12TH INTERNATIONAL SCIENTIFIC CONFERENCE ON LYME DISEASE AND OTHER SPIROCHETAL & TICK-BORNE DISORDERS

APRIL 1999, NEW YORK CITY/NEW JERSEY, USA

Abstract for Oral & Poster Presentations

Deadline: Oral Abstract - December 22, 1998
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DISEASE Lyme Disease/Babesiosis

PREVENTION & BASIC SCIENCE
- ___ Ecology, Entomology
- ___ Vaccine
- ___ Animal models
- ___ Microbiology
- ___ Pathogenesis
- ___ Pathology
- ___ Other:

PATIENT MANAGEMENT
- X Clinical manifestations
- X Laboratory diagnosis
- ___ Early disease management
- X Late disease management
- X Chronic disease management
- ___ Other:

ANTIBIOTICS AND ATOVAQUONE FOR LYME CO-INFECTIONS: Improvement of Neurologic Signs Including Paralysis: Three Case Reports
OBJECTIVE: To present cases of three separate patients (with video documentation) to support effective reversal of severe neurologic signs with treatment protocols for Lyme disease and Babesiosis.
METHODS: Patients were diagnosed and treated in a Lyme-specialized practice.
Patient 001: A 26-year-old Caucasian male presented in September 1997 with swollen joints, cervical stiffness, numbness, twitching, dizziness, fatigue and mood swings. Multiple neurologists supported a presumptive MS diagnosis and gave corticosteroids. MRI showed no white matter lesions but showed an enhancing spinal cord lesion from the cerebromedullary junction to C5. Complete quadriplegia (consistent with posterior column defect) ensued by January 1998. Lyme ELISA was negative. Western blot showed positive IgG bands at 18, 41 and 58 kDa; positive IgM bands at 23-25 and 34 kDa. Lyme urine antigen test (LUAT) was borderline positive (23 ng/mL). Ehrlichiosis (HGE/HME) tests were negative. Ceftriaxone 2g qd IV and azithromycin 500 mg qd PO were started in August 1998; he walked 10 steps 3 weeks later. Babesia microti-RNA FISH (October 1998) showed multiple spores. Atovaquone 1500 mg bid PO was added; 1 week later he walked 16 steps. November 1998 MRI showed a normal cord; lesion had resolved. Patient 002: A 10-year old Caucasian female, normal from birth to age 2 but with seizures worsening to paralysis by age 5 presented in March 1997 with dystonia and a history of a tick bite. Lyme ELISA was negative. Western blot showed highly positive IgG bands at 18, 30, 34, 41, 66 and 93 kDa and positive IgM bands at 39 and 41 kDa. LUAT showed 232 ng/mL on Day 3. HGE/HME tests were negative but questionable. AST and GGT were 152 and 75 IU, respectively; LFTs normalized with treatment. Azithromycin 250 mg PO qd was started; before next visit (041898) she had stood up from her wheelchair. Cefixime 200 mg PO qd was added, then changed to cefuroxime axetil 250 mg PO qd. She currently is walking, has no seizures and has stopped taking levodopa. EEGs done 040297 and 091598 show complete normalization. Babesia microti IgM/IgG as well as RNA FISH (since last visit 111498) show positive 1:80/1:80 titers and multiple spores, respectively; patient is being started on atovaquone.
Patient 003: A 14-year-old Caucasian male presented in December 1997 with severe spastic paralysis (started dantrolene sodium 100 mg PO qid, Mg and B_{12} IM); 2 months earlier he had suddenly become extremely tired and quickly lost the ability to stand. Multiple neurologists' suspicion of "conversion disorder" was ruled out by a psychiatrist. LUAT and ELISA were negative. Initial Western blot was negative; later positive IgM bands appeared at 18, 34 and 41 kDa. Grepafloxacin 400 mg PO qd was started 012098; doxycycline 100 mg PO bid was added 012398, switched to azithromycin 500 mg PO qd 021398. Atovaquone 750 mg PO bid was also started that date (on suspicion; subsequent B. microti IgG and RNA FISH of 100498 showed positive 1:80 titer and multiple spores, respectively). He improved until 042098 (about to resume school), suddenly relapsed, became flaccid and developed diarrhea (Western blot of 100498 showed IgM bands at 34 [highly positive], 41 and 58 kDa, but no IgG bands, suggesting recent re-exposure). Vancomycin 500 mg PO bid was started 042898. All medications were stopped 051198 when AST rose to 107 IU and ALT to 243 IU. LFTs had normalized by June 1998; he then walked again. In September 1998 tremors, secondary paralysis and severe myalgia recurred. Azithromycin was restarted at the same dose, plus cefuroxime axetil 250 mg PO bid; ceftriaxone 2 g IV qd was started October 1998. Doxycycline was added at the previous dose and raised to 400 mg/ day together with stopping azithromycin. and restarting of atovaquone. Cefuroxime 1 g IV qd was started 111098. HGE/HME remain negative; Rocky Mountain spotted fever has been consistently positive. Since September 1998 his ability to stand has increased from 5 seconds to currently > 11 minutes.
RESULTS: Substantial neurologic sign amelioration (if not reversal) often occurs with concurrent Lyme treatment with intravenous and oral antibiotics and Babesia co-infection treatment with oral atovaquone (at ≥ 33 mg/kg/day).
CONCLUSION: Co-infections probably amplify signs of Lyme disease (particularly neurological ones), thereby confounding both its diagnosis and treatment. Once co-infections are cleared, Lyme disease can probably be more easily and completely treated and its neurological manifestations more easily reversed. Lyme disease and its co-infections should be definitively identified or ruled out in any patient presenting with neurological signs .

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TREATMENT OF ADOLESCENTS WITH NEUROPSYCHIATRIC LYME DISEASE
A Model for Collaboration Between Mental Health Practitioners and Lyme-Treating Clinicians
S. K. Berenbaum, CSW-R; M. L. Canon, CSW-R; K.B. Liegner, M.D.; J. Kochevar, FNP-C

Objective: To resolve diagnostic and treatment problems in adolescents with Lyme Disease who manifest primary neuropsychiatric symptoms, and are often highly resistant to medical treatment.
Methodology: Seven patients, ages 13 to 23, were treated for Lyme Disease. The practitioner and clinicians conferenced weekly, at a minimum, to develop and modify treatment plans.

PHASES OF TREATMENT

A. Referral and Initial Evaluation Phase:
Objective: To promote engagement, minimizing complications to that process. Mental Health Practitioner screens for Lyme Disease, using Berenbaum-Canon Lyme Disease Screening Protocol, prepares adolescent and parents for first medical appointment, and makes referral call to medical office. Lyme treating clinician conducts a comprehensive intake examination. Mental health and medical practitioners share information following medical exam, discussing clinical questions and preliminary findings.

B. Treatment Phase:
Objective: To facilitate effective medical and mental health treatment. Mental health practitioner educates the patient and parents regarding Lyme Disease, provides supportive therapy regarding further medical diagnostic testing, and necessary medical treatment, and provides individual, conjoint and family treatment as needed for DSM IV diagnoses. Ancillary professionals are included in treatment team, as needed. Psychiatrist provides adjunctive management, with complex high risk adolescents. School representative is included when an adolescent has a need for special accommodations or modifications in his/her school program.

C. Termination Phase
Objective: To provide a smooth transition for patient and family, minimizing problems that planned or unplanned termination's may present. Mental Health and medical practitioner communicate upon notification of patient's treatment termination, addressing risk level of termination decision, medical and psychiatric prognoses, and recommendations for post-discharge support.

Handling Crisis
Crises need to be anticipated when working with adolescents. Communication among professionals is essential in developing and implementing strategies when a crisis occurs. Safety is always a primary concern.

Results: Patients and families were more committed to both medical and psychotherapeutic treatment, ancillary substance abuse as well as psychiatric issues improved, risk of suicidality and self-mutilation lessened. Patients tolerated antibiotics well, and compliance with Lyme Disease treatment improved significantly
Conclusions: A team approach is needed for adolescents with Neuropsychiatric Lyme Disease, with parallel medical and psychotherapeutic treatment. Coordination of medical and psychotherapeutic treatment planning promotes resolutions of both medical and psychiatric diagnoses.

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DISEASE Co Infection

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Co-infection with *Borrelia burgdorferi*, *Babesia microti*, and the agent of HGE in field samples collected on Long Island, NY

Edward M. Bosler and Jason R. Campbell*
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Since *Ixodes scapularis* is the vector for *Borrelia burgdorferi*, *Babesia microti*, and the agent of human granulocytic ehrlichiosis (HGE), it is likely that a single tick can harbor and transmit multiple pathogens. *Peromyscus leucopus*, the white-footed mouse, is considered the primary rodent reservoir for all three pathogens. To determine possible sources of tick infection, we examined the role of *P. leucopus* in the zoonotic cycles of these tick-borne pathogens on Long Island.

During peak larval tick activity, we conducted a mark-recapture field study in order to monitor temporal aspects of these infections in mice. Twenty eight *P. leucopus* recruits were captured and variously recaptured for a total of 48 captures. Traps were set weekly so that sequential captures of a single animal were never less than one week apart. Blood and tissue samples were collected upon each capture. DNA was extracted from blood and tissue was cultured for *B. burgdorferi* in BSK media. Blood extracts were analyzed by PCR for all three pathogens. Additionally, animals were held for 24 hours during which time replete larval ticks that dropped off were collected. Ticks were allowed to molt and analyzed for infection by PCR.

Our findings indicate that the zoonotic cycles for all three of these pathogens exist and overlap considerably on Long Island. Blood and tissue samples from mice yielded PCR and culture data which indicated co-infection in the mouse population. *B. burgdorferi* was detected alone in 3/48 captures and in combination with at least one other pathogen in 7/48 captures. Likewise, *B. microti* was found alone in 16/48 and as a co-infection in 15/48 captures and HGE was found alone in 1/48 and as a co-infection in 10/48 captures. These data are striking in several respects. First, *B. microti* infection is present alone in one third of the samples, and in combination with other pathogens in nearly another third. *B. burgdorferi* and the agent of HGE are rarely found alone in any mouse and were never found together in the absence of *B. microti*. Analysis of *Peromyscus*-derived ticks showed infection rates of 45%, 10%, and 29% for *B. burgdorferi*, *B. microti*, and HGE, respectively as well as co-infections with every combination of these organisms. Finally, co-infection with all three pathogens did occur in at least 4% of captured mice and 7% of mouse-derived ticks. In conclusion, co-infection appears to play an important role in the zoonotic cycles of *Ixodes*-borne pathogens on Long Island.

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Detection of Phenoloxidase in *Ixodes scapularis* Ticks

Sacha P. Sidjanski, Jason R. Campbell* and Edward M. Bosler*
Department of Medicine, Division of Infectious Diseases, HSC-T15, SUNY, Stony Brook, NY 11794

Our studies were undertaken to examine the presence or absence of phenoloxidase (PO) in *Ixodes scapularis* ticks, the vector of *Borrelia burgdorferi, Babesia microti* and the rickettsial agent of Human granulocytic Ehrlichiosis in the Northeastern and Midwestern United States. In numerous crustaceans and insects, PO is one of the terminal components of a cascade of proteolytic reactions that may form an integral part of the cellular and humoral defense system. Recognition of a foreign agent is followed by a series of steps leading to the conversion of prophenoloxidase (proPO) into active PO, a key enzyme required for encapsulation and melanization of pathogens and parasites. First, a monohydroxylated substrate such as tyrosine methyl ester is hydroxylated by a monophenoloxidase (MPO) to form a dihydroxylated product. This product is further oxidized by a diphenoloxidase (DPO) forming a quinone which will lead eventually to melanin formation.

We attempted to detect PO by gel electrophoresis of tick organ extracts followed by assaying specifically for MPO and DPO activity, the ability to oxidize a spectrum of phenolic substrates and the ability of specific inhibitors to preclude PO activity.

Initial attempts to detect PO in crude tick extracts were unsuccessful. Therefore, we subsequently dissected and assayed specific organs from ticks. Furthermore, we determined that without activation PO could not be detected in tick organ extracts. Following activation with a detergent we able to visualize a band that exhibited MPO and DPO activity. Positive controls and specific PO inhibitors were run in parallel. Our results provide evidence of the existence of PO in *I. scapularis*.

Not all hard tick species can transmit *B. burgdorferi*. *Amblyomma americanum* and *Dermacentor variabilis* are considered to be inefficient vectors. A possible explanation for the high vector efficiency of *I. scapularis* may be directly related to the inability of proPO to be readily activated under natural conditions. Future work should focus on the existence and function of defense mechanisms within other potential vectors of *B. burgdorferi* as well as incompetent vectors.

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The Prevalence of Antibodies Against *B. burgdorferi* in Dogs in Southeastern Michigan

Jason R. Campbell* and Edward M. Bosler
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Human cases of Lyme Disease have been reported throughout the state of Michigan, including both the upper and lower peninsulas; however, those originating on the upper peninsula comprise a majority of these cases (Michigan Department of Public Health). Additionally, *Ixodes scapularis*, the tick which transmits Lyme, has been collected primarily on the upper peninsula, though it does occur on the lower peninsula as well (Michigan Department of Public Health). In a passive 12 year study Walker et. al. (1998) demonstrated that *I. scapularis* is far more prevalent on the upper peninsula than it is on the southern peninsula. This study also included results from a canine serosurvey conducted in 1992 and 1993 which showed antibodies to *Borrelia burgdorferi* in 8% of sera collected from the upper peninsula and only 0.1% of the sera from the lower peninsula. In 1996 and 1997 we conducted a canine serosurvey in southeastern Michigan that examined incidence of *B. burgdorferi* transmission in the counties of Livingston, Macomb, Oakland, and Wayne. Local veterinarians provided blood samples along with pertinent information on each dog with owners' consent. Dogs that had been vaccinated against *B. burgdorferi* or with travel history to known endemic areas were excluded from the study. Samples were refrigerated and sent on wet ice to the testing facility where they were analyzed blindly. Sera were screened by enzyme-linked immunosorbent assay (ELISA) and positive sera were analyzed by Western Blot (*B. burgdorferi* Marblot, MarDx Diagnostics, Inc.). 1399 samples were collected and analyzed over a one year period. ELISA positive samples were obtained from each of the four counties surveyed; 23/284 (8.1%) from Livingston, 16/220 (7.3%) from Macomb, 42/592 (7.1%) from Oakland, and 36/303 (11.9%) from Wayne for a total of 117/1399 (8.4%) positive samples. Reactivity of Western blots was determined using the MarDx-recommended criteria of 5/10 major bands present to indicate exposure to *B. burgdorferi*. Analysis of Western Blots again yielded positive results from all four counties surveyed. Samples that were ELISA positive and subsequently reactive upon Western Blot analysis were considered to be seropositive to *B. burgdorferi*. Using this two-tiered method, we determined seropositivity rates of these four counties to be 4.6% (Livingston), 1.8% (Macomb), 3.5% (Oakland), and 3.3% (Wayne) for an overall 3.4% rate of exposure to B. burgdorferi in this area. The marked increase in canine seropositivity between 1993 and 1996 indicates a possible emerging public health threat in southern Michigan.

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Lyme Borreliosis Seronegativa

Lyme borreliosis is **a diagnosis based on consultation**, where case history is highly important, the data obtained during clinical examinations and treatment are inevitable, and verification in the laboratory is of critical importance. Diagnosing Lyme borreliosis is made difficult by diverse clinical manifestations, the number of diseases and syndromes of unknown origin that can be associated with it, the low number of specific symptoms and the fact that specific symptoms are often missing. It is through a complex process that we have to decide whether we usually diagnose Lyme borreliosis less or more frequently than its natural prevalence. In my opinion, **it is impossible to overdiagnose them,** as the cumulative prevalence of the disease in Hungary could reach as much as one million. The situation could be the same worldwide.

Seropositivity itself, however, cannot be the exclusive basis for verifying Lyme borreliosis, because
•Seronegative Lyme borreliosis is part of the natural process of the disease, due to its special immunological properties. Diagnostic difficulties may be caused by seronegativity that can appear any time, not only at the beginning but also at the end of the disease proved by several clinical case histories.
•It has been widely known with diseases caused by Spirochetes that early antibiotic treatment prevents antibody production for a lifetime and causes a seronegative picture.
•It has just been proven that the generation of an autoantibody binding to LFA-1 could cause different immune disturbances in the body and difficulties in the serological diagnosis of Lyme borreliosis.

The practical solution is modifying the classic dark-field microscopic method with a new reagent **to verify seronegative Lyme borreliosis**. I have been running these examinations for thirteen years now; my results can be summarized as follows:
•I have **video documentary** about Spirochetoid forms being present in the blood of the patients. I can show their different forms, motion, duplication, shedding, and even forms after their shedding.
•As a **negative control**, there are always present different cells of blood with hardened membrane by a reagent in the same preparation, not allowing the cells to produce any kind artificial products, the so-called myeloid figures.
•As a **positive control**, there are immunocytological results with monoclonal antibodies against OspA and flagellin kindly gifted by **Professor Barbour**.
•**Further control experiments** were run with the new reagent and blood, and cultivated *Borrelia burgdorferi s.l.* kindly donated by **Professor Stanek**. It could be seen the hardened membrane of different cells and free moving of Borrelias.
•**The results of parallel examinations** of 714 seropositive samples with different reagents proved the results of microscopical investigations.
•**By interpreting the microanatomical changes of *Borrelia burgdorferi*** we have been enabled to give new answers to the questions asked during the pathogenesis and clinical course of Lyme borreliosis.

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LYME IN THE 21ST CENTURY: MULTIDISCIPLINARY MANAGEMENT OF LATE-STAGE AND PERSISTENT LYME BORRELIOSIS PATIENTS

Authors: *Courtney N. Ganz, M.A., Independent Researcher, Berkshire Brain Injury Support Group; Sarah Rozehnal Ward, M.S., C.C.C.\SLP., Massachusetts General Hospital, Center for Neurological Recovery; Suzanne D. K. Doswell, Massachusetts Brain Injury Association, Berkshire Brain Injury Support Group

OBJECTIVES:

Evidence that Lyme Borreliosis generates moderate to severe cognitive impairments in adults and children, sometimes lingering in nature, is accumulating. While treatment for Borreliosis may resolve neurological and physical complaints in time, scant options exist for coping with the disease in the interim for physicians and patients.

Often patients complain of impaired cognitive-emotional issues such as irritability, memory impairment, organizational problems, poor attention, etc. leading to difficulties with expressive and receptive communication, attending to a variety of tasks of daily living, sustaining employment and/or pursuing former activities.

Patients who have experienced acquired brain injury, whether trauma, stroke, etc., in actuality seem to share similar cognitive impairments or symptomology and often benefit from comprehensive rehabilitation therapies, when few other options are available to improve their condition.

Such patients, in already established care plans, are a potentially useful control group and offer a reasonable avenue for the Lyme disease specialist to pursue in offering new management options for the care of their patients. The thrust of this approach would be in conjunction with other standard therapies for the disease and for the purposes of enhancement.

METHODS:

This poster presentation will evaluate and compare the standard treatment protocol of cognitive-based deficits secondary to traumatic brain injury and acquired brain injury (such as stroke, encephalopathy, tumor, etc.) and the efficacy in utilizing such specialized rehabilitation therapies to treat the cognitive impairments in individuals with late-stage or persistent Lyme Borreliosis.

The intent is to familiarize the physician with the current rehabilitation services available to teach patients compensatory strategies to maximize their performance on tasks of daily living. Resources for referrals, bibliographies and educational materials distinguishing between the sometimes misleading cognitive and communication issues versus other complaints will be made available.

RESULTS:

Many Lyme patients could benefit from an integrated cognitive rehabilitation program to learn strategies to improve overall functioning on tasks of daily living and to compensate for their current deficits. This avenue of rehabilitation warrants consideration and further research by those involved in treating individuals living with Lyme disease.

CONCLUSION:

Most physicians are perplexed by the type and degree of cognitive problems in Lyme disease patients. By obtaining a greater understanding and familiarity with the types of cognitive deficits and rehabilitation approaches available to minimize the impact of such deficits (while in recovery and/or treatment), the physician may gain new angles of perspective on these patients, leading to a) a more valuable understanding of the disease process, and b) the ability to better integrate effective treatment and management approaches to relieve the patient and physician alike.

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12TH INTERNATIONAL SCIENTIFIC CONFERENCE ON LYME DISEASE AND OTHER SPIROCHETAL & TICK-BORNE DISORDERS

APRIL 1999, NEW YORK CITY/NEW JERSEY, USA

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DISEASE Lyme disease

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- [ ] Microbiology
- [ ] Pathogenesis
- [ ] Pathology
- [ ] Other:

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Significant economic loss and illness results from the activities of the man-biting tick, *Ixodes scapularis*, which transmits the etiological agent which causes Lyme disease, *Borrelia bergdorferi*, as well as a number of other serious pathogens. Although acaricides are commercially available for use in the control of *I. scapularis*, concerns about the environmental impact and safety of the use of such chemical compounds has limited their use by the public. Entomopathogenic nematodes are promising biological control agents for a number of economically important insect pests. Thirteen species or strains of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* were tested in vitro against unfed and replete larvae, nymphs, and adults of *I. scapularis*. Nematodes were pathogenic to replete female ticks, but not to unfed or replete larvae, nymphs, males, and unfed females. *Steinernema riobravis* (355) and *H. megidis* (M145) killed replete female ticks most rapidly, with mean day of death post infection of 2.5 and 3.5 days, respectively. However, all nematode strains and species were lethal to replete female ticks within 7.5 days. We then tested and compared the efficacy of 2 exotic variants of *Steinernema riobravis* (355 and Osca), originally isolated from the Rio Grande Valley, against replete *I. scapularis* females. Wooden box plots ($1m^2$) along the meadow/woodland interface were seeded with 10 replete female *I. scapularis*, and the plots were sprayed with 1×10^5, 5×10^5, or 1×10^6 *S. riobravis* 355 or Osca. *S. riobravis* 355 and Osca were both highly pathogenic to *I. scapularis* replete females, resulting in killing within 5 days of 93 and 100%, respectively, in plots treated with 1×10^6 nematodes. These data suggest that entomopathogenic nematodes may be useful as an alternative management method for *I. scapularis* populations, and may be more acceptable than acaricidal chemicals for use in tick infested areas.

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HIGH DOSE TRIMETHOPRIM –SULFAMETHOXAZOLE THERAPY:A USEFUL ADJUNCT TO COMBINATION THERAPY IN TREATMENT RESISTANT BABESIOSIS

Background: Krause described persistent parasitemia after acute babesiosis when patients were given Cleocin and Quinine (C + Q) (NEJM 7/98 Vol 339,160-165). Horowitz described significant clinical improvement among chronic Lyme patients co-infected with Babesia microti when given Atovaquone And Azithromycin (M&Z) (Abstract, 11th International Scientific Conference on Lyme disease, NYC, 4/98), however persistence of Babesia DNA & RNA has been noted with both regimens despite repeated courses of both antibiotics (Horowitz, R I : Abstract, 12th International Conference on Lyme Disease and Other Spirochetal and Tick-borne Disorders, NYC , NY 4/99). Gupta et al describe parasitemia resolving with the addition of high dose Trimethoprim-sulfamethoxazole (T/S) to a regimen of Atovaquone, Cleocin & Quinine (Am.J.Hemat. 50:60-62 ,1995). This report describes an improved treatment regimen for resistant Babesiosis using high dose T/S in combination therapy.

METHODOLOGY

Babesia microti infections were diagnosed among a cohort of 30 chronic Lyme disease patients using both IFA and PCR/RNA analysis (27/30 patients were PCR and/or RNA positive). Patients were divided into 4 treatment groups, using either low dose T/S (Bactrim DS, one QID).or high dose T/S (two QID). Most patients had already received either C+Q or M+2 alone, and patients self selected their treatment regimen based on their prior tolerability of the drugs. Group 1 received Cleocin 600 mg. TID + Quinine 650 mg. TID (C+Q) for 7 days with low dose T/S for 7-10 days. Group 2 received C+Q + high dose T/S for 7-10 days. Group 3 received Atovaquone 750 mg. PO BID + Azrithromycin 600 mg. PO QD (M+Z) for 21 days with low dose T/S during the last 10 days of treatment, with Group 4 receiving the same regimen with high dose T/S. PCR and/or RNA analysis was performed post-treatment in all 4 groups, and success or failure was defined according to persistent parasitemia as evidenced by PCR/RNA positivity post treatment.

RESULTS

22/30 patients (73%) completed the treatment regimen, with 2 patients switching from high dose to low dose T/S secondary to GI intolerance. 7 patients (23%) dropped out secondary to nausea, vomiting, rashes and/or flaring of symptoms. One patient was lost to follow-up. PCR and/or RNA analysis was able to be obtained on 18/22 patients completing the study. Group 1 (low dose T/S) had 3 failures and 1 success. Group 2 (high dose T/S) had 1 success and no failures. Group 3 (low dose T/S) had 5 failures, and no success. Group 4 (high dose T/S) had 7 successes and only 1 failure. Overall the high dose T/S regimen (groups 2 & 4) had an 89% success rate, while the low dose T/S regimen had an 89% failure rate.

CONCLUSION:

High dose T/S when added to either C+Q or M + Z significantly improved the eradication rate of Babesia microti. Lyme disease patients with chronic persistent symptomatology may be co-infected, and those patients who cleared their parasitemia as evidenced by PCR/RNA negativity post-treatment showed significant sustained clinical improvement. GI tolerance was generally poor, and routine use of anti-nausea agents is recommended, with M+Z being better tolerated than C+Q. M+Z with high dose T/S may therefore represent an effective, better tolerated 1st line treatment for chronic persistent babesiosis.

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BABESIOSIS IN UPSTATE N.Y.: PCR AND RNA EVIDENCE OF CO-INFECTION WITH BABESIA MICROTI AMONG IXODIDAE TICKS IN DUTCHESS COUNTY, NY.

Background: Until recently, Babesiosis has not been recognized as existing in upper NY State. Meldrum et al (Clin.Infect. Dis. 1992 Dec; 15/(6):1019-230) reported epidemiological data on 136 cases of human babesiosis reported from laboratories and clinicians in the State of NY from 1982 to 1991. All but two patients who had traveled to Nantucket Island, in Massachusetts, acquired the disease in Suffolk County, Long Island. This report constitutes the 1st evidence of co-infection among Ixodidae ticks with Babesiosis in upstate NY.

Methodology: 30 ticks preserved in ethanol were sent from the laboratory of Dr. Richard Ostfeld at the Institute of Ecosystems Studies in Millbrook, NY to Igenex laboratories in Palo Alto, California where PCR analysis was performed, in November 1998. 192 serum specimens were sent from the medical office of Dr. Richard Horowitz to Igenex laboratories from January 1998 until November 1998 for PCR (DNA) and /or RNA analysis for Babesia microti.

Results: 13/30 ticks were PCR positive for Babesia microti, yielding an infection rate of 43.3%. 72 of 189 serum specimens were PCR positive, 38 of 58 serum specimens tested were RNA positive, and 15 were both PCR and RNA positive, yielding infection rates of 38% and 66% respectively among the population tested.

Conclusion: Ixodes ticks in Dutchess County, NY are co-infected with Babesia microti. Ostfelt et al (Journal of Medical Entomology 35:901-903) reported data from adult ticks collected at the Institute for Ecosystem Studies in Autumn 1996, where 188 ticks were examined for both Borrelia and Ehrlichia, but not for Babesia. 66% were infected with Borrelia, 42.6% with Ehrlichia, and 28.2% with both organisms. Lyme disease patients with chronic persistent symptomatology in upstate NY therefore need to be tested and appropriately treated for multiple co-infections, including Babesiosis.

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CHRONIC PERSISTENT BABESIOSIS AFTER ACUTE TREATMENT WITH CLEOCIN AND QUININE, AND ATOVAQUONE AND AZITHROMYCIN

Background: Persistent parasitemia after acute babesiosis was described by Krause (NEJM 7/98, Vol 339, 160-165) when patients were given Cleocin and Quinine (C+Q), and an experimental regimen with Atovaquone and Azithromycin (M+Z) was noted to possibly cure human babesiosis. Horowitz described significant clinical improvement in a cohort of chronic Lyme patients co-infected with babesia when given Atovaquone + Azithromycin, but relapses were seen at the completion of therapy, and PCR studies were needed to elucidate the eradication rate of the organism (Horowitz, R.I.: Atovaquone and Azithromycin therapy: A new treatment protocol for Babesiosis in co-infected Lyme patients, in Abstracts of the 11th International Scientific Conference on Lyme disease, NYC, NY April 1998).This report describes PCR + RNA evidence of persistent parasitemia with both antibiotic regimens.

Methodology: 192 serum specimens were sent from the medical office of Dr. Richard Horowitz to Igenex laboratories from January 1998 until November 1998 for IFA,PCR, and RNA analysis for Babesia microti. Patients who were positive for either IFA and/or PCR-RNA analysis were treated with either Cleocin 600mg TID + Quinine 650mg TID for 7 days (C+Q), or Azithromycin 600mg QD with Atovaquone 750mg PO BID with meals for 21 days. Patients received multiple courses of the above regimens depending on their clinical response or PCR/RNA positivity post treatment, and crossed over to the opposite antibiotic regimen if clinical failure or PCR/RNA positivity occurred. CBC + liver functions were checked on day 10 +21 of the M+Z, and one 1 month post C+Q.

Results: 72 of 189 serum specimens were PCR positive, and 38 of 58 specimens were RNA positive. 33 charts were analyzed among patients who received one or more courses of M+Z or C+Q and remained PCR and/or RNA positive post treatment. PCR testing and RNA testing remained positive up to 9 months and 5 months respectively, with several patients who were both IFA and PCR negative turning PCR positive after treatment. The majority of patients clinically improved while on the regimens but relapsed shortly after the antibiotics were stopped, with flares occurring often during treatment. Only 4 out of 27 patients became PCR/RNA negative post treatment. Crossing over from one regimen to the other was generally ineffective as PCR/RNA values remained positive, except in 2 cases. M+Z was better tolerated than C+Q, and lab values generally remained within normal limits with both regimens, with an occasional mild elevation of liver functions.

Conclusion: Persistence of babesial DNA and RNA was noted with both C+Q and M+Z, despite repeated courses of both antibiotics. Patients who eventually cleared their parasitemia as evidenced by negative PCR/RNA values had significant clinical improvement, including improvement in cognitive functioning and decreased paresthesias, not usually associated with acute babesiosis. Co-infection with babesia microti may therefore play an important role in chronic ongoing symptomatology among co-infected Lyme patients. Improved therapeutic regimens are required.

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METRONIDAZOLE THERAPY IN THE TREATMENT OF CHRONIC LYME DISEASE

Background: Borrelia burgdorferi has been shown to be capable of persisting in human hosts despite extensive antibiotic treatment (Preac-Music V, et al: Survival of Bb in antibiotically treated patients with Lyme Borreliosis. Infection 1989; 17:355-359). Persistent illness is due to a combination of factors including sequestration in antibiotic and immunologically privileged sites (Luft et al: Invasion of the CNS by Bb in acute disseminated infection, JAMA 1992; 267:1364-1367). This report describes the use of Metronidazole (Flagyl) in a cohort of chronic Lyme patients resistant to standard antibiotic regimens.

Methodology 57 patients with Lyme disease, and/or Ehrlichiosis and Babesiosis were previously treated with either oral, IM or IV regimens prior to being started on metronidazole. Flagyl was given at a dose of 250mg. TID for the 1st week irrespective of body weight, and patients between 121-150 lbs had their dose increased at the 2nd week to 1000 mg/day, with patients greater than 150 lbs. increased to 1500 mg/day for the next month. Dosages were decreased or temporarily stopped and restarted for severe Jarish-Herxheimer type flares, or for increased paresthesias of the extremities. Most patients took Flagyl alone, but several patients added it to their prior antibiotic regimens if they had shown no further improvement of symptoms. Patients filled out a Karnofsky performance scale before and after treatment, and were monitored with a CBC and liver function testing at the completion of therapy.

Results: Jarish-Herxheimer type flares were common during the 1st few weeks of treatment (32/57 patients). 47% of patients reported a significant decrease in arthralgias, with joint pain disappearing completely in 5 patients after only 7 days of therapy. Fatigue improved in 19/57 patients (33%) and neurocognitive symptoms improved in 28 patients (49%) including decreased headaches, paresthesias, and improved memory and concentration. Several patients did not show clinical improvement until the Metronidazole was stopped. The median and mean percent improvement by the Karnofsky performance scale was 13% after 1 month of treatment. An additional 26% mean percent improvement was reported among 7 patients completing a 2nd month of treatment.

Conclusion: Flagyl appears to have anti-borrelial activity and its effectiveness has also been documented in human infections with syphilis (Davies AH., Br.J.Vener Dis 1967; 43:197-200). Median and mean improvement in Lyme disease patients was 13% at one month, but several patients showed dramatic clinical improvement, and those patients with an inadequate clinical response, often had PCR and RNA evidence of ongoing co-infection with Babesiosis. The clinical effectiveness of Metronidazole may be explained by its high bioavailability, good cellular penetration and tissue distribution with good penetration into the CSF, and the formation of redox intermediate metabolites which target the RNA, DNA, or cellular proteins of the micro-organisms irrespective of replication. Further studies need to be done to evaluate the spectrum of Flagyl's role in chronic Lyme disease.

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BICILLIN THERAPY AND LYME DISEASE: A RETROSPECTIVE STUDY OF THE SAFETY AND EFFICACY OF HIGH DOSE INTRAMUSCULAR BICILLIN IN THE TREATMENT OF CHRONIC RESISTANT LYME DISEASE.

BACKGROUND: Patients with Lyme disease often have incomplete clinical responses to oral antibiotic regimens. Cimmino et al described 2 patients with chronic Lyme arthritis resistant to recommended antibiotics regimens who were cured by long term treatment with benzathine penicillin (Ann Rheum Dis 1992; Aug;51 (8)1007-8). Steere et al performed a double blind study comparing IM Bicillin with placebo in the treatment of arthritis and reported a complete resolution of Lyme arthritis in 35% of those patients (NEJM, 1985; 312:869-874). This study describes the results of high dose IM Bicillin in a cohort of chronic Lyme disease patients.

METHODS: 35 Patients were diagnosed with Lyme disease and other tick borne disorders (Ehrlichiosis and /or Babesiosis) from Dutchess County, NY. Patients were started on either oral antibiotics (Amoxicillin, Doxycycline, Ceftin or Suprax) plus a macrolide (Zithromax 500mg/d or Biaxin 500 mg t.i.d.) or low Bicillin (1.2 - 2.4 million/units IM weekly) plus a macrolide. Patients returned weekly for shots and monthly for a medical followup where a CBC, renal and liver function testing was performed. Patients filled out a Lyme disease screening questionnaire at baseline (Burriscano 1995) with positive symptoms evaluated at each subsequent visit. They were also asked to evaluate their percentage of normal functioning before, during ,and after treatment. (0-100, 100=normal baseline functioning). Patients who reported no improvement of symptoms after 1-3 months of oral therapy (plateaued) were switched to low dose Bicillin therapy (1.2-2.4 million units IM weekly) with a macrolide, for another 1-6 months. Patients in the low dose Bicillin group who initially improved but subsequently plateaued, were switched to higher dose Bicillin therapy, (4.8 million units IM weekly) in combination with a macrolide, and continued on this regimen for another 1-6 months.

RESULTS: Patients tolerated high dose Bicillin. Local side effects were common with muscle soreness, and occasional local erythema and pruritis. Laboratory values remained within normal limits with rare elevations of the AST-ALT. Patients often reported Jarish-Herxheimer type flares during the 48 hours post injection, followed by significant improvement in chronic symptomatology, including decreased fatigue, myalgias, arthralgias, headaches, paresthesias and cognitive difficulties. 24 of the 35 patients reported changes in their % of normal functioning. The mean percent perceived clinical improvement switching from oral antibiotics to a low dose Bicillin regimen was 34%. Another 17% improvement was noted upon switching to the high dose Bicillin regimen.

CONCLUSION: High dose IM Bicillin is well tolerated and is a viable alternative to oral antibiotic regimens in treatment resistant Lyme patients. The efficacy of Bicillin may be related to its long half life and elevated serum levels without the associated peak and troughs of oral antibiotics.

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TITLE: CLINICAL STUDY OF BOTANICAL FORMULA IN LYME URINE ANTIGEN TESTING

AUTHOR: *David A. Jernigan, D.C.

RESEARCH LOCATION: International Nutraceutical Research Group (I.N.R.G.I.). Wichita,Kansas

AFFILIATIONS: Jernigan Nutraceuticals,Inc. - Founder

OBJECTIVE. Determine if the herbal formula called Borrelogen is effective in providing positive Lyme Urine Antigen Tests.

METHODOLOGY: Thirty-nine subjects suffering from various chronic illnesses were selected through positive clinical Bio-Resonance Testing and probable case history who had no previous diagnosis of lyme disease. Subjects were given Borrelogen to be taken daily for one week prior to taking a three day urine collection for the LUAT. Frozen urine was submitted to IGeneX Reference Laboratories.

RESULT: Reporting only the highest LUAT score for each three day series, the mean score was 154 ng/ml., median of 125 ng/ml., with a standard deviation of 101 ng/ml. for the range of 33 ng/ml. to >400 ng/ml.

CONCLUSION: In Lyme Urine Antigen Testing, one must kill some Borrelia burgdorferi bacteria so that the dead protein particles can be detected by a combination of the antibody and florescent ELISA used by IGeneX. Normally, prescription antibiotics are given to the patient the week prior to the urine collection to increase the likelihood of a positive test. A positive LUAT as established is in the range of 32-45. Any score over 45 is considered highly positive. When Borrelogen is used instead of prescription antibiotics the majority of cased were reported over 100 and as high as >400, demonstrating that this herbal formula may be an effective alternative to antibiotics by effectively assisting the body in the elimination of the spirochetes, resulting in increased probability of highly positive Lyme Urine Antigen Test confirmations. Borrelogen has been verified by independent testing to not cause a false positive LUAT.

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- __ Vaccine
- __ Animal models
- __ Microbiology
- __ Pathogenesis
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- __ Other:

PATIENT MANAGEMENT
- __ Clinical manifestations
- __ Laboratory diagnosis
- __ Early disease management
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- __ Chronic disease management
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IMPLICATIONS OF THE RECENT LYME CULTURE TECHNIQUE FOR THE DIAGNOSIS OF SYPHILIS

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Objective: To assess whether the culture technique recently developed to isolate *Borrelia burgdorferi* from patients with chronic lyme disease would improve the sensitivity of the diagnosis of syphilis, especially in HIV infected individuals at high risk for re-exposure to *Treponema pallidum*.

Methods: A review of the historical literature on cell-wall deficient forms of various spirochetes, especially *T. pallidum*, was undertaken in an attempt to trace parallels between the apparent insensitivity of serologic and gene amplification techniques used in lyme and what may be a similar insensitivity in syphilis diagnosis.

Results: The sensitivity of non-treponemal tests to measure the mortality of syphilis has historically been questioned. Many pre-1970 authors repeatedly stated that latent syphilis was most likely maintained in part by a spirochetal life cycle distinct from the easily demonstrated binary fission of *T. pallidum*. This research was abandoned in light of the efficacy of penicillin. The culture of *B. burgdorferi* from treated lyme patients, however, suggests the existence of sero-negative chronic lyme disease, and re-opens the old question of a life cycle for *T. pallidum*.

Conclusion: Syphilis and HIV may interact at levels that cannot be presently measured with standard syphilis serology, especially non-treponemal tests. If serology and direct detection by gene amplification are as insensitive for latent syphilis as they seem to be for late lyme, then it may be useful for syphilis researchers to attempt to parallel the *B. burgdorferi* culture technique with the venereal treponematoses (keeping in mind that *T. pallidum* loses virulence quickly in culture), in order to establish a new gold standard in this field as well.

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12TH INTERNATIONAL SCIENTIFIC CONFERENCE ON LYME DISEASE AND OTHER SPIROCHETAL & TICK-BORNE DISORDERS

APRIL 1999, NEW YORK CITY/NEW JERSEY, USA

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DISEASE Babesiosis

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DETECTION OF *BABESIA MICROTI* - PANEL APPROCH

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Babesiosis is caused by an intraerythrocytic parasite, *Babesia microti*. Babesiosis is usually diagnosed by the examination of Giemsa-stained thin blood smears. In addition, there are indirect immunofluorescence assays (IFAs) using polyclonal antibodies directed against *B. microti*. However, all these tests lack specificity and sensitivity. Isolation and culture of these parasites as a diagnostic method would provide adequate specificity and sensitivity, however it is not considered technically or economically feasible.

We have developed two nucleic acid based diagnostic tests for direct detection of *B. microti* DNA from whole blood. Polymerase chain reaction (PCR) test is a highly specific and sensitive assay for direct detection of *B. microti* specific DNA. The "fluorescent in-situ hybridization (FISH) test is a non-amplified, highly specific assay for direct detection of *B. microti* RNA in whole blood smears. These two tests were performed on 221 EDTA treated whole blood samples, from patients with Babesiosis-like symptoms. FISH was considered positive if either the ring form or the merozoite form or both forms were present in the red cell. In addition, IFAs for detection of *B. microti* IgG and IgM antibodies were performed on serum, from all the patients according to the manufacturer's recommendations (MRL). Samples were considered positive by IFA if the *B. microti* antibody titers were 1:80 or greater.

Of the 221 samples, 38 samples (17.2%) were positive by IFA, 73 samples (33%) were positive by FISH and 83 samples (37.6%) were positive by PCR. 56 samples (25.3%, 11 IFA positive and 45 IFA negative samples) were positive by both, FISH and PCR. After discrepant analysis, 38 samples (17.2%) were considered IFA positive, 58 samples (26.2%) FISH positive and 83 samples (37.5%) PCR positive. Based on the data presented here, and the fact that the FISH and the PCR assays are independent of the host's immune response schedule, much earlier detection is possible. Thus, the panel approach , when used in conjunction with clinical symptoms for Babesiosis could improve patient diagnosis. In addition, it provides a tool to follow patients during treatment.

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