**Keynote Speaker Saturday April 25, 1998**

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## Increase Evidence Of Mosquito/Spirochete Associations

Willy Burgdorfer, Ph.D.

The recent demonstration and isolation of spirochetes (including the Lyme disease agent, *Borrelia afzelii)* in and from *Aedes* and *Culex* mosquitoes in southern Moravia (Czech Republic) promoted this short review of literature pertaining to the association of spirochetes with mosquitoes.

Reports as early as 1904 describe the presence of spirochetes in intestinal tracts of larval mosquitoes as well as in malpighian tubules and salivary glands of adult *Culex*, *Anopheles* and *Aedes* mosquitoes. Yet, there is no information as to the ability of these insects to transmit spirochetes by bite. An exception is a report on the successful transmission of *Borrelia anserina* by experimentally infected *Aedes aegypti*. Similar experimental infection and transmission studies of the Lyme disease agent *Borrelia burgdorferi sensu stricto* in three species of mosquitoes (*Ae. aegypti, Ae. atropalpus*, *Ae. triseriatus*) showed the duration of spirochetal infections in the intestinal tracts of these insects to be ephemeral and not involving salivary gland tissues. In contrast are the recent reports from southern Moravia where over-wintering *Aedes* and *Culex* have been found naturally infected with spirochetes. One strain isolated from *Ae. vexans* was identified as *B. afzelii,* two other strains from *C. pipiens molestus* appeared to be new hitherto undescribed spirochetes. Studies are in progress to determine whether these spirochetes produce in their mosquito vectors systemic infections including the tissues of salivary glands from where they could be transmitted via saliva during feeding on animal hosts and possibly humans.

**Notes:**

**Presentation Sunday April 26, 1998**

**Paul Duray, M.D.**
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## Host And Mammalian Histopathology In Borreliosis

Paul H. Duray, M.D.

Basic histopathologic alterations in human target tissues in LD involve, with some geographic differences, the presence of T lymphocytes with subsets, abundant plasma cells and precursors, macrophages, dendritic antigen-presenting cells, variable mast cells, synovia, chronic skin and soft tissue sites, particularly striated muscle, and infrequently, peripheral and in the acute stages, some neutrophiles. Over time, the immunopathologic infiltrates aggregate in joint nerves and interstitial lymphohistiocytic inflammation. Myocarditis is thought to be transient, but when present is comprised of transmural interstitial lymphohistiocytic inflammation. There are some geographic differences: lymphocytic nodular aggregates of skin and muscle are found in European cases, with proliferative synovitis resembling rheumatoid arthritis more common to U.S.

Acrodermatitis chronica atrophicans with a wide spectrum of histopathologic changes is nearly exclusive of U.S. cases, more commonly seen in Europe. Unusual cases of CNS involvement, splenitis, progressive uveitis, eosinophilic fasciitis, tenosynovitis, and morpheaform skin lesions have been associated with infection, but too infrequent to be considered components of human borreliosis at this stage of current knowledge.

Domestic pet infections mimic human lesions regards autonomic gangliitis, myocarditis, and synovitis. Rodents and lagomorphs in boreal sites have infrequent inflammatory foci in livers, but no synovial, CNS, or soft tissue alterations that are consistently seen. White tailed deer examined from Westchester County, NY, had lymphoid hyperplasia of spleen with stainable spirochetes, and hepatic nodular lymphocytic infiltrates. These deer harbor stainable spirochetes in the ocular vitreous. Southern Wisconsin deer have similar findings. Regards induced infection in laboratory animals, Lewis rats incur skin and joint inflammation, myocarditis and soft tissue inflammation in NIH-3 mice, and erythema migrans in lagomorphs.

This experience has been accumulated from human samples where there had not been prior antimicrobial treatment before tissue sampling. In general, spirochete searches microscopically, are unsuccessful in patients having had prior antibiotic therapy.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Complete Genome Sequence of *Borrelia burgdorferi*

Claire M. Fraser, Ph.D.

Spirochetes from the genus *Borrelia* were found to be the etiologic agent of Lyme disease in the United States in 1982. Lyme disease is a multi-systemic disease, which can become chronic if left untreated, and its causative agent, *B. burgdorferi*, is carried by ticks, mostly in the genus *Ixodes*. Lyme disease is currently the most prevalent tick-borne disease in the United States. The type strain, B31 was isolated from an *Ixodes scapularis* tick on Shelter Island, New York. We report here the complete genome sequencing of *B. burgdorferi* isolate B31. The genome contains a linear chromosome of 910,725 bp and 21 linear and circular plasmids with a combined size of more than 600,000 bp. The chromosome contains 853 genes encoding a basic set of proteins for DNA replication, transcription, translation, solute transport, and energy metabolism, but no genes for cellular biosynthetic reactions, similar to *Mycoplasma genitalium*. Since *B. burgdorferi* and *M. genitalium* are distantly related eubacteria, we suggest that their limited metabolic capacities reflect convergent evolution by gene loss from a more metabolically competent progenitor. Eight hundred thirty eight genes were identified on 21 plasmids, the majority of which have no known biological function. Seventy percent of plasmid genes are paralogs that comprise 47 gene families; a large number of these genes encode putative lipoproteins. The biological significance of the multiple plasmid-encoded genes is not clear, although they may play a role in antigenic variation or immune evasion.

**Notes:**

**Presentation Saturday April 25, 1998**

**Jon T. Skare, Ph.D.**
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## Identification and Characterization of Virulent Strain Associated Outer Membrane Proteins of *Borrelia burgdorferi*

Jon T. Skare, Ph.D.

Virulent strain associated (VSA) outer membrane proteins of *Borrelia burgdorferi* mediate the initial borrelial-host interaction and, as such, are candidate virulence determinants. To identify such proteins, we used serum from rabbits immune to re-infection with virulent *B. burgdorferi sensu stricto* strain B31 adsorbed against avirulent *B. burgdorferi* to obtain a reagent consisting of antibodies specific for VSA antigens. This VSA-specific serum was then used to screen immunoreactive plaques from a *B. burgdorferi* expression library. Sequence analysis indicated that the 16 immunoreactive *B. burgdorferi* antigens were encoded by 9 genes, including the locus required for decorin binding (*dbpAB*) and an additional lipoprotein antigen containing 22 consecutive 9 amino acid repeats that we have designated *vraA* for VSA repetitive antigen A. Of the 9 genes identified in this screen, 8 were encoded on plasmids known to be lost during *in vitro* cultivation with a concomitant loss of infectivity in animal models of Lyme borreliosis. We have subsequently determined that DbpA, DbpB and VraA are derepressed at 37°C relative to cells grown at either 23°C or 32°C, suggesting that they are expressed at high levels within mammalian hosts.

These results, along with other previous observations, indicate that *B. burgdorferi* alters its protein and antigenic profile within the mammalian host(s) relative to cells cultivated *in vitro*. As such, the identification of antigens expressed at higher levels within infected mammals, such as DbpA, DbpB, and VraA, provide additional logical vaccine candidates to complement the current OspA vaccine regimen. Finally, with the advent of the *B. burgdorferi* genome project, it should be possible to assess the global regulation of genes encoding VSA antigens to determine the environmental cues that regulate *B. burgdorferi* VSA gene expression.

**Notes:**

**Presentation Saturday April 25, 1998**

**Maria M. Picken, M.D., Ph.D.**
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## Survey for *Borrelia* Species Among Reservoir Animals Captured in Forested Areas of Greater Metropolitan Chicago.

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**Objectives:** To survey the small animal reservoir of forested areas in northwestern and north suburban Chicago for the presence of *B. burgdorferi*, to isolate spirochetes in culture, and to characterize the resulting isolates by molecular genotypic analysis.

**Methods:** During summer 1996, 126 small animals were trapped in northwestern Cook Co. and cultured for *B. burgdorferi*. These comprised 106 *Peromyscus leucopus,* 8 *Tamias striatus,* 5 *Microtus pennsylvanicus,* 3 *Spermophilus tridecemlineatus,* 1 *Marmota monax,* 1 *Peromyscus maniculatus*, 1 *Sorex cinereus,* and 1 *Zapus hudsonius.* During summer 1997, 56 animals were trapped in Lake County, north of Chicago, and cultured. These comprised 16 *P. leucopus*, 34 *M. pennsylvanicus*, 3 *P. maniculatus* , and 3 *Z. hudsonius* . *Borrelia* isolates were characterized by pulsed-field gel electrophoretic (PFGE) analysis of their plasmid content, and chromosomal macrorestriction patterns as well as nucleotide sequence determination of the *rrf* (5S)-*rrl* (23S) intergenic spacer region.

**Results:** Two isolates were obtained from Cook Co. in 1996, from *P. leucopus* and *M. pennsylvanicus.* In 1997, 8 isolates were obtained form 5 animals captured in Lake Co., comprising 4 *M. pennsylvanicus* and 1 *Z. hudsonius.* In the case of 3 *M. pennsylvanicus*, isolates were obtained from both urinary bladder and ear snips. PFGE analysis of *Mlu*I-digested genomic DNA from the isolates demonstrated two patterns of high molecular mass fragments identical to those of *B. burgdorferi* strains DN127 (2 Cook Co. isolates) and 25015 (8 Lake Co. isolates). Plasmid patterns and low molecular mass fragments showed differences between the 10 strains. Sequence analysis of the *rrf-rrl* intergenic spacer region demonstrated closely similar sequences to those reported for DN127 and 25015, but also showed individual nucleotide differences between strains.

**Conclusions:** These studies demonstrate the presence of DN127-group *B. burgdorferi* infecting the rodent population in northwestern and north suburban forest areas of Chicago. The prevalence of these organisms is clearly higher than was previously supposed. Whether DN127-group borrelia represent a human Lyme disease risk has yet to be determined.

**Notes:**

**Presentation Saturday April 25, 1998**

**Mark J. Cartwright**
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## Toxins of *Borrelia burgdorferi*

Mark J. Cartwright*, Suzanne E. Martin, and Sam T. Donta, Boston University Medical Center.

The mechanisms responsible for many of the symptoms in patients with Lyme disease remain to be delineated. The organism can only rarely be found following the initial infection and dissemination, suggesting that for persistent infection the reservoir is intracellular. Because so many of the symptoms appear to be related to the nervous system, it is postulated that the Lyme spirochetes reside in neurons themselves, glial cells, or endothelial cells that provide the nervous system with its blood supply. It is further postulated that toxins are released by the borrelial organisms that then interfere with normal neurochemical function.

The purpose of this research program is to identify toxins of *B. burgdorferi* that may directly or indirectly impact on normal neurophysiology. Towards that goal, we designed "degenerate" primers to highly conserved regions within toxin sequences. We used these primers for PCR to identify genes that express proteins analogous to existing toxins. To date, we have identified and cloned *B. burgdorferi* genes derived from cholera, diphtheria, and pertussis toxins. The putative toxin genes are identical to several unidentified genes contained in the recently published complete DNA sequence of *B. burgdorferi*. In addition, one of the putative toxin genes is conserved to a *Treponema pallidum* gene of undefined function. As a second approach to identify possible toxins, assays (e.g. ribosylation) were performed on both the *B. burgdorderi* bacteria and its conditioned media, in which enzymatic activity was detected.

**Notes:**

**Presentation Saturday April 25, 1998**

**David W. Dorward**
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## *In Vitro* Evidence for Lymphocytic Membrane Cloaking By *Borrelia burgdorferi*

David W. Dorward* and Elizabeth R. Fischer. NIH/Rocky Mountain Labs, Hamilton, MT

*In vitro* studies have demonstrated that Lyme disease spirochetes including *Borrelia burgdorferi* and *B. garinii* attach to, invade, and kill subsets of human B and T lymphocytes. To further understand such interactions, temperature-regulated co-incubation mixtures were examined by immunofluorescence and electron microscopy. Low passage (<8) *B. burgdoferi* Sh-2-82 and SKW 6.4 B cells were mixed at 10:1 ratios at 4 ° C for 1 hr, then warmed to 37°C and followed. Spirochetes attached to nearly 50% of B cells in all mixtures. Whereas, attachment peaked after 1 hr at 37°, invasion appeared to peak at 4 hrs. Addition of 0.1-1% carboxymethycellulose dramatically enhanced the rate of spirochetal motility, but did not increase cell invasion. No evidence for degradation of nor damage to intracellular spirochetes was detected or observed. Emergence form B cells was either lytic or non-lytic. Emergent spirochetes frequently retained lymphocytic membrane envelopes. Video microscopy revealed that enveloped spirochetes had normal motility. After 24 hrs one third of co-incubated spirochetes labeled with antibodies to human B cell antigens. Relatively few spirochetes remained enveloped after 48hrs. Immune electron microscopy showed that although such enveloped spirochetes exhibited surface-exposed B cell antigens, anti-OspA antibodies failed to bind. Furthermore, spirochetes incubated with B cells acquired a time-dependent resistance to classic complement-mediated killing. Such results suggest that *in vitro* interactions with cultured human B cells result in *B. burgdorferi* retaining one or more layers of lymphocytic membrane that mask spirochetal antigens, and possible interfere with antibody-mediated recognition and neutralization. If such interactions occur *in vivo*, such a process could represent a previously unrecognized bacterial virulence strategy.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Tick Vectors of *Borrelia burgdorferi*

John Anderson, Ph.D.

There are about 839 different species of ticks in the world, of which almost 80 percent are hard bodied ticks. A few of these, namely *Ixodes scapularis* and *Ixodes pacificus* of North America, and *Ixodes ricinus* and *Ixodes persulcatus,* and possibly *Ixodes ovatus* in the Old World, are the principal vectors of spirochetes that cause Lyme disease. These species have similar natural histories, feed on a wide variety of animals including mammals, birds and lizards, and all readily feed on humans. Reservoir hosts of borreliae are primarily rodents. Birds also harbor borreliae and can transport these pathogens and tick vectors relatively long distances during migration. *Ixodes scapularis* remains abundant in eastern and mid-western United States because of the ever increasing number of white tail deer. Personal protection measures are important to reducing exposure to borreliae.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Co-infection of Mammals and Ticks with Emerging Tick-borne Pathogens

Edward Bosler, Ph.D.

Over the past several years emerging and re-emerging tick-borne infections are being diagnosed in humans with greater frequency and may represent a serious public health problem. In the Northeast and Midwest the agents of Babesiosis (*Babesia microti*) and human granulocytic ehrlichiosis (*Ehrlichia phagocytophila*) are transmitted by the same vector tick, *Ixodes scapularis*, as the Lyme disease bacterium. Evidence from human patients suggests that co-infection with these causal organisms occurs frequently and empirically that infection with one or more organisms may exacerbate or alter the symptoms of another.

During 1997 we assessed the presence of co-infection with these organisms in potential rodent reservoir hosts, ticks feeding on these hosts and in free ranging ticks collected from selected areas of Long Island. Free ranging adult ticks were also examined from areas of Connecticut and from along the eastern seaboard from Massachusetts to South Carolina. Pathogens were directly detected by *in vitro* cultivation and indirectly by PCR. PCR proved more practical for analyzing large numbers of field derived samples. The rates of co-infection in both hosts and ticks indicate widespread distribution of the pathogens and that the zoonotic cycles of each are established on Long Island. These data also indicate a strong need to establish laboratory "zoonotic" models for co-infection.

**Notes:**

**Presentation** **Saturday April 25, 1998**

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## Clinical and Laboratory Characterization of the Canine Monocytic Ehrlichiosis Syndrome

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When first described six decades ago in Africa, canine ehrlichiosis was a mild disease of dogs associated with tick bites. The diagnosis was clinical and parasitological and the condition was relatively self-limiting. Subsequently, the classical disease evolved into a fulminating infection typified by fever, pancytopenia (with a predominance of thrombocytopenia), a hemorragic crisis which may include epistaxis, naso-ocular discharge, polyarthritis and a host of multisystemic metabolic and pathologic disturbances. The latter may include cardiovascular and CNS complications, thus making CME, an *imitator,* next only to Lyme disease. A breed predisposition has been postulated. The distribution of cases co-incides with the presence of tick vectors although mechanical transmission cannot be precluded. These findings prompted specialists in human infectious diseases to search for a similar human syndrome.

With improved technology *Ehrlichia canis*, the prototype etiologic agent was cultured and isolated and better diagnostic techniques (e.g. The Indirect Immunofluorescence, Polymerase Chain Reaction) have been devised. In addition, clinicians and the lay public have become more cognizant of potential cases of the disease and seek to differentiate it from syndromes such as RMSF, Lyme Disease, Babesiosis and a wide array of autoimmune diseases. The diagnosis has been complicated by emergence of atypical ehrlichiosis putatively attributed to different strains of *E. canis and* /or *E. risticii.* Definitive diagnosis is based on a combination of laboratory findings and a mosaic of clinical observations consistent with ehrlichiosis. The treatment of choice is tetracycline and the ideal method of control is elimination of ticks and other biting vectors. Laboratory studies have been constrained by reliance on canine models in the absence of a readily available good murine model.

Profiles of the syndrome derived from extensive laboratory experimentation and anecdotal clinical observations highlight the rapidly evolving nature of this re-emerging syndrome.

**Key-words:** Canine ehrlichiosis, etiopathology, diagnosis, epidemiology prevention, control.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Lyme Disease in Dairy Cattle

S.L. Bushmich, M.S., D.V.M.

In this talk we will summarize findings on bovine Lyme borreliosis gleaned from several studies, some of which are in progress. Lyme disease has been reported in dairy cattle (Post et al. 1986, Wells et. al. 1993, Burgess et al. 1986, Bushmich 1992). The most prevalent clinical sign is lameness; erythematous skin rash has also been described. Serologic diagnosis is hampered by cross reactivity with other flagellated flora, as well as a high level of subclinical infection. Our laboratory has conducted several studies to help define this disease in cattle. Our initial study involved experimental infection of neonatal calves with *Borrelia burgdorferi (Bb)* culture. Infected calves developed a positive serological response to *Bb* erythematous skin rash at the injection site from which *Bb* were cultured, and shed live *Bb* in the urine. Aside from the skin rash, they were clinically normal. *Bb* were detected (by culture and/or PCR) in urine of all 4 infected calves, as well as synovial fluid from one calf and blood from another. Necropsy cultures from infected calves were positive for *Bb* in spleen and synovial tissue of one calf, and kidney and bladder of another. Control calves were negative serologically and by culture/PCR. A later detailed case study involves a mature Holstein cow with initial clinical sign of severe lameness. Western blot demonstrated *Bb* specific antibodies, and skin biopsy was *Bb* culture and PCR positive. Physical examination revealed no other cause of lameness. The cow responded well to a short course of oxytetracycline treatment, then became lame again. This cow was then moved to a research facility and treated with alternating penicillin and oxytetracycline for over 50 days. Although she improved clinically and returned to the herd, she became severely lame again 2 months later and was euthanised. *Bb* was found in synovial tissue, lymph node, bladder and uterus at necropsy. Studies of natural *Bb* infection in bred Holstein heifers are presented as a separate poster presentation. Preliminary results of experimental infection of bred dairy heifers with *Bb* infected and non-infected control *Ixodes scapularis* ticks will also be presented.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Oral Corticosteroid Treatment of Dogs Infected with *Borrelia burgdorferi*

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Corticosteroids are powerful drugs often used to abrogate clinical signs of inflammation. Therefore, administration of these drugs during Lyme disease might be beneficial. However, they could be detrimental as well, since corticosteroids impair the immune system. To investigate the effects of corticosteroids on subclinical persistent *B. burgdorferi* infection we treated persistently infected dogs with oral prednisolone.

Four specific-pathogen-free beagles were infected by tick challenge. Dogs were maintained for 505 to 581 days after infection. During this time, all dogs were monitored daily for clinical signs and body temperature changes. Serum antibody titers against *B. burgdorferi* were measured every two weeks, and skin punch biopsy samples were obtained every month for culture. At day 413 after infection, two dogs (2 and 3) received oral treatment with Prednisolone (2 mg/kg body weight) twice a day for 14 consecutive days. The same prednisolone treatment regimen was administered starting on day 566 to dogs 3 and 4 which was 15 days before tissues were cultured for the presence of *B. burgdorferi.*

One month after tick exposure, all four dogs showed infection as documented by seroconversion and by spirochete-positive skin biopsy cultures. Clinical signs of acute lameness developed between 50 and 169 days after infection. Dogs 1-3 showed two, three, and one episode of lameness, respectively, while dog 4 did not become lame. Onset of lameness was sudden and clinical signs resolved usually after five days. Within 90 days after infection, serum antibody titers reached maximal levels and remained unchanged throughout the experiment. Besides weight gain, no adverse effects were observed during the course of the corticosteroid treatment. However, 5 and 8 days after the treatment had stopped, dog 2 and 3 developed severe polyarthritis, which resolved without treatment after an additional seven days. At the end of the experiment, 25 tissues of each dog were cultured in BSK II medium. Dog 1, which received no corticosteroids, had 14 positive tissues, while dog 2 which had received prednisolone three months earlier had only one positive tissue (fascia of the right hind leg). Dog 3 and 4, which received corticosteroid treatment shortly before testing, had 10 and 19 tissues positive, respectively.

In summary, dogs persistently infected with *B. burgdorferi* show no adverse affects during oral corticosteroid treatment. However, once the treatment was terminated, severe polyarthritis occurred, probably due to reactivated persistent infection triggering local joint inflammation and an enhanced immune response against *B. burgdorferi.*

**Notes:**

**Presentation Saturday April 25, 1998**

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## Overview of Testing in Lyme Disease

No abstract submitted.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Immunodetection of *Borrelia burgdorferi in vivo* Expressed Antigens

S.E. Schutzer, M.D.

Precise immunodiagnosis of infectious diseases is based upon identification of an immune response to unique antigens of the suspected agent. Temporal issues such as recent or past infection require understanding and application of the immune response. Rapidly multiplying bacterial infections may provoke an early IgM response which may suggest acute infection. Early expression of antigens unique to the organism may also provoke an antibody response which may suggest acute infection. *Borrelia burgdorferi (Bb)* infection which causes Lyme disease has a behavior different than a fast growing bacterial disease. The immune response is often delayed by weeks, well beyond the optimal clinical utility of current immunologic tests. However the spirochete produces several antigens that are unique to *Bb*, permitting selective diagnosis of this organism. In addition to the Osp proteins, more recently discovered ones such as 22 kd, 35, and 37, are produced only in *vivo* infections as opposed to the *in vitro* cultured organisms. The detection of antibody bound to unique antigens in *Bb* specific immune complexes is a marker for active disease as opposed to past infection. This has important diagnostic and therapeutic uses.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Antigen Detection of *Borrelia burgdorferi* in Urine.

Nick S. Harris, Ph.D.

This is a review of the relationship of the bladder and the urine as a unique area for the detection of *B. burgdorferi.* Since 1986, there have been reports on the presence of *B. burgdorferi* spirochetes or antigen in urine, or cultured from the bladder, of mice, rabbits and dogs in experimentally and naturally induced Lyme infection. A variety of detection techniques have been used: 1) biopsy; 2) culture; 3) PCR; and 4) antigen-capture assays. In human systems, both PCR and antigen-capture with monoclonal or polyclonal antibodies have been used.

While the PCR technique seems to be more useful prior to antibiotics or after antibiotics have stopped, in those patients with persistent disease, the detection of antigen in urine by Antigen-Capture may actually be enhanced during antibiotic treatment. There have been studies, using nested PCR, showing the consistency of detecting DNA antigen in urine, early in disease, during the time of the EM. In another study, multiple primers sets have been used to detect *B. burgdorferi* DNA in patients with chronic (persistent/recurrent) Lyme disease. The LUAT (**L**yme **U**rine **A**ntigen **T**est), developed in 1990, utilizing a unique polyclonal antibody and a variant of the fluorescent ELISA has detected antigen in patients with early disease as well as those with persistent/recurrent disease.

Data from the PCR and Antigen-Capture studies will be used to illustrate the fact that the urine is an important tissue for the clinical detection of laboratory markers for Lyme disease.

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## New System for *Borrelia in vitro* Cultivation

Paul H. Duray, M.D., Steven Hatfill, M.D., Ph.D., Adriana Marques, M.D.,
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Most *in vitro* cultivation systems are unicell type cultures involving one cell lineage type or another. The current technology is limited concerning the feasibility of growing whole tissue fragments with integral mesenchymal stroma, extracellular matrix, blood vessels, and epithelial structures, although some investigators have maintained thin tissue fragments for a limited time in matrigels. NASA engineers at the Johnson Manned Spaceflight Center designed and developed a fluid filled Bioreactor called a Rotating Wall Vessel Bioreactor (RWV), a double-walled instrument which operates under microgravity conditions. The instrument was originally developed to conduct experiments in space on living cells and tissue samples. Rotation and microgravity conditions afford the efficient transport of cell nutrients and the withdrawal of toxic metabolites, enabling the growth of integral stroma and parenchymal tissues for extended periods of time. Basic construction is a cylindrical growth chamber containing an inner co-rotating cylinder with a gas exchange membrane. Viscous coupling causes the fluid medium in the inner vessel to accelerate until the entire fluid mass is rotating at an angular rate equal to that of the outer wall, resulting in beads, cells or tissue chunks to remain suspended in the fluid at a given rotational speed. We and others have learned that mammalian and human tissue samples are maintained viable for extended periods of time, permitting the feasibility of conducting infectious disease and carcinogenic experiments without resorting to laboratory animal experimentation. Examples of human tissue that can be maintained viable in the RWV include spleen, lymph node, tonsil, salivary, skeletal muscle, synovial, lung, skin, and prostate tissue. For reasons unknown, we have been unsuccessful with CNS and renal tissue. We recently cocultured *Borrelia burgdorferi* with human tonsil, skin, and synovial tissue obtained from routine surgery excisions, and showed that the spirochetes preferentially invaded the rotating tissue samples, and grow in numbers in the tissue that far exceed that seen in such tissue samples under conditions of natural infection. This system enables the maintenance of *Borrelial* spirochetes in mammalian and human tissue samples, ex vivo, and may be used as an adjunct to isolate miroorganisms from clinically derived biopsy samples of erythema migrans, joint and soft tissue, and other sites.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Improved Culturing and Sensitivity to Antibiotics of *Borrelia burgdorferi* Isolated from patients with Early Lyme

Charles Pavia, Ph.D.

Despite dissemination during early Lyme disease, the yield of blood cultures for detecting *B. burgdorferi* is often 5% or less. The volume of blood cultured influences the yield in other bacteria infections. Whether increasing the volume of blood or serum inoculated into media would improve the yield of blood cultures in early Lyme disease is not known. In our initial studies, three 3cc aliquots of whole blood or serum were inoculated into BSK media from 31 patients presenting with erythema migrans. Eight (25.8%) of the 31 patients had a positive whole blood or serum culture, including 3 of 6 (50%) with multiple lesions compared to 5 of 25 (20%) with a single lesion (P=).16). Whole blood was culture-positive for 3 of 30 (10%) evaluable patients compared to serum which was positive for 6 of 31 (19.4%) patients. In subsequent studies, six 3cc aliquots of serum were inoculated into BSK media from 26 untreated patients with EM. Seven (27%) patients were culture-positive including 2 of 7 (28.5%) with multiple lesions and 5 of 19 (26%) with a single lesion. We conclude that *B. burgdorferi* can be recovered from peripheral blood in 25% of patients presenting with the EM rash if sufficient volume is inoculated into culture media. Some of these new patient isolates were tested for their sensitivity *in vitro* to selected antibiotics. When compared to standard laboratory-adapted strains, such as B31 and CA287, these new isolates were highly susceptible to antibiotics at concentrations equal to or less than 0.5 ug/ml.

**Notes:**

**Presentation Saturday April 25, 1998**

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## Tick-borne Disorders

Julie Rawlings, M.P.H.

The risk for acquiring tick-borne diseases increases as the weather gets warmer and ticks begin looking for blood meals. Health care providers should be cognizant of the many tick-borne diseases that occur in the United States, including babesiosis, caused by protozoa of the genus *Babesia*; Colorado tick fever, caused by a double stranded RNA virus; Lyme disease and tick-borne relapsing fever, caused by *Borrelia* spirochetes; and Rocky Mountain spotted fever, ehrlichiosis, and tularemia caused by small gram-negative coccobacilli. Vectors for the agents of these diseases include the hard ticks *Amblyomma americanum, Dermacentor andersoni, Dermacentor variabilis, Ixodes pacificus*, and *Ixodes scapularis* and soft ticks *Ornithodoros hermsi* and *Ornithodoros turicata*. Signs and symptoms for the various tick-borne diseases may be similar; these, as well as their transmission cycles, laboratory diagnosis, and treatment will be discussed.

**Notes:**

**Presentation Saturday April 25, 1998**

**William T. Golde, Ph.D.**
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## Coinfections in Patients on Long Island

William T. Golde, Ph.D.

Ixodid ticks are known to be the vectors of *Borrelia burgdorferi* and the piroplasm *Babesia microti.* In addition, these tick species are also believed to be the vector of the agent of human granulocytic ehrlichiosis (HGE). We conducted a prospective study in 1997 to analyze patients presenting with erythema migrans, and therefore *Borrelia burgdorfer*i infection, for co-infection with two other pathogens transmitted by the vector of Lyme disease on Long Island, *Ixodes scapularis.* In the course of our study, we enrolled 18 patients from which we obtained serum and whole blood samples and 16 of which submitted to a skin punch biopsy. Serum samples were assayed for antibody to all three pathogens, whole blood was tested for the presence of these infectious agents by PCR and direct culture, and biopsies were cultured as well as analyzed by PCR. Results indicate that the co-infection rate for *B. burgdorferi* and the agent of HGE is very high where as the rate of infection with *B. microti* is low. The continued development and improvement of detection methods and recruitment of a larger patient population are ongoing in order to confirm these preliminary findings.

**Notes:**

**Presentation Saturday April 25, 1998**

**Richard C. Tilton, Ph.D.**
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## Confirmatory Testing for Non-Lyme Tick Borne Diseases

Richard C. Tilton, Ph.D.

A variety of tests are available for laboratory diagnosis of *Babesia* and both types of *Ehrlichia* infections. The mainstay of antibody screening for both *Babesia* and *Ehrlichia* is an indirect fluorescent antibody test (IFA). IFA slides have become commercially available but if the situation is analagous to Lyme disease testing, a confirmatory test should be performed.

Results are presented on a variety of IFA test for *Babesia* antibody as well as a confirmatory Western Blot for IgG and IgM antibodies to *Babesia.*

Over 500 Patients were tested for antibodies to both ehrlichial species during the summer of 1996. All reactive IFAs were reflexed to a Western blot. Results indicate that a Western Blot confirmatory test is indicated for HGE but, at this time, not for HME.

**Notes:**

# End of Day 1

You are cordially invited to join us for the conference reception tonight, April 25.

**Time**: 6:00 p.m. - 8:00 p.m.
**Place**: Equitable Conference Center-Atrium