West Nile in Connecticut

John F. Anderson, PhD
Connecticut Agricultural Experiment Station

West Nile Virus (WNV) occurs naturally in birds and is transmitted by mosquitoes. It is primarily tropical/subtropical and is widely distributed through Africa, parts of western Asia, and has been recovered from several European countries. It was first isolated from a febrile woman living in the West Nile District, Northern Province of Uganda in 1937. While this virus has been isolated from a sick rock pigeon and a dead goose in the Old World, major bird mortality has not been documented. Outbreaks of human disease caused by WNV have been reported in Europe, but this virus seemingly did not persist .

An outbreak of arboviral encephalitis in humans was recognized to be occurring in late August 1999 in New York City and was initially reported on 4 September. We began trapping mosquitoes for the testing of viruses the following day on 5 September in Greenwich, the Connecticut town closest to New York City. Traps were subsequently placed in 13 other towns in southwestern, CT. American crows were reported dying in southwestern Connecticut in the second week of September.

Virus isolations were attempted from mosquitoes and from brain tissues from dead birds. All isolation attempts were made in Vero cells grown in 5% CO_2 at 37°C. Isolates were initially screened serologically against known North American viruses associated with mosquitoes, and subsequently their genomes were sequenced and analyzed genetically by comparative phylogenetic analysis.

Virus was isolated from two species of mosquitoes, *Culex pipiens* and *Aedes vexans*, captured on the Greenwich/Stamford town line on 14 September. Virus was also isolated from brain tissues of 28 of 31 crows that died from 13 September through 12 October and from brain tissues of a Cooper's hawk. CDC isolated virus from the brain of a sandhill crane that died at the Beardsley Zoo in Bridgeport, CT. The birds all died along a 62-mile corridor from Greenwich on the New York border eastward to Madison, CT, in towns bordering directly on Long Island Sound or island about 15 miles.

All virus isolations were similar to one another and were closely related to a WNV isolate from mosquitoes captured in Romania in 1996. CDC demonstrated their isolates from birds and mosquitoes also to be related to the Romanian isolate but to be almost identical to a then unreported WMV isolate from a goose that died in Israel in 1998.

No human or horse cases of West Nile Virus Fever were recorded in Connecticut.

Our isolates and those by CDC are the first WNV isolates to be recorded in North America. It is unknown how WNV was introduced into the New World, but, if established in North American this virus will likely have severe effects on the health of humans, horses, and birds.

Overview of Human Ehrlichioses and Rocky Mountain Spotted Fever in the US

Christopher Paddock, MD
Centers for Disease Control and Prevention

In contrast to Lyme disease, rickettsial and ehrlichial infections assume relative obscurity among infectious diseases recognized by most physicians. Although existing state and national surveillance systems for tickborne illnesses report substantially fewer cases of Rocky Mountain spotted fever (RMSF) and ehrlichiosis than of Lyme disease, these diseases are important with respect to their broad geographic distributions throughout the US and their potential for severe and fatal illnesses in otherwise healthy persons. The differential diagnoses for these diseases are extensive, and the early clinical signs and symptoms resemble various other infectious and noninfectious etiologies. These infections respond quickly to appropriate treatment. Most antimicrobials used as empirical therapies for presumed bacterial infections in febrile patients are characteristically ineffective in treating rickettsial and ehrlichial diseases; appropriate treatment is seldom provided unless the diagnosis is suspected. Physician awareness of these diseases and early recognition of their salient epidemiologic and clinical features form the foundation of successful patient outcomes.

RMSF has been recognized as a clinical entity for over a century, and cases have originated from almost every state in the continental US. During the last decade, approximately 400 to 800 cases of RMSF were reported each year; >90% of these cases occurred during April through September. Age-specific incidence is highest in children < 10 years, although case-fatality ratios are highest in persons >60 years. Hospitalization occurs in 50%-70% of patients. During 1981-1996, the mean annual case-fatality ratio for RMSF was 3.4%. The ehrlichioses comprise at least 3 clinically similar but epidemiologically distinct diseases in the US, caused by *Ehrlichia chaffeensis*, *E. phagocytophila*, and *E. ewingii*. These diseases have only been recognized in the last 14 years and fall under the rubric of emerging infections. Surveillance data for the ehrlichioses are sparse. The current best estimates report approximately 1,200 US cases during 1986 through 1997. Cases have been reported from almost every state, although these diseases are most frequently identified in the southeastern, northeastern and upper midwesternm regions of the US. Different tick vectors transmit different species of ehrlichia, reflected by the generally distinct geographic distribution of each type of ehrlichiosis. Age-specific incidences for the ehrlichioses are highest in persons >40 years of age, although severe and occasionally fatal cases have occurred in young adults and children. Laboratory confirmation of RMSF and the ehrlichioses requires serologic, molecular, or culture-based methods. Indirect immunofluorescence assays (IFA) remain the most available and best recognized method of confirming these diseases; however, diagnostic antibody levels typically do not appear until 7-14 days after the onset of illness. In this context, treatment decisions should be based on a presumptive diagnosis developed from epidemiologic and clinical clues and should never be delayed while waiting for laboratory confirmation. Tetracyclines (especially doxycycline) are the antibiotics of choice in treating most adult and pediatric patients with these infections.

Babesiosis

Peter Krause, MD
University of Connecticut School of Medicine

Babesiosis is an emerging malaria-like disease caused by an intraerythrocytic protozoan that is transmitted by the deer tick, the same tick that transmits Lyme disease. The causative protozoan was first described in cattle in 1891 by the Hungarian microbiologist Babes. At least three Babesia species have been found to cause disease in humans, *B. microti* (a rodent species), *B. gibsoni* (WA-1, a dog species), and *B. divergens* (*B. bovis*, a cattle species). Since the first documented case of human babesial infection was reported in 1957, infection by *B. divergens* has been demonstrated in Europe and infection by *B. microti* has been documented increasingly in the northeastern and upper midwestern United States. A parasite very closely related to *B. gibsoni* (WA-1), appears to infect humans along the Pacific coast. The clinical manifestations of babesiosis range from subclinical illness to fulminant disease resulting in death. The case fatality rate has been estimated at 5%. Although infection is common in endemic areas, most is mild or asymptomatic. In clinically apparent cases, symptoms of babesiosis begin after an incubation period of one to nine weeks from the beginning of tick feeding. Typical symptoms include intermittent temperature to as high as 104°F and one or more of the following: chills, sweats, myalgia, arthralgia, nausea, and vomiting. The findings on physical examination generally are minimal, often consisting only of fever. Mild splenomegaly, hepatomegaly, or both are noted occasionally but rash seldom is reported. Abnormal laboratory findings include moderately severe hemolytic anemia, an elevated reticulocyte count, thrombocytopenia, proteinuria, and elevated blood urea nitrogen and creatinine. The symptoms of babesiosis usually last for a few weeks to several months. Patients at risk for severe disease include those who lack a spleen, are infected with *B. divergens*, are over the age of 50 or are coinfected with the agents of HIV or Lyme disease. Moderate to severe babesiosis may occur in children but infection generally is less severe than in adults. Diagnosis of *B. microti* infection is made by microscopic demonstration of the organism using Giemsa-stained thin blood films. In experienced laboratories, the polymerase chain reaction (PCR) can be a sensitive and specific test for detection of *Babesia* DNA but great care must be taken to prevent false positive test results. Serological testing is useful, particularly in diagnosing *B. microti* infection. The indirect immunofluorescence serologic assay (for both IgG and IgM antibody) is sensitive and specific when performed in qualified laboratories. The current therapy of choice for babesiosis consists of the combination of clindamycin and quinine for 7 to 10 days. Adverse reactions are common, however, especially transient hearing impairment and abdominal distress. Exchange blood transfusion should be reserved for patients with severe infections including those with high parasitemia (over 5%) and coma, hypotension, congestive heart failure, pulmonary edema or renal failu*re. In combination with clindamycin and quinine, it is the treatment of choice for all cases of B. divergens* babesiosis.

Prevelance of Infection in Ticks Submitted to the Human Tick Test Kit Program of the U.S. Army Center for Health Promotion and Preventative Medicine, 1999

Ellen Stromdahl, MS
United States Army Center For Health Promotion and Preventative Medicine

Ticks removed from humans are sent by Department of Defense medical personnel/clinics to the Tick-Borne Disease Laboratory of the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) for identification and testing for pathogens by polymerase chain reaction (PCR). Results of tick identification are reported by telephone within 1 day to the tick bite patient's healthcare provider and results of analysis are subsequently reported by telephone within 3-8 working days. *Amblyomma americanum* are tested for *Ehrlichia chaffeensis* and *Borrelia burgdorferi*, *Ixodes scapularis* are tested for *Borrelia burgdorferi* and the agent of human granulocytic ehrlichiosis (HGE), and *Dermacentor variabilis* are tested for *Rickettsia rickettsii*. Since different species of ticks transmit different diseases, and since most tickborne diseases have very similar early symptoms, knowing the species and infection status of the tick enhances the physician's ability to accurately diagnose and treat the patient. In 1999, analysis by PCR found 1/444 (0.2%) *A. americanum* positive for *B. burgdorferi* and 7/444 (2%) positive for *E. chaffeensis*. One hundred twelve of 390 (29%) *I. scapularis* were positive for *B. burgdorferi* and 26 (7%) were positive for the agent of HGE. Ten (3%) *I. scapularis* were coinfected with both organisms. Fourteen of 304 (5%) *D. variabilis* were positive for spotted fever group (SFG) rickettsiae. Restriction fragment length polymorphism (RFLP) analysis identified all as *Rickettsia montana*, a nonpathogenic SFG rickettsia.

– New Ehrlichia agent – deadly if unrecog

Analysis of Southern Borrelia

Angela James, PhD
Centers for Disease Control and Prevention

Lyme disease is the most common tick-borne disease reported in the United States. The majority of the cases occur in northeastern and mid-Atlantic regions. *Borrelia burgdorferi* sensu lato, the etiological agent, is transmitted by *Ixodes* spp. ticks. *Borrelia burgdorferi* has been isolated from several locales throughout the southern United States and these isolates appear to be genetically more diverse than isolates from the northeastern regions. *Borrelia* sp. isolates have been made from mammals, birds, and ticks from Georgia, Florida, North Carolina, South Carolina, Virginia, Missouri, Oklahoma, and Texas. *Borrelia* sp. isolates have been cultured in BSKII from *Ixodes scapularis*, *I. affinis*, *I. dentatus*, and *I. minor*. It appears that *I. affinis*, *I. minor*, and *I. dentatus* maintain enzootic cycles and that *I. scapularis* acts as a bridge vector to humans. A variety of vertebrate species serve as reservoirs of *Borrelia* sp., including the cotton mouse, cotton rat, eastern woodrat, and cottontail rabbit. However, few human cases have been reported from this region. This low number may be a result of ecological factors such as the variety and number of hosts, precipitation, and the one or two year life cycle of the black-legged tick (*I. scapularis*) in this region. Moreover, accurate diagnosis of Lyme disease and tick-associated erythemas in general may be complicated by the possibility of multiple ixodid tick/*Borrelia* sp. transmission cycles in the southern US. For example, *B. lonestari* was recently described; it was detected via PCR in the metastriate tick, *Amblyomma americanum*. The lone star tick (*A. americanum*) is the most prevalent tick species found in the southeastern United States and it frequently bites humans. The prevalence and association of *Borrelia burgdorferi*, *B. lonestari*, and other, possibly new *Borrelia* genospecies to human illness remains unclear in the southern United States. Thus, a reevaluation of Lyme disease in the south is warranted.

B. burgdorferi pathogenic - Yes

Isolates from Fla, Ga, SC.

Human isolate -
Cat flea -
Lone star -

Coinfections

Louis A. Magnarelli, PhD
Connecticut Agricultural Experiment Station

Humans, domesticated animals, and wildlife, such as white-tailed deer and white-footed mice, may be exposed to one or more infectious agents transmitted by *Ixodes scapularis* ticks. Although Lyme borreliosis is more prevalent in Connecticut and in other northeastern and upper midwestern states, monocytic and granulocytic ehrlichiosis, and babesiosis also can occur there.

Serologic analyses were conducted to determine prevalence of infections. Results revealed the presence of antibodies to different pathogens in humans, horses, dogs, white-tailed deer, and white-footed mice in Connecticut.

Coexistence of antibodies to *Borrelia burgdorferi* and the human granulocytic ehrlichiosis agent was most prevalent. In many instances, however, it was unclear if there were simultaneous infections by multiple agents or if hosts were exposed to different disease organisms in separate incidents that occurred over several weeks or months.

If one of these diseases is clinically suspected or diagnosed, laboratory testing should be extended to determine if there are other tickborne infections.

Ehrlichiosis — febrile, influenza-like illness

Fairfield - 7.7%

Middlesex 3%

Windham - 4% -

Babesia - mostly coastal areas

2 or 3 TK borne dz - 26% / 180 pts.

& diagnosed pts. Can be asymptomatic

Preliminary *in vitro* and *in vivo* Findings of Hyperbaric Oxygen Treatment in Experimental *Bb* Infection

Charles Pavia, PhD
NY Medical College School of Medicine
NYCOM Microbiology and Immunodiagnostic Laboratory of NYIT

In these studies, we evaluated repeated HBOT for its ability to kill *Bb* in vitro, and in vivo, in a murine model of Lyme disease. Several North American tick-derived and recently obtained patient isolates were studied separately in our assay systems. To test for in vitro susceptibility, one-half to one million *Bb* were cultured in a small volume (0.1 - 0.2 ml) of BSK media using small snap-cap test tubes. With the caps removed, these cultures were then exposed, for one hour (twice daily for 2 consecutive days), to pure, filtered oxygen pressurized to 2-3 times normal atmospheric conditions. This was achieved using a specially constructed, miniaturized cylindrical chamber (length = 12 inches; diameter = 8 inches), equipped to accept any pressurized gas mixture through its portal opening. After the final HBOT, all cultures received an additional 0.5 ml of BSK media (making the final volume now 0.6 - 0.7 ml), and their caps were snapped shut. Matching control cultures received no HBOT. All cultures were incubated at 33° C for 2-3 days and were examined microscopically for live *Bb*. Our results showed that 14 of 17 strains of *Bb* had their growth inhibited by 33-94%, while there was little or no inhibition of 3 *Bb* strains. For the in vivo studies, separate groups of C3H or CD1 mice were infected intradermally with 100,000 *Bb*. Two to 4 weeks later, one group of infected mice received two, 1.0-1.5 hour HBO exposures, for two consecutive or alternating days. The treated mice were sacrificed one day after the last treatment, and extract cultures of their urinary bladders were prepared in BSK media. It was found that no *Bb* grew out of 80% of these extract cultures, whereas live *Bb* organisms were recoverable from 90% of extract cultures prepared from matched, infected control mice not treated with HBO. These data suggest that HBOT may be considered as a clinically useful form of adjunct therapy in the treatment of Lyme disease.

Immunity against Host-adapted *B. burgdorferi* in the Rabbit

Presenter:
James N. Miller, PhD
Department of Microbiology and Immunology, *UCLA School of Medicine*, Los Angeles, CA

Major Contributors:
Ellen S. Shang, PhD
Department of Microbiology and Immunology, *UCLA School of Medicine*, Los Angeles, CA
David R. Blanco, PhD
Department of Microbiology and Immunology, *UCLA School of Medicine*, Los Angeles, CA
Michael A. Lovett, MD, PhD
Division of Infectious Diseases, Department of Medicine, *UCLA School of Medicine*, Los Angeles, CA

Studies by Barthold and his colleagues have underscored the significance of host-adapted *B. burgdorferi* in the attempt to understand the immunology and pathogenesis of Lyme disease. Utilizing the rabbit model and the *B. burgdorferi* B31 strain, we have demonstrated by skin implantation challenge, a complete infection-derived immunity to large numbers of host-adapted homologous spirochetes (1.38×10^8). Further, we have shown that heterologous challenge with host-adapted organisms from strain 297 results in rapid clearance of skin and disseminated infection. Immunization of rabbits with outer membrane vesicles (OMV) isolated from the B31 strain and its avirulent derivative lacking OspA and DbpA (B313) conferred highly significant protection against challenge with 6×10^4 cultivated B31 spirochetes but not against challenge using host-adapted organisms. Analysis of the antibody responses to OM proteins among infected rabbits immune to skin implant challenge suggests that the remarkable protection induced by infection is due to antibodies directed against antigens unique to or markedly up-regulated in host-adapted *B. burgdorferi.* The rabbit model thus provides the opportunity to identify *B. burgdorferi* surface molecules up-regulated during rabbit infection and ultimately to determine their relationship to the basis of infection-derived immunity.

worked w/ Norris, Nowakowski, Steere

Immunologic Aspects of VlsE, a *B. burgdorferi* Antigenic Variation Protein

Steve J. Norris, PhD
University of Texas - Houston Medical School

Lyme disease borrelia can persistently infect Lyme disease patients and infected animals for months to years despite a vigorous host immune response. Our laboratory recently discovered the vls (VMP-like sequence) antigenic variation system in *B. burgdorferi* B31 that may in part explain the ability of this organism to evade an active immune response. The vls locus consists of the expression site for a surface lipoprotein, VlsE, and 15 'silent' cassettes that represent variations of the central cassette region of the vlsE gene. Segments of the silent cassettes begin to recombine into the vlsE gene within 4 days following infection, and this ongoing process could produce as many as 1030 different sequence variations. Lyme disease patients consistently produce a strong antibody response against VlsE, which may actually be useful in the diagnosis of Lyme disease. Although immunization with a single form of VlsE fully protects mice against infection with *B. burgdorferi* expressing the identical protein, it is only partially protective against variants expressing slightly different versions of VlsE; this finding demonstrates the role of VlsE sequence variation in immune evasion. DNA sequences homologous to vlsE have been identified in every infectious Lyme disease isolate examined to date, and may represent a common immune evasion mechanism in Lyme disease borrelia. Further characterization of the vls system may lead to improved immunodiagnosis and additional immunoprotective vaccines against Lyme disease.

Properties of Bb Pathogens –
- Local, disseminated & late
- 1° invasive w/ little or NO toxigenesis
- Persist infect despite active immune resp.
- Loss of plasmids during in vitro culture correlate w/ decr. infectivity
- Invasiveness vs. Toxigenesis.

Mechanisms of Persistence
- Protective niches
- Hidden antigen (masking)
- Inhibition of immune respons
- one more

vls silent cassettes only occurs in infection,

Invariable Regions of VlsE, the Variable Surface Antigen of *Borrelia burgdorferi*: Their Application in Diagnosis and Immunoprophylaxis of Lyme Disease

Mario Philipp, PhD
Tulane University Medical Center
Tulane Regional Primate Research Center, Department of Parasitology

Antigenic variation is an effective strategy evolved by pathogenic microbes to avoid immune destruction. Variable antigens such as the variable major protein (Vmp) of *Borrelia hermsii* and the variant surface glycoprotein (VSG) of African trypanosomes include an immunodominant variable domain and one or more invariable domains which are not antigenic. Short, nonantigenic, invariable regions also may be present within the variable domain.

VlsE (variable major protein-like sequence), the variable surface antigen of *Borrelia burgdorferi*, the Lyme disease spirochete, also contains both variable and invariable domains. In addition, interspersed within the VlsE variable domain there are six invariable regions ($IR1_{1-6}$) which together amount to one half of this portion's primary structure. We show here that these IRs are conserved among strains and genospecies of the *B. burgdorferi* sensu lato complex. Unlike the invariable regions of Vmp and VSG, which are not antigenic in natural infections, the most conserved of the IRs, IR_6, is immunodominant in Lyme disease patients and in monkeys infected with *B. burgdorferi*. The utilization of IR_6 as a universal diagnostic reagent for Lyme disease and the possible application of other IRs as vaccines will be discussed.

Serodiagnosis –
C6 Elisa simple better than Std Elisa
– specific Gold
All genospecies detectable in acute & chronic infect
– Inexpensive to manufacture
–

Antibiotic Treatment of Lyme Borreliosis: A Review of Studies with Dogs

R. K. Straubinger [1]*, A. F. Straubinger[1], B. A. Summers[2], R. H. Jacobson[3]
James A. Baker Institute for Animal Health[1], Department of Pathology[2], and Department of Population Medicine and Diagnostic Science[3],
NYS College of Veterinary Medicine Cornell University, Ithaca, New York 14853

takes 25 samples per dog.

Objective: Determine whether therapy with the antibiotics amoxicillin, ceftriaxone, doxycycline and azithromycin, commonly used in Lyme disease patients alleviate clinical signs of canine Lyme borreliosis, and whether the infectious agent, *Borrelia burgdorferi*, can be eliminated from the host.

Design: In three separate experiments over a four-year period, 24 beagle dogs infected with *B.burgdorferi* by tick exposure were treated with antibiotics for 30 consecutive days. Ten infected dogs were not treated and served as positive controls. After treatment, dogs were housed in P-2 units for an additional 70 to 350 days. Serum samples were collected in 2-week, and skin biopsy samples in monthly intervals. Detection of *B.burgdorferi* was attempted by culture and PCR in tissue samples.

Results: After tick exposure, 33 of 34 dogs became infected as shown by serology, culture, and PCR. Clinical signs of Lyme borreliosis included elevated temperature for one to two days and lameness, caused by acute mono- or oligoarthritis of large joints such as the shoulder, elbow, carpal, and knee joints. After antibiotic therapy, dogs improved clinically, and only once was posttreatment lameness observed in a dog. At the end of the experiments, 25 tissue samples of each dog were cultured. Live spirochetes were recovered from three single tissues samples from three treated dogs, while multiple tissues samples were positive in all non-treated dogs. By PCR 20/24 treated and 10/10 non-treated dogs were positive for *B.burgdorferi* DNA. Serologically, the level of peak antibody titers was dependent on the time when therapy was initiated. Short time intervals between infection and treatment (50 days) resulted in moderate peak antibody titers, and during the post-therapeutic observation period antibodies against *B.burgdorferi* disappeared almost completely. However, treatment initiated 120 days post infection allowed antibody levels to develop to maximum titers and beyond 300 days following therapy dogs still had moderate antibody titers. Histologically, 3/24 treated dogs showed mild monarthritis or meningitis in just single tissue samples, while 8/10 non-treated dogs had mild non-suppurative polyarthritis, sometimes combined with periarteritis.

Conclusions: Treatment with high doses with commonly used antibiotics for a 30 day period resulted in clinical improvement and arthritis was prevented or cured. However treatment was not sufficient to eliminate the persistent infection in dogs and impeded the development of maximum antibody titers.

Tetra – Bacteriostatic - lets immune system
= extracellular & intracellular
- can interfere w/ metabolism

Macrolides – Azith – Bacteriostatic – affect placenta
- intracell & extracell.

= Doxy – 2 of 10 pos – PCR pos despite antibody levels low
Azith – PCR pos

A *Borrellia burgdorferi* Repetitive Antigen that Confers Protection against Experimental Lyme Disease

Jon T. Skare, PhD
Texas A & M University System Health Science Center

We have previously described the expression cloning of nine *Borrelia burgdorferi* antigens, using rabbit serum enriched for antibodies specific for infection-associated antigens, and determined that seven of these antigens were associated with infectious *B. burgdorferi* strain B31. One of these infection-associated antigens encoded a 451 amino acid lipoprotein containing 21 consecutive and invariant 9 amino acid repeat sequences near the amino terminus that we have designated VraA for virulent strain associated repetitive antigen A. The *vraA* locus (designated BBI16 by The Institute for Genomic Research [TIGR]) maps to one of the 28 kilobase linear plasmids (designated lp28-4) that is not present in noninfectious isolates. The absence of lp28-4 alone from *B. burgdorferi* strain B31 results in a 15-fold decrease in the infectious dose (ID_{50}) relative to wild-type *B. burgdorferi*, suggesting that genetic loci found on lp28-4 are involved in the pathogenesis of *B. burgdorferi* infection. We have also determined that VraA is a surface exposed lipoprotein based on protease accessibility assays of intact whole cells. Furthermore, VraA is synthesized greater when cells are grown at 37° C relative to cells grown at either 32°C or 23°C, suggesting that VraA is a host inducible antigen. Homologues cross-reactive to *B. burgdorferi* strain B31 VraA with distinct molecular masses were identified in several *B. burgdorferi* sensu lato isolates as well as other *Borrelia* spp. including *B. andersonii*, suggesting that the immunogenic epitope(s) present in VraA are conserved between *Borrelia* spp. In protection studies, five of six mice immunized with recombinant glutathione-S-transferase (GST)-VraA were protected from infectious challenge with 10^2 infectious *B. burgdorferi* strain B31 whereas naïve mice or mice immunized with GST alone were completely susceptible. Furthermore, the protection elicited by VraA immunization provides an additional testable vaccine candidate to protect against Lyme disease and perhaps other *Borrelia* spp related diseases.

DONE!!!!!!!!! signed off

Use of the Borreliacidal Assay in the Serodiagnosis of Lyme Disease

Ronald Schell, PhD
University of Wisconsin School of Medicine

Lyme Neuroborreliosis - The Role of PCR and Culture in the Diagnosis and in the Confirmation of Relapse after Antibiotic Treatment

Jarmo Oksi, MD, PhD
Turku University Central Hospital, Department of Medicine, Finland

The pathogenetic mechanisms of Lyme neuroborreliosis (LNB) are poorly understood. They may be caused either by direct action of *Borrelia burgdorferi* (*Bb*) or by indirect immunologic reactions of the host. The diagnosis of LNB has usually been based on nonspecific findings, serologic testing and other indirect methods. However, evidence for the presence of *Bb* can also be obtained by culture or polymerase chain reaction (PCR) of cerebrospinal fluid, plasma, or brain tissue specimens. Furthermore, the direct demonstration of *Bb* in the brain lesions indicates that direct invasion of the spirochetes has obviously been the pathogenetic mechanism in these cases. The low number of spirochetes in tissue samples and body fluids is one of the reasons for the difficulties to demonstrate *Bb* by culture or PCR. Therefore, a negative result obtained by these methods can never exclude LNB. However, a positive result can confirm the diagnosis or treatment failure independently of results of antibody tests. PCR offers a practical means to differentiate patients with "Post-Lyme syndrome" or with "serological scars" from those who definitely need retreatment.

We report on 13 patients with clinical relapse of disseminated LB after antibiotic treatment and culture or PCR positivity. These patients were a subgroup of a total of 165 patients with disseminated infection with *Bb*, which were followed after antibiotic treatment. Before the initial antibiotic treatment 11 of the 13 patients had neurologic symptoms or findings including meningitis in 3, encephalitis in 3, radiculitis or neuritis in 2, and other neurologic symptoms in 5 of the patients. Brain MRI was abnormal in 5 of 8 studied. 8 of the patients had culture or PCR-proven initial diagnosis; and the diagnosis of the remaining 5 patients was based on positive serology only. The microbiologic confirmation of relapse was based on blood culture in 3 patients, on brain biopsy PCR in 1 patient, and on plasma PCR in 9 patients. All 13 patients were initially treated for more than 3 months with iv and/or oral antibiotics. Antibody levels decreased or changed to seronegative in 6 of the 13 patients after the first treatment. However, this did not guarantee a succesful eradication of the spirochete as shown by PCR and culture. None of the patients was PCR or culture positive after the retreatment.

PRESENTATIONS MARCH 25, 2000 SATURDAY

Laboratory Testing Panel

Mark Golightly, MD *SUNY at Stony Brook School of Medicine*
Eli Mordechai, PhD *Medical Diagnostic Laboratories*
Ronald Schell, PhD *University of Wisconsin School of Medicine*
Jyotsna Shah, PhD *Igenex Laboratories*
Richard Tilton, PhD *BBI Clinicial Laboratories*
Steven Schutzer, MD *University of Medicine and Dentistry of New Jersey*

1. What tests do you recommend be done? Why?
2. Do you see any correlations between EIAs and Western blot?
3. Regarding Western blots: What criteria do you use for a positive? Can a single specific reaction be compatible with Lyme disease?
4. Of what value is PCR-DNA testing for Lyme disease?
5. Do you do urine antigen testing? Why/why not?
6. What is accuracy of testing for babesiosis? Blood smears? Antibodies? Western blot? PCR?
7. What is accuracy of testing for ehrlichiosis? Blood smears? Antibodies? Western blot? PCR?

Looking for antigen Unique to Bb.-

Tilton - Clear DNA when dead in 2-3 wks

Igenex - Whole blood better than serum
- ✓ in urine
- Prefer panel approach -