorferi has been found in the dog tick (D.variabilis) [7], common in Michigan. Although we do not know whether D.variabilis is an efficient vector

of spirochetes, all motile stages of this tick feed on mammals; along with I.dammini, D.variabilis may play a role in the ecology of Lyme disease [7]. The low prevalence of infected specimens of D.variabilis suggests that this tick probably ingests spirochetes from infected hosts but has a minor role in ecology of Lyme disease [7]. In addition, there are no convincing reports indicating an association between D.variabilis bites and the development of erythema migrans in humans. Therefore, adults of this species do not appear to be vectors of B.burgdorferi. All D.alpobictus that have been examined were negative. The absence of B.burgdorferi in this species suggests that this tick, like D.variabilis, has a minor, if any, role in the ecology of Lyme disease. D.alpobictus has been reported to harbor the spirochete, but at a very low rate (0.6% of 157) [84]. B.burgdorferi was not found in I.cookei, I.marxi, I.muris, or I.texanus. These tick species have not been reported to harbor the spirochete.

Most of the infected ticks (16 of 18) were taken from white-footed mice trapped in Menominee county; however, some (2 of 18) were taken from humans in the same county. Infected I.dammini and D.variabilis coexisted on white-footed mice. This reinforces the epidemiological significance of this rodent in Lyme disease.

Although the tick is the only proven vector of Lyme disease, the distribution of confirmed cases of Lyme disease in Michigan doesn't correlate well with the known distribution of I.dammini. In the Upper Peninsula, I.dammini is not uncommon and Lyme disease is endemic. In the Lower peninsula, I.dammini is not common. However, Lyme disease is reported. Records at Michigan Department Of Public Health show that adult female I.dammini has been found in four different counties in the Lower peninsula; namely Jackson county (2 ticks),

Kent county (1 tick), Clinton county (3 ticks), and Lapeer county (1 tick). The only I.dammini examined from these four counties was from Lapeer county and B.burgdorferi was not detected. Finding I.dammini in the Upper Peninsula indicates that the collecting methods being used are appropriate. The lack of success in finding I.dammini in the Lower peninsula may be due to the spotty distribution of I.dammini. Despite the apparent rarity of the deer tick in the Lower peninsula , 142 of 201 confirmed cases of Lyme disease were from the Lower peninsula. This suggests two possibilities; the possibility of other arthropod vectors, and the possibility of overdiagnosis of Lyme disease due to increased awareness of Lyme disease and increased press coverage. B.burgdorferi has been isolated from mosquitoes, deer flies, and horse flies [82]. But the presence of the bacteria in these other arthropods doesnot mean they are capable of spreading Lyme disease. Further studies are required before any link between other vectors and Lyme disease can be proven.

I.dammini has the highest infection rate among the tick species collected in Michigan. In addition, the presence of I.dammini in the Upper peninsula correlates well with the distribution of confirmed cases of Lyme disease. These observations suggest that I.dammini is likely to be involved in the transmission of Lyme disease and is a principal vector of the disease in Michigan.

We conclude that I.dammini is present in Michigan and is most likely to be involved in the transmission of Lyme disease. D.variabilis and D.alpobictus have a minor role in the ecology of Lyme disease. Most tick species are found in Michigan. The possibility of other vectors for Lyme disease in Michigan requires further investigation.

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