Karen Forschner KV-F
#14. 4/19-20/96

IX Annual International Conference on Lyme Borreliosis & other tick-borne disorders

CHRONIC LYME DISEASE:
Basic Science & Clinical Approaches

+ published in "CID" special edition

April 19 & 20, 1996

Wyndham Copley Plaza Hotel, Boston, MA

Conference Co-Chairs
Elisabeth Aberer, M.D. University of Graz (Austria)
Sam T. Donta, M.D. Boston University Hospital

Poster Session Chair
Jonathan A. Edlow, M.D. Mount Auburn Hospital

Awarded 15 Credit Hours Category 1
by Mount Auburn Hospital, Cambridge, MA

HOSTED BY:
Lyme Disease Foundation, Inc. (860) 525-2000
One Financial Plaza, Hartford, CT 06103

Conference Faculty

E. Aberer, M.D., University of Graz, Austria
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S.W. Barthold, Ph.D., D.V.M., Yale University School of Medicine
J.J. Burrascano, Jr., M.D., Southampton Hospital
L.A. Corsaro, M.D., Northern Westchester Hospital
P.K. Coyle, M.D., State University of New York, School of Medicine
S.T. Donta, M.D., Boston University Medical Center
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M.T. Philipp, Ph.D., Tulane University Primate Research Center
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R.C. Tilton, Ph.D., BBI-North American Clinical Labs
J.J. Weis, Ph.D., University of Utah
J. Zahradnik, M.D., Connaught Laboratories

Acknowledgements

The Lyme Disease Foundation wants to thank to an outstanding group of Massachusetts volunteers for their help with the preparation of the conference. This group includes Catherine Belden, Claire D'Andrea, Linda and James Hilliard, Lisa Lainer, Kathy Larkin, and Glen and Margaret Mannke. Thank you all!

Scientific Program -- Schedule of Presentations

Both days: Continential Breakfast 7:30 - 8:30 am; Lunch 12:00 pm.

Friday, April 19, 1996

8:30 am Welcome & Introduction

Animal Models of Chronic Lyme Disease: Chair: S.W. Barthold, Ph.D., D.V.M.

8:35 am - Protective and disease-modulating antibody mediated immunity to *Borrelia burgdorferi* antigens expressed *in vivo* - *S.W. Barthold, Ph.D., D.V.M.*
9:05 am - Hamster model of Lyme borreliosis - *R.F. Schell, Ph.D.*
9:25 am - Chronic Lyme disease in the rhesus monkey - *M.T. Philipp, Ph.D.*
9:45 am - Discussion

Pathogenesis of Chronic Lyme Disease: Chair: A.G. Barbour, M.D

10:00 am - The borrelia's strategies for survival: Implications for chronic disease - *A.G. Barbour, M.D.*
10:30 am - Correlation of severity of arthritis with level of persistence of spirochetes in murine Lyme disease - *J.J. Weis, Ph.D.*
10:50 am - Acquisition and induction of enzymes which digest the extracellular matrix by *Borrelia burgdorferi* - *M.S. Klempner, M.D.*
11:10 am - Effects of *Borrelia burgdorferi* on human B- and T- cells - *D.W. Dorward, Ph.D.*
11:30 am - Why is chronic Lyme borreliosis chronic? - *E. Aberer, M.D.*
11:50 am - Discussion

Laboratory Diagnosis of Chronic Lyme Disease: Chair: C.S. Pavia, Ph.D.

1:00 pm - Anti-borrelial activity of serum from patients with late Lyme disease - *C.S. Pavia, Ph.D.*
1:20 pm - Western immunoblot for Lyme disease: Determination of sensitivity, specificity, and interpretive criteria using commercially available performance panels - *R.C. Tilton, Ph.D.*
1:40 pm - Use of PCR assays to monitor the clearance of *B. burgdorferi* DNA from blood following antibiotic therapy - *M.M. Manak, Ph.D.*
2:00 pm - PCR in the diagnosis of patients with early and late Lyme borreliosis: Comparison of methods - *B.L. Schmidt, Ph.D.*
2:20 pm - Discussion

Prevention of Lyme Disease

3:00 pm - Progress in the clinical development of a Lyme disease vaccine in the U.S.A. - *F. Meurice, M.D.*
3:20 pm - Overview of Lyme disease vaccine trials - *J. Zahradnik, M.D.*
3:40 pm - Discussion

Emerging Tick-borne Diseases: Chair: D. H. Persing, M.D., Ph.D.

3:50 pm - *Ehrlichia equi* in *Ixodes scapularis:* Relevance to Lyme borreliosis - *L.A. Magnarelli, Ph.D.*
4:10 pm - The cold zone: A convergence of tick-transmitted diseases in areas endemic for Lyme disease - *D.H. Persing, M.D., Ph.D.*
4:30 pm - Is Human Granulocytic Ehrlichiosis a new Lyme disease? - *S.J. Dumler, M.D.*
4:50 pm - Discussion

Scientific Program -- Schedule of Presentation

Saturday, April 20, 1996

Clinical Diagnosis of Chronic Lyme Disease **Chair: N.A. Shadick, M.D., M.P.H.**

8:30 am - Multivariate analysis of 160 patients with Lyme disease - *L.A. Fein, M.D., M.P.H.*
8:50 am - The long term follow-up of Lyme disease: A population-based retrospective cohort study - *N.A. Shadick, M.D., M.P.H.*
9:10 am - Disseminated Lyme disease and pregnancy -
9:30 am - Discussion
9:40 am - Lyme disease and the clinical spectrum of antibiotic-responsive chronic meningoencephalomyelitides - *K.B. Liegner, M.D.*
10:00 am - Neurologic complications of late and chronic Lyme disease - *P.K. Coyle, M.D.*
10:20 am - Lyme disease vs. depression vs. somatization: Cognitive tests and functional imaging - *B.A. Fallon, M.D., M.P.H.*
10:50 am - Discussion
11:00 am - General Discussion: Issues in the diagnosis of chronic Lyme disease - Moderators: *P.K. Coyle, M.D.; K.B. Liegner, M.D.*

Poster Session 11:30 am - 12:00 pm

Treatment of Chronic Lyme Disease **Chair: L.A. Corsaro, M.D.**

1:00 pm - Chronic Lyme disease: An evolving syndrome - *B.J. Luft, M.D.*
1:20 pm - The antimicrobial agent, melittin, exhibits powerful *in vitro* inhibitory effect on the Lyme disease spirochete - *C.F. Garon, Ph.D.*
1:40 pm - Tetracycline therapy of chronic Lyme disease - *S.T. Donta, M.D.*
2:00 pm - Intramuscular bicillin for persistent pediatric Lyme disease - *L.A. Corsaro, M.D.*
2:20 pm - Use of vancomycin in the treatment of chronic persistent Lyme disease - *J.J. Burrascano, Jr., M.D.*
2:40 pm - Discussion

3:20 p.m. A New NIH Intramural-Extramural Collaborative Study of Chronic Neuroborreliosis *A. R. Marques, M.D.*, National Institutes of Health, Bethesda, MD

3:30 p.m. General Discussion: Issues in the therapy of chronic Lyme disease- Moderators: *S.T. Donta, M.D.; B. J. Luft, M.D.*

4:30 - 6:00 p.m. Public Forum (Panel from both days) - Moderator: *J. Rawlings, M.P.H.*

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Overview of Lyme disease vaccine trials - *J. Zahradnik, M.D.*

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Chair: D. H. Persing, M.D., Ph.D. 3:50 - 5:00 p.m.

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Is Human Granulocytic Ehrlichiosis a new Lyme disease? - *S.J. Dumler, M.D.*

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Multivariate analysis of 160 patients with Lyme disease - *L.A. Fein, M.D., MPH*

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NIH - 5% minimum overhead

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Public Forum (Panel from both days) - 4:30 - 6:00 p.m.
Moderator: *J. Rawlings, MPH*

CONFERENCE FACULTY

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AGENDA INCLUDES

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Prevention of Lyme Disease

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Clinical Diagnosis of Chronic Lyme Disease

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Lyme Disease Foundation, Inc.
[redacted]

NEWS RELEASE

(Chronic LD)

For Release:
Immediate

For more information contact:

Chronic Lyme Borreliosis: Finding Solutions to the Puzzle

PRESS CONFERENCE:
Friday, April 19, 12:00 - 1:00 PM
Saturday, April 20, 12:00 - 1:00 PM

PUBLIC FORUM:
Saturday, April 20, 4:30 - 6:00 PM
Admission is free to the public

HARTFORD, Conn. 02,96 — The Lyme Disease Foundation, Inc. (LDF) has scheduled its **Ninth Annual International Conference on Lyme Borreliosis & other Tick-borne Disorders** for April 19 and 20 in Boston, Massachusetts. This LDF conference will be the first and only international scientific forum dedicated specifically to the topic of chronic Lyme borreliosis (disease).

The future of chronic Lyme disease (LD) research and clinical management will be significantly impacted by this important event. For the first time, a faculty of 30 international scientists, researchers, and clinicians, all leading experts, will gather to discuss all currently available knowledge on chronic LD. Existing approaches and philosophies about the management of the disease will be debated. The goal of the conference is to plan a collective effort to find a cure.

Presentations will include data on pathogenesis (disease origin and development) and co-infections. A **two-day press conference is scheduled for 12 noon, on both Friday April 19 and Saturday April 20** to provide media access to the experts in these fields.

Friday Press Conference

Patients & Chronic LD

Every aspect of a person's life is changed by chronic LD. Patients will speak on how LD affects their personal, professional, financial and social lives. Learn how they adapt to these challenges.

Chronic LD: An evolving syndrome

Although LD is fairly young, our understanding of this disease has evolved during the past decade. LD, originally viewed as a simple disease effecting mostly the joints, is now considered a multisystemic disorder with chronic, sometimes debilitating, potential. Not much is known about the full clinical spectrum of chronic LD, however, a growing interest and collaboration in this area hopefully will bring the answers.

-- more --

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Guest Editorial

B. burgdorferi—Seek and Ye Shall Find Expanding the Envelope

Kenneth B. Liegner

The borreliae, present on this earth for eons, evolved alongside mammalian life forms in a host-parasite relationship, no doubt long before the appearance of humankind. It should not surprise us, then, that we have much to learn about the range of diseases that the borreliae may underlie and the true scope of infection of the human inhabitants of this planet.

We are at the threshold of a new and exciting era in the understanding and conquest of Lyme borreliosis. Direct antigen detection methods may strike a discordant note with antibody testing's "perfect music of the Spheres," but when clinically validated and honed to optimal sensitivity and specificity, these will be powerful tools in the exploration of the pathogenesis and clinical manifestations of borrelial disease. Researchers as well as rank and file physicians will be able to diagnose with confidence and will have measurable indices of disease activity as these tests begin to become commercially available.

However, with these advances, we will begin to perceive just how daunting an adversary the borreliae really are and how commonly affected the populace of endemic regions. It is my prediction that a much wider range of human disease, now only hinted at, and maybe even totally unanticipated, will eventually be linked to borrelial infection in humans. The borreliae are resilient, phoenixlike. Cutting-edge direct antigen detection methods will corroborate the conclusions already quite apparent from the relatively few reports of culture isolation of borreliae from human subjects following antibiotic treatment now in the worldwide peer-reviewed literature (1–5). The rarity of such isolations should not lead to the conclusion that this phenomenon is rare but only that this has been difficult to prove conclusively with methods available until recently. Nocton et. al. (6) demonstrated polymerase chain reaction (PCR) positivity in serial synovial fluids of 100% of patients treated with conventional oral antibiotics and in 37% of those treated with longer oral and/or intravenous antibiotic regimens. The authors opined that the presence of Bb-DNA implied viable organisms. Bradley et. al. (7) had similar findings and conclusions in their study of serial synovial fluids using Bb-specific DNA detection. Borreliae are, however, tissue tropic and the absence of detection of Bb-specific DNA in body fluids does not exclude their presence in interstitial, intracellular, and parenchymal sites. The Rocky Mountain Laboratory antigen capture method, because it depends on the direct detection of the myriad blebs shed by each living borrelial spirochete, promises to have a far higher yield in tissues and fluids than DNA detection relying on genomic or even of multitarget probes (8, 9). Systematic application of direct antigen detection methods to suspect populations will reveal the true extent of the disease and define the ratio of seropositive to seronegative cases. Seronegativity may be due to T cell anergy and not only as a result of early application of antibiotic therapy (10). Claims that seronegative Lyme disease is rare or that it is common are currently unverifiable.

A tissue repository should be established for tissues from humans suspected of borrelial disease, including autopsy materials, for in depth study using all currently available classic histologic as well as cutting-edge research methods. The pathologist is, after all is said and done, the final arbiter of truth in clinical matters (11–13).

With recognition of chronic persistent infection, we will begin to look at disease pathogenesis quite differently. A persisting pathogen may induce noxious injury over not just days, weeks, or months, but years and decades, and even the natural life of the host. Slowly simmering infection can induce a wide variety of host responses, both direct and immune-mediated. The treatment approach may need to be very different in this circumstance than for a readily extirpated bacterium such as staphylococcus or streptococcus (14–17). Chronic infection may require chronic treatment. Definitive cure, while theoretically possible, may not be achieved using currently available methods in chronically infected patients. This dilemma should prompt a determined effort to develop definitive means of curing the infection (18, 19).

A focus on problems associated with prolonged antibiotic therapy (20) has diverted attention from the much more ominous and insidious spread of borrelial disease in the general population. This vast *de facto* and unintended "Tuskegee" experiment of nature has far greater long-term societal impact in terms of personal suffering, economic loss (21), disability, and death (13, 22) than complications of intensive treatment for a serious disease that are, to some degree, unavoidable.

Often, sequelae of borrelial disease are treated as independent disease entities without being traced back to the inciting etiology. For example, Goodman and colleagues found an incidence of seropositivity for *Borrelia burgdorferi* four times higher amongst patients awaiting cardiac transplantation for chronic congestive cardiomyopathy compared to a control population (23). Yet, this very costly and debilitating illness and the fabulously expensive cardiac transplantation and its aftermath is not counted in the economic impact of Lyme disease. Dementing diseases are amongst society's costliest illnesses. What percentage of patients requiring placement in long-term care facilities for organic brain syndromes really represent unrecognized and untreated end-stage chronic neuroborreliosis (24)? This question deserves to be answered.

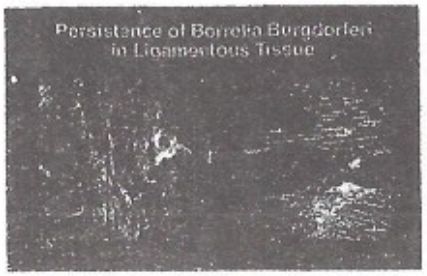

FIG. 1. Persistence of *B. burgdorferi* in the ligamentous tissue. (Reprinted with permission from *Arthritis and Rheumatism.*)

Lyme disease should be included in the differential diagnosis of a wide range of neurologic syndromes. The National Neurologic Research Bank holding tissues for various neurologic diseases including multiple sclerosis, Alzheimer's disease, and motor neurone disease should begin to be systematically evaluated for evidence of borrelial etiology and pathogenesis using the newer direct antigen detection methods (25). Theoretic conceptualizations already developed for other "slow" infections of the central nervous system (CNS) may find application in borrelial disease of the CNS (26). In depth evaluation of some patients previously thought to have multiple sclerosis has uncovered significant evidence of CNS and/or systemic borrelial infection and good response to *intensive* antibiotic treatment (27, 28).

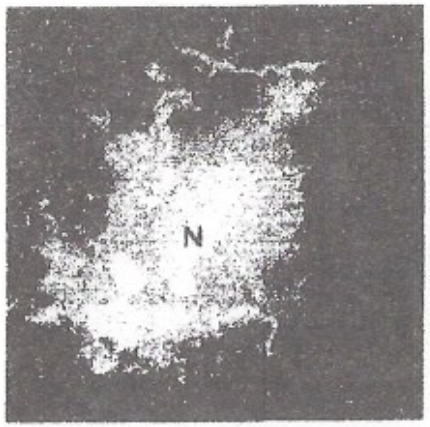

FIG. 2B. Representative confocal microscopic image of optically sectioned fibroblast cocultured with *B. burgdorferi* for 24 hours. Serial section 2.4 m below cell surface shows clear intact spirochete adjacent to perinuclear region of fibroblast. Typical periodicity of spiral shape of *B. burgdorferi* is apparent. Nucleus, nucleoli (N), and mitochondria are visible. (Reprinted with permission from *Journal of Infectious Diseases.*)

The role of borrelial infection also needs to be systematically studied using modern methods in psychiatric syndromes (29) (including derangements leading to domestic violence, suicide, and homicide), attention deficit disorder, various arthritides, "idiopathic" or "autoimmune" diseases, chronic fatigue syndrome, and fibromyalgia. Stunning electron photomicrographs of Haupl and colleagues demonstrate Lyme spirochetes nestled parallel to collagen fibers from synovial tissue removed from a patient previously intensively treated for Lyme disease (3) (see Fig. 1). Klempner and colleagues' beautiful confocal photomicrographs conclusively prove intracellular localization of borreliae within human fibroblasts (30) (see Fig. 2). Is it not likely then, that Lyme disease-associated fibromyalgia with pain localized to fibroblast-produced collagen-rich fasciae and entheses may not be due to the persistence of living borreliae? Although their conclusions were otherwise, the data of Dinerman and Steere clearly demonstrated antibiotic responsiveness of symptoms in their series of patients with Lyme disease-associated fibromyalgia (31).

While the reality and extent of chronic persistent infection needs to be more widely recognized, it also must be acknowledged that there may be self-perpetuating immune-mediated mechanisms of injury that may coexist with active infection or be operative following eradication of the pathogen. For the latter circumstance, antibiotic treatment of a prolonged nature would be futile. Sorting out which cases are due to chronic persistent infection and which are not will be a major achievement of direct antigen detection methods. Creative immune-modulating interventions may prove more effective in inducing remission and averting ongoing injury than antibiotic treatment for this subset of patients, or these may have a combined role with antibiotic therapy in patients having active infection (32).

We should be humble before this disease. Until there is general agreement on a "gold standard" for diagnosis of active Lyme disease, presently available standard and research assays must be viewed as approximations of the truth only (33). Likewise, limits placed on the geographic range of the infection must be greeted with extreme skepticism, as success in documenting the nearly ubiquitous borreliae in a given natural setting depends largely on the determination and experience of the investigator.

At the present time, there exists no substitute for the clinical judgement of an experienced treating physician knowledgeable about the manifold presentations of the disease, adept in listening to the patient and in observing, as in other natural phenomena, the interaction of host and pathogen. Skillful application of antibiotics continues to be the mainstay of treatment for what is, first and foremost, an infectious disease.

Science is all about measuring things. Once objective measures of disease activity are widely available, rational approaches to treatment will replace those based on convention or blind obedience to authority, and the medical neglect now so frequent in chronic Lyme borreliosis will take its well-deserved place in the history of medicine, and not in modern practice.

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Hamster Model of Lyme Borreliosis

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Animal models of Lyme borreliosis are extremely important for elucidating the mechanisms of pathogenesis, chronicity and immunity. When adult inbred LSH hamsters are injected in the hind paws with 10^6 *Borrelia burgdorferi sensu lato*, clinical manifestations of Lyme arthritis (synovitis) are induced. Inflammation and swelling of the hind paws are detected 7 days after infected, peak on day 10 and gradually decline. A chronic synovitis characterized by hypertrophic villi, focal erosion of articular cartilage and subsynovial mononuclear infiltrate persists for approximately 1 year. When hamsters are vaccinated with whole cell preparation of inactivated *B. burgdorferi* in alum, severe destructive arthritis develops after challenge with representative isolates of six seroprotective groups of *B. burgdorferi* sensu lato. *B. burgdorferi*-specific T lymphocytes, especially $CD4^+$ T lymphocytes, are responsible for the development of the severe destructive Lyme arthritis. When vaccinated hamsters are depleted of $CD4^+$ T lymphocytes by administration of monoclonal antibody GK1.5 and challenged, they failed to develop severe destructive arthritis. Similarly, nonvaccinated hamsters with or without depletion of $CD4^+$ T lymphocytes failed to develop severe destructive arthritis. These studies illustrate the importance of cell-mediated immunity in controlling or preventing the induction of events leading to development of synovitis in nonvaccinated hamsters and severe destructive arthritis in vaccinated hamsters. Our results also suggest that as more protective antigens are added to develop a comprehensive Lyme vaccine, the ability of these proteins to induce or elicit adverse effects may increase.

NOTES: T-Lymph. play an important role in ch LD
might add to chr. LD.

recog Heat shock proteins 6 & 7.

T-cell from Treated non-resp. pats.

Suggest CMI resp for Severe destr. arthrits.

"Cell-mediated resp. are involved in Severe Destruct. Arthrits"

["Although these results conflict w others "they are important because..."]

Osp A Leady candidate for vaccine — We tested it's ability.

Assume! 1. Borreliacidal antibodys = protection.

Low & barely protective - only 1/80 dilut.

Booster - Incr ELISA level no activity borreliacidal.

only B-31 killed. (Not any others)

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Chronic Lyme Disease in the Rhesus Monkey

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Tulane University Primate Research Center, Department of Parasitology, Lousiana[1]; Louisiana State University, Department of Neurology, Louisiana [2].

We investigated the appearance of arthritis and neuroborreliosis, as well as humoral and cellular immune responses in rhesus macaques inoculated with *Borrelia burgdorferi sensu stricto* (strain JD 1) during 3, 6, and 46 months post-inoculation (PI). Sixteen animals were inoculated by the bite of *Ixodes scapularis* nymphs, 3 by needle inoculation and 6 were controls. Signs of arthritis were investigated clinically by physical examination, and post-mortem both at the gross and microscopic levels. Signs of neuroborreliosis were sought for in the same way and, in addition, by nerve conduction studies and nerve biopsies. Longitudinal analysis (> 52 weeks PI) of serum antibody indicated a gradual increase in the number of antigens recognized by IgG antibodies on Western blots and a high anti-p39 IgG antibody ELISA titer that reached a plateau of 1:8700 (geometric mean titer) by 10 wks PI. Blastogenesis of peripheral blood mononuclear cells qualified in response to whole killed spirochetes revealed that animals undergo periods of responsiveness interspersed with prolonged intervals of unresponsiveness (10-30 weeks), in face of a persistent antibody response. At the gross level, no joint abnormalities were observed 3 months PI, whereas all of the infected animals showed gross joint abnormalities 6 months PI. Microscopic lesions were apparent at both time points in all animals, most frequently in the knee and elbow joints. Forty-six months PI , 1 of 6 animals examined post-mortem showed marked cartilage destruction with synovial cell hyperplasia and periarticular fibrosis in several joints. Peripheral neuritis involving multiple nerves was the most prominent and consistent neurologic manifestation at 3 months PI. In contrast, by 6 months PI, inflammation was rarely seen, whereas axonal degeneration was prominent. Neuropathologic changes were observed also in the CNS, but to a lesser extent. Sural nerve biopsies indicated a reduction in the number of myelinated nerve fibers in animals which showed, by nerve conduction studies, a pattern and type of peripheral neuropathy best characterized as primarily an axonal-degeneration subtype of mononeuropathy multiplex. Nerve conduction eventually returned to normal in all animals and, concomitantly, regenerative changes such as neuroma or fibrosis were observed in biopsies of some animals. Possible etiologies of the neuropathy observed include localized direct infection within nerve, focal immune-mediated attack on nerve, or focal ischemia of nerve.

NOTES: 50% new. - 1° Neuroborreliosis.

Bb JD1 - Sensu stricto

All animals get full disease.

EM - 4/6 animals clinically

Microscopic 6/6 tick animals (Inflammation of infiltrates remission stain purple)

Subcutaneous circulatory syst involved in initial infec.

LYMEarthritis

9 anim. 0 clinically Gross - None Micro NDet.

(Mononeuropathy)	5 -	1	5	5
Sural 2	5	0	6	5

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Intramuscular Bicillin For Persistent Pediatric Lyme Disease

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Steere et al ('85) reported that 3 weeks of IM benzathine penicillin led to a complete resolution of Lyme arthritis in 35% of patients. Cimmino and Accardo ('92) reported two cases of adult patients with chronic Lyme arthritis resistant to the recommended antibiotic regimens who were cured by 2-6 months of treatment with benzathine penicillin. Based solely on these reports, the use of IM Penicillin among patients with persistent Lyme disease has become common. As a preliminary study of efficacy, we conducted a chart review and follow-up of all patients with seropositive Lyme disease treated with IM Bicillin in a private pediatric out-patient office in a Lyme endemic area between 1993 and 1995.

Methods: The diagnosis of Lyme disease was based on at least one seropositive test and typical articular or neurological symptoms. Treatment consisted of either Bicillin LA or CR 1.2-2.4 million units administered weekly. Relapse was defined as the return of any symptoms which required greater than two weeks of Abx treatment. To assess efficacy, the longest period without symptoms prior to IM Bicillin was compared to the symptom free interval after bicillin.

Results: 61 charts were reviewed of which 25 met study criteria for seropositive Lyme disease. Mean age at time of chart review 11.9 +/- 4.4 years. Mean age at time of Lyme disease onset was 9.4 +/- 4.3 years. Mean duration of symptoms prior to the administration of antibiotics was 16 +/- 32 weeks. All patients failed to sustain improvement after courses of oral antibiotics ranging from 4 to 22 weeks (mean 25.9 +/- 29 wks; median 14 wks). Five children received IV antibiotics and all failed to sustain improvement despite having received 4-27 weeks of IV treatment (mean 9.6 +?- 9.8 wks; median 6 weeks). The longest period free of clinically significant Lyme disease since symptom onset and prior to receiving IM Bicillin ranged from 0-76 wks (mean 60.2 +/- 84.2 wks.; median 8 wks). Of the twenty-five patients given IM PCN, the mean duration of IM treatment was 4-38 weeks (mean 14.5 +/- 8.9 wks; median 10.5 wks). One was still receiving treatment at the time of follow-up and another was symptom free having just completed treatment. Of the 23 children available to assess relapse after treatment, 19 were symptom free, 3 had mild symptoms that did not require treatment, and 1 relapsed and was being retreated. Among the 22 relapse free children, the follow-up period ranged from 2 to 62 weeks (mean 27 +/- 15.5 wks; median 22 wks). Seventeen of the 22 children had exceeded their longest relapse free interval prior to IM Bicillin, 14 of whom had been relapse free by more than twice the duration of their pre-Bicillin relapse free interval.

Conclusion: A chart review and follow-up studies suggest that intramuscular Bicillin may be a particularly effective treatment for children with antibiotic refractory persistent Lyme disease whether previously treated orally or with intravenous antibiotics.

NOTES:

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The Antimicrobial Agent, Melittin, Exhibits powerful *in vitro* Inhibitory Effects on the Lyme Disease Spirochete

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Borrelia burgdorferi has demonstrated an extraordinary capacity to resist the effects of powerful eukaryotic and prokaryotic replication inhibitors. Biologically significant concentrations of inhibitors such as: aphidicolin, Ara C, cis Platinum, CPX, Hydroxyurea, Mimosine, Nalidixic Acid, Trioxsalen and Boromethyglycine showed little or no effect on growth when added to BSK II cultures. The effects of the various inhibitors were monitored by dark field, transmission and field emission scanning electron microscopy as well as DNA precursor incorporation and OD. In contrast, however, the antimicrobial agent, melittin, a 26 amino acid, cationic, amphipathic, peptide contained in honeybee venom, showed almost immediate and profound inhibitory effects. At concentrations of 100 microM melittin, spirochete motility ceased within minutes and the culture was effectively killed. Similarly treated cultures without melittin were unaffected and grew normally. Ultrastructural examination of spirochetes revealed interesting alterations apparently limited to bacterial surface components. Transmission electron microscopy revealed distinct pores along the spirochetal surface with an absence of intact endoflagella. Field emission scanning electron microscopy showed bled-like protrusions over the surface of the spirochete with a large collection of bleb-like material in the background. While surface damage appeared widespread rather than limited to a specific area of the cell, no osmotic lysis was visible. Melittin is an antimicrobial peptide thought to work by inserting into the bacteria (and eukaryotic) membrane causing aggregation and membrane micellization. The extraordinary sensitivity of *Borrelia burgdorferi* to this small peptide may provide both a useful research reagent and important clues to the development of effective drugs against Lyme disease.

NOTES:

IX Annual International Scientific Conference on Lyme Borreliosis

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LYME BORRELIOSIS IN NORTH-EASTERN POLAND

Robert Flisiak*, Danuta Prokopowicz

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Lyme borreliosis become a serious diagnostical and therapeutical problem particularly in regions with high exposure of residents to ticks, such as some forestry parts of north-eastern Poland. To evaluate possible risk of Lyme borreliosis in this region we analysed seasonal changes of prevalence of specific antibodies against Borrelia burgdorferi and clinical symptoms of Lyme borreliosis in Białowieża (forestry area of north-eastern Poland) inhabitants.

Antibodies against 41-kDa antigen of B. burgdorferi B31 strain were found with an EIA assay in 71 among 143 (49,7%) of Białowieża inhabitants examined in September 1993. Prevalence of antibodies was significantly ($p<0,01$) higher than in control group of blood donors from urban population of Białystok (18,8%). Significant differences were also observed between optical density values of IgM and IgG antibodies. The highest prevalence (60%) was observed in age 41-50 and in forestry workers (70,6%). Symptoms related to Lyme borreliosis (according to Centers for Disease Control) were found in 54 from 71 seropositive persons (76,5%), that was significantly ($p<0,001$) higher than in group of seronegative persons (11/72 = 15,2%). Further observations revealed the highest prevalence of IgM antibodies in September 1993 (43,4%) decreased during winter and spring (15,7% in April), accompanied by the highest prevalence of IgG antibodies (17,7% in April 1994). Quantitative dynamics of antibodies level expressed as corrected optical density revealed similar tendency. Moreover in 40 asymptomatic persons, that were seropositive in September 1993, additional control was performed in May 1994. From 33 persons, that revealed IgM antibodies in September 1993, three (9%) were still seropositive, four (12%) cleared IgM antibodies and became IgG - seropositive, and remained 26 persons (79%) had no antibodies. Specific IgG antibodies found in 7 persons in September, were still present in 4.

Prevalence of specific antibodies against Borrelia burgdorferi and Lyme borreliosis morbidity are higher in north-eastern Poland, than in other parts of Central Europe. These results motivate to recognize this area as a region of a high risk for Lyme borreliosis. Moreover obtained serological results indicate necessity of taking into consideration seasonal dynamics, when comparison of seroconversion rate against Borrelia burgdorferi is performed between different areas and different populations.

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HUMAN EHRLICHIOSIS IN TEXAS, 1986-1995

Julie Rawlings
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Although human ehrlichiosis will not become a notifiable disease in Texas until mid-1996, epidemiologic data for 38 cases occurring from 1986-1995 has been collected. Nearly one-third (12) of the cases were reported during the last two years. Of these, eight patients were male and four were female, and ages ranged from 13 to 72. Months of onset were April (2), May (2), June (4), July (2), September (1), and October (1). Ten patients were hospitalized, usually for five to seven days. Four persons, who had more severe illnesses, were hospitalized for longer periods of time. Signs and symptoms included fever (12), headache (10), malaise (10), myalgias (10), thrombocytopenia (10), nausea/vomiting (7), anorexia (5), and rash (5). The patients with more severe illness experienced severe respiratory symptoms, renal failure, clinical hepatitis, meningoencephalitis, confusion, and/or seizures. Six persons recalled a tick bite prior to onset of symptoms. One person was bitten in Eastern Oklahoma; three were bitten in Arkansas.

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GASTROINTESTINAL DISEASE IN CHILDREN WITH LYME

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PURPOSE- Lyme disease can affect a wide range of organ symptoms producing dermatologic, musculoskeletal, neurologic, genitourinary, repiratory, cardiovascular and ocular manifestations. Reports of gastrointestinal (GI) manifestations have been limited to liver and spleen thus far. To address the possiblity of direct involvement of the GI tract, a retrospective study was made of children who had Lyme and chronic abdominal pain.

METHODS-All patients included in our study had an erythema migrans rash, clinical manifestations of Lyme disease (arthritis, Bell's palsy, heart block) and confirmatory enzyme-linked immunosorbent assay or positive western blot. The records of 400 children were examined and ten patients satisfying the above criteria were retrospectively identified. Each case record included a history, physical examination, complete blood cell count, liver function tests, ultrasonography of the abdomen, upper GI X-ray series with small bowel follow through, esophagogastroduodenoscopy (EGD), or colonoscopy. Stool samples were examined for occult blood, Salmonella, Shigella, Yersinia, Campylobacter, ova, parasites, and Clostridium difficile toxin. Biopsy specimens were reviewed to assess the GI mucosa by microscopy and to the presence or absence of H. Pylori (on EGD) and spirochetes. Spirochetes were detected in biopsy specimens using a modified Dieterle stain.

RESULTS-Biopsy evidence of inflammation was found in the stomach, duodenum and colon. Pathologies included gastritis, duodenitis, gastric ulcer and colitis. Spirochetes with the microscopic appearance of Borrelia were found in five patients with chronic inflammatory conditions of the GI tract.

CONCLUSION- The inflammation may have been due to the spirochete organism itself, a product related to their presence in the GI tract or as a consequence of medications used to treat Lyme.

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Small Slaughter – immune-syringe-prot.
Tick – NO prot., but induc. arthritis reac.
Transplant – No prot.

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Scientific Program, Friday April 19, 1996

Karen – Long-term implicat
10 million of $ on vaccine

Protective and Disease-modulating Antibody-mediated Immunity to *Borrelia burgdorferi* Antigens Expressed *in vivo*.

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Borrelia burgdorferi is a dynamic pathogen with a propensity for persistent infection in immunologically responsive hosts, including humans. We have utilized a well-defined mouse model of Lyme disease, in which mice infected by syringe of tick develop heart and joint disease that undergo spontaneous resolution and episodic recurrence over the course of persistent infection. Spirochetes appear to persist extracellularly within collagenous connective tissue, particularly the skin. Using the mouse model, we have found that *B. burgdorferi* undergoes dramatic changes in protein expression in different environments. For example, OspA is abundantly expressed on spirochetes in the midgut of unfed ticks, but is rapidly down-regulated when ticks begin to take a blood meal. Spirochetes entering the host do not express OspA, and are therefore not vulnerable to OspA immunity. During infection, other proteins are expressed that are probably required for tissue invasion. Several proteins have been identified that are expressed exclusively *in vivo* and recognized by the host immune response. Based on adoptive and passive transfer studies, host humoral, but not cellular, responses directed against *in vivo*-expressed proteins have varying degrees of protective activity and are also involved in resolution (and recurrence) of disease. Compartmentalization of host immunity to antibody facilitates efforts to incriminate responsible antigens by screening a genomic expression library with serum from infected mice. Incrimination and characterization of the genes and proteins involved in persistent infection and disease modulation are the frontier of Lyme disease research, with the potential for development of second generation preventive as well as therapeutic vaccines. This presentation will provide an overview of this work.

NOTES: Mice get infec. 2-3 wks after swelling of joints
3 days later – dies [illegible] to tissue. & joint gets inflam.

Same pathology occurs in dogs, & humans & monkeys.
Same pathogen gets diff resp. in diff tissue.
"Skin is highly immunologic but no real inflammation. Tell us imm. resp. no help" bladder, skin, spleen, lung & no severe inflam'n as in joints.
"No doubt some humans who did not get treatment became persistently infected".
SCID mouse – Can not clear spir. from joint tissue. mice Severely arthrit
Talks about shape of Bb you see in tissue depends on slice & what plane the Bb is in.
1993 – Barlow disc. OspA. – when people (researchers) fo[und] Bb shuts down OspA in host. So people don't get OspA imm. resp.
Vaccine work OK but the Bb in host lose OspA "Host adapted spirochetes"

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- ☐ MICROBIOLOGY
- ☐ PATHOGENESIS
- ☒ DIAGNOSIS
- ☐ CLINICAL MANIFEST.
- ☐ TREATMENT
- ☐ VETERINARY ISSUES
- ☐ EPIDEMIOLOGY
- ☐ OTHER SPIROCHETAL & TICK-BORNE DISEASES
- ☐ OTHER (LIST)

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HUMAN EHRLICHIOSIS IN TEXAS, 1986-1995

Julie Rawlings
Texas Department of Health

Although human ehrlichiosis will not become a notifiable disease in Texas until mid-1996, epidemiologic data for 38 cases occurring from 1986-1995 has been collected. Nearly one-third (12) of the cases were reported during the last two years. Of these, eight patients were male and four were female, and ages ranged from 13 to 72. Months of onset were April (2), May (2), June (4), July (2), September (1), and October (1). Ten patients were hospitalized, usually for five to seven days. Four persons, who had more severe illnesses, were hospitalized for longer periods of time. Signs and symptoms included fever (12), headache (10), malaise (10), myalgias (10), thrombocytopenia (10), nausea/vomiting (7), anorexia (5), and rash (5). The patients with more severe illness experienced severe respiratory symptoms, renal failure, clinical hepatitis, meningoencephalitis, confusion, and/or seizures. Six persons recalled a tick bite prior to onset of symptoms. One person was bitten in Eastern Oklahoma; three were bitten in Arkansas.

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Small Triangle – immune-syringe – prot.
Tick – NO prot., but induc. arthritis weak.
Triangle – NO prot.

Stephen W. Barthold, Ph.D., D.V.M.
Professor
Yale University School of Medicine
P.O. Box 208016
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Scientific Program, Friday April 19, 1996

Karen – Long-term implicat
10 million of $ on vaccine

Protective and Disease-modulating Antibody-mediated Immunity to *Borrelia burgdorferi* Antigens Expressed *in vivo*.

Stephen W. Barthold. Section of Comparative Medicine, Yale University of Medicine, New Haven, CT.

Borrelia burgdorferi is a dynamic pathogen with a propensity for persistent infection in immunologically responsive hosts, including humans. We have utilized a well-defined mouse model of Lyme disease, in which mice infected by syringe of tick develop heart and joint disease that undergo spontaneous resolution and episodic recurrence over the course of persistent infection. Spirochetes appear to persist extracellularly within collagenous connective tissue, particularly the skin. Using the mouse model, we have found that *B. burgdorferi* undergoes dramatic changes in protein expression in different environments. For example, OspA is abundantly expressed on spirochetes in the midgut of unfed ticks, but is rapidly down-regulated when ticks begin to take a blood meal. Spirochetes entering the host do not express OspA, and are therefore not vulnerable to OspA immunity. During infection, other proteins are expressed that are probably required for tissue invasion. Several proteins have been identified that are expressed exclusively *in vivo* and recognized by the host immune response. Based on adoptive and passive transfer studies, host humoral, but not cellular, responses directed against *in vivo*-expressed proteins have varying degrees of protective activity and are also involved in resolution (and recurrence) of disease. Compartmentalization of host immunity to antibody facilitates efforts to incriminate responsible antigens by screening a genomic expression library with serum from infected mice. Incrimination and characterization of the genes and proteins involved in persistent infection and disease modulation are the frontier of Lyme disease research, with the potential for development of second generation preventive as well as therapeutic vaccines. This presentation will provide an overview of this work.

NOTES: Mice get infec. 2-3 wks after swelling of joints
3 days later – dies fibro tissue. & joint gets inflam.

Same pathology occurs in dogs, & humans & monkeys.
Same pathogen gets diff resp. in diff tissue.
"Skin is highly immunologic but no real inflammation. Tell us imm. resp. no help" bladder, skin, spleen, lungs & no severe inflam'n as in joints.
"No doubt some human who did not get treatment become persistently infected."
SCID mouse – Can not clear spir. from joint tissue. mice Severely arthrit
Talks about shape of Bb you see in tissue depends on slice & what plane the Bb is in.
1993 – Barlow disc. OspA. – when people (researchers) to Bb shuts down OspA in host. So people don't get OspA imm. resp.
Vaccine work ok until. Bb in host OspA" "Host adapted spirochetes"

Ronald F. Schell, Ph.D.
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Hamster Model of Lyme Borreliosis

E.L. Munson, B.K. DuChateau, L.C.L. Lim, S. M. Callister and R.F. Schell. Wisconsin State Laboratory of Hygiene and Department of Medical Microbiology and Immunology, Gundersen Medical Foundation, LaCrosse, Wisconsin.

Animal models of Lyme borreliosis are extremely important for elucidating the mechanisms of pathogenesis, chronicity and immunity. When adult inbred LSH hamsters are injected in the hind paws with 10^6 *Borrelia burgdorferi sensu lato*, clinical manifestations of Lyme arthritis (synovitis) are induced. Inflammation and swelling of the hind paws are detected 7 days after infected, peak on day 10 and gradually decline. A chronic synovitis characterized by hypertrophic villi, focal erosion of articular cartilage and subsynovial mononuclear infiltrate persists for approximately 1 year. When hamsters are vaccinated with whole cell preparation of inactivated *B. burgdorferi* in alum, severe destructive arthritis develops after challenge with representative isolates of six seroprotective groups of *B. burgdorferi* sensu lato. *B. burgdorferi*-specific T lymphocytes, especially $CD4^+$ T lymphocytes, are responsible for the development of the severe destructive Lyme arthritis. When vaccinated hamsters are depleted of $CD4^+$ T lymphocytes by administration of monoclonal antibody GK1.5 and challenged, they failed to develop severe destructive arthritis. Similarly, nonvaccinated hamsters with or without depletion of $CD4^+$ T lymphocytes failed to develop severe destructive arthritis. These studies illustrate the importance of cell-mediated immunity in controlling or preventing the induction of events leading to development of synovitis in nonvaccinated hamsters and severe destructive arthritis in vaccinated hamsters. Our results also suggest that as more protective antigens are added to develop a comprehensive Lyme vaccine, the ability of these proteins to induce or elicit adverse effects may increase.

NOTES: T-Lymph. play an important role in ch LD
might add to chr. LD.

recog Heat shock proteins 6 & 7.

T-cell from Treated non-resp. pats.

Suggest CMI resp for Severe destr. arthrits.

"Cell-mediated resp. are involved in Severe Destruct. Arthrits"

["Although these results conflict w others "they are important because..."]

Osp A Leady candidate for vaccine — We tested it's ability.

Assume! 1. Borreliacidal antibodys = protection.

Low & barely protective - only 1/80 dilut.

Booster - Incr ELISA level no activity borreliacidal.

only B-31 killed. (Not any others)

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James H. Oliver, Jr.: Ticks, Lyme Disease, and a Golden Gloves Champion

MARLIN E. RICE

James H. Oliver, Jr. is Fuller E. Callaway Professor of Biology Emeritus at Georgia Southern University and a Fellow of the American Association for the Advancement of Science. Oliver is widely recognized as an international expert in medical entomology and acarology, especially the biology and cytogenetics of pathogen-transmitting ticks and parasitic mites.

Oliver was born 10 March 1931 in Augusta, Georgia, but his boyhood was spent near Waynesboro, where his father farmed 2,000 acres. Oliver earned a B.S. (Biology, 1952) from Georgia Southern College (now Georgia Southern University), and an M.S. (Zoology, 1954) from Florida State University. He allowed himself to be drafted by the U.S. Army during the Korean War. He was assigned to the Army Chemical Corp in Ft. Dietrich, Maryland, at a top-secret biological warfare facility. There he was fortunate to work on a complex group of animals, which inspired his lifelong interest and determined the direction of his academic career. Using the G.I. Bill to further fund his education, Oliver earned a Ph.D. (Entomology, 1962) studying mites and parthenogenesis from University of Kansas. Upon graduation,

James Oliver at 10 years old, 1941.

he accepted an assistant professor position at University of California–Berkeley, but also was offered a National Science Foundation Postdoctoral Fellowship at Melbourne University in Australia. He convinced the department chair, Robert Usinger, to let him take a year's sabbatical leave to Australia before beginning his appointment at UC–Berkeley.

Oliver supervised the research of 30 master's students, nine Ph.D. students, and 30 postdoctoral students from 11 countries. He published 12 book chapters and more than 250 refereed papers, and has been awarded more than $12 million in research grants and contracts. During his career, he was a popular speaker and gave lectures in 24 states, 19 countries, and at numerous universities including Cornell, Harvard, Texas A&M, and Yale.

Oliver was instrumental in getting the U.S. National Tick Collection transferred from the Smithsonian Institution to Georgia Southern University. The collection has over one million specimens, 300 type specimens, and nearly all of the world's 850 recognized species. But news of the collection moving to Statesboro was not without controversy, as the locals complained that Georgia had enough ticks and didn't need any more brought into the state. He leveraged the National Tick Collection as a resource to conduct extensive research on Lyme disease, *Borrelia burgdorferi*, and the ticks that vectored the debilitating pathogen. Oliver and his colleagues found 300 southern

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genetic strains of *Borrelia*; 57 of them nearly identical to the northern pathogen and are classified as *Borrelia burgdorferi* sensu stricto. Additionally, they discovered two new species of Lyme disease bacteria in the South: *Borrelia americana* and *Borrelia carolinensis*. If these two new species are found to cause illness in humans, then the discovery will help in understanding the debilitating Lyme disease in the South. Internationally recognized for his research contributions, Oliver has received numerous honors including the Honorary Gold Medal of Achievement, Warsaw Agricultural University (Poland); J. G. Mendel Medal, Czechoslovakia Academy of Sciences; Medal of Honor, Entomological Foundation; and Honorary Doctoral Degree (Doktor Honoris Causa), University of South Bohemia, Czech Republic.

The Entomological Society of America presented the Founders' Memorial Award to Oliver for honoring the late Robert L. Usinger, and he was elected an Honorary Member and Fellow in 1985 and 1995, respectively. Oliver served as president of several societies including the Southeastern Society of Parasitologists, Acarological Society of America, and in 1990, the Entomological Society of America. In honor of his lifetime achievements and dedication to science, Georgia Southern University created the James H. Oliver, Jr. Institute of Arthropodology and Parasitology, where he served as director for 19 years.

This interview occurred 13 May 2016 at Oliver's home in Statesboro, Georgia. We casually talked about the jasmine in bloom, the thick Georgia humidity, and a litter of red fox pups that played on his porch that morning. His wife, Sue, occasionally joined in the conversation. Oliver was 85 years old.

Oliver: Good morning.

Rice: ***Yes it is. Pleasure to see you again.***
Welcome. Good to see you again.

Let's start with your boyhood. Did your parents encourage your interest in biology or natural history?
No. Not a thing. My father was a farmer—a dairyman—and also a barber. My mother was one of the old South ladies who enjoyed the social life of a small Southern town. Neither one of them went to college. So there was no boyhood interest in biology from my parents. I always liked being in the woods and going hunting in the fields. But I was leaning toward medical school because my grandfather was a physician and his father was a physician.

James H. Oliver, Jr., Fuller E. Callaway Professor of Biology Emeritus, Georgia Southern University, 2016 (photo by Kay Rice).

You mentioned hunting in the woods. What did you like to hunt?
Quail, quail. Yeah.

At some point in your life, you decided you wanted to attend university. How did you make that decision?
My mother and father divorced. I was in high school and I didn't know what I wanted to do.

Were you a good student?
Jim: No, not very. I was a good school athlete and party guy. [*Laughs*.]

Sue: But you were more than that; you were the class president and you were a leader.

Jim: Oh yeah, I was captain of the football team, basketball team, and ran track and played tennis in state [tournaments].

Sue: It was a small country school. He had maybe 50 in his class. The football coach would take Jim out of math class to go over the plays. I came from New York City area [and] had 500 in my graduating class. Ninety-five percent of them went to college. I couldn't even believe what he didn't get [in high school]; prior to college [he] was sort of a blank slate [*laughs*] in education.

Jim: My brother was an electrical engineer at Georgia Tech—honor roll the whole time. He says to me, "I want you to enroll in Georgia Tech after you finish school as a senior." But I don't like physics and math. But I felt like I had to do something athletically. I really wasn't big enough to do the [sports] I wanted to do in university. But I was frustrated and decided to go to the University of Georgia. I like animals, so maybe I'll be a veterinarian. That was acceptable to everybody but the trouble was you had chemistry and a lot of other meaty courses. And I thought I wouldn't do well, [but] I was into boxing and I could compete that way.

You went into boxing at the University of Georgia? Were you a good boxer?
Yeah. I won the state championship in my weight—the Golden Gloves.

Did you ever get knocked out boxing?
No.

Did you ever knock out an opponent?
Yeah. [*Laughs*.]

What's the quickest round you ever won?
Probably second or third round. I was so good at it because I was in good physical condition—*great* physical condition. I had a coach that said the one's that's in the best physical condition and can keep his left jab going all the time and don't try for a knockout—just hammer him [would win]. It was very good advice, because after the second round my opponent would usually get arm weary and I'd block him by keeping my hands up. That's how I won most of my fights, out of pure physical condition, and I was coordinated and fast. So then I found what I wanted to do; I'm not going to be a veterinarian, I'm going to be a boxer—a professional boxer! Well, that wasn't well thought out. [*Laughs*.] My brother, and he was always a scholar, said, "My god, you can't do that. You're going to have a brain concussion!" "Yeah, but I'm quick." I was finally talked into not doing that and leaving the University of Georgia. I went to Georgia Southern 'cause it was only 50 miles away from home and I liked teaching as well. So

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I'll become a high school biology teacher and coach. That was my goal for several years until I decided I don't want to do that. I think I can do more interesting things—I'll just become a college teacher. All of a sudden, I thought "I'm going to turn myself into a scholar." I joined a classic book club 'cause I didn't have any exposure to the classics.

Do you have a favorite classic?
Not really. That was short-lived! But it actually stimulated me into going to graduate school.

Did you have any challenges as an undergraduate student? You came from a small rural school to a state university.
A lot of time, I cut classes. But I realized that if you were going to go on, you had to get a graduate degree.

If you cut classes, you probably didn't do well academically?
Not until about my sophomore year, and then I decided that this was foolish, and I was wasting everybody's time. Once I decided that, I made the dean's list from then on until I graduated. Majored in biology. Was a student assistant teacher. Published my first scientific paper.

What was your first scientific paper?
It was in ornithology.

Ornithology?
Yeah. I really liked bird watching.

Well, you certainly liked shooting them.
And eating them! [*Laughs*.] But we had to do a student project in ornithology class. That was my first scientific paper: unusual nesting behavior of the brown-headed nuthatch. So then I thought, I'll just go on to graduate school and study ornithology [at] Florida State University. But the ornithologist was not particularly outstanding and I thought, well *damn*. So there was a parasitologist there—Bob Short—and he studied blood flukes. I was impressed with him. He had an NIH grant, which I didn't know what it was, [but] I did my master's degree on one of the life stages of a parasitic worm. But I was starting to get more academically interested. And what do you have to do to do this stuff? So I looked at successful people, and what did they do to distinguish themselves? They published scholarly papers and have

James Oliver with his 1950 Golden Gloves Light Weight Championship trophy (photo by Marlin Rice).

foreign experience. At the same time, I was drafted.

Drafted while you were at Florida State?
Yep. For the Korean War. The draft board had been giving me extensions because I was getting good grades. I just let myself get drafted in the Army. I told the draft board I didn't need any more deferments. So when my number comes up, call me. And they did. After I took some tests, [the Army] sent me to a biological warfare research center. It was a new program called Specialists and Professional. The idea behind it was that [the Army] didn't want entomologists to be cooks and mechanics. They were going to have this special program if [the draftees] have various characteristics and were bright enough.

What year was basic training?
1954-1955. The army told me to go to Frederick, Maryland. It was a secret biological warfare facility and that intrigued me: studying ticks, mosquitoes, and the pathogens they transmitted.

Human pathogens?
Yeah. We knew the Russians were involved in biological warfare. So I did that and that's where I met Sue. She was at a girl's college in Frederick. She couldn't get married until she finished college, so I went to Johns Hopkins for a year, but I wanted to do a worldwide, broader program, and so, when she graduated, we married, and then I moved to [University of] Kansas.

What were you doing at Johns Hopkins?
Medical entomology. I also was waiting on Sue for a year to finish Hood College. I was working with Lloyd Rozeboom and he was a mosquito person in the School of Public Health. I was only at Johns Hopkins for a year. I wanted to broaden myself and get more entomology, so I went west to Kansas.

Let's go back to the biological warfare program. What was one of the most interesting aspects your Army team was investigating?
It's a really interesting camp with Civil Service people and 500 soldiers like me that had special training and aptitude.

All working on biological warfare?
Of some kind, in crops and bacteriology.

You said crops?
Crops. Yeah, plants. Just use your imagination. These are the things that would be very important in a war. If you want to bring a nation, like Russia, to their knees, destroy their agriculture. We felt more threatened, more so than we needed to be, but they didn't put me in crops or bacteriology. They put me in the entomology branch and I had never had entomology.

This sounds typical of the Army. They put you in an area where you have no expertise.
But I am a biologist, so that's where I started working on ticks and mosquitoes—how to produce a lot of them. Drop them out of airplanes. Everything was very hush-hush, very secret. I'm still leery talking about it, because I think they might put me in jail because I'm delivering secrets. [*Laughs*.] It was a crazy time.

How do you do a defensive application of ticks and mosquitoes?
We would run all kinds of distribution tests on where these things go when you release them and what were the factors that would cause the migration. Can we drop them out of airplanes and how do we get the bugs to the enemy? That was the thing we did. So I did my two years, plus a year at Johns Hopkins, then we got married and moved to Kansas.

Who was your advisor at University of Kansas?
Ralph Barr—a mosquito guy. But when I got there, they had hired Joe Camin,

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James Oliver and Jerry Rozen trapping raccoons for ticks on St. Catherine's Island, Georgia, 1983.

who was an acarologist, so I transferred professors from Ralph Barr to Joe Camin [because] I got interested in ticks and mites.

Harvard biologist Stephen Jay Gould, in an essay published in* Hen's Teeth and Horse's Toes*, cited your doctoral research on the mating biology of an earthworm mite. What was the element of fascination with this mite that parasitizes earthworms?
From a biological standpoint, it's a very interesting life history. As a behavior, earthworms deposit eggs in the soil and these little mites get onto the earthworm when it makes its cocoon; it would encapsulate these little mites. Under a microscope, [you could see] these mites swimming around in the [earthworm] yolk. I had an NIH [National Institutes of Health] grant to go to Michigan to study these mites. Joe Camin said I was the only student he knew that had gotten their own NIH grant to do the work, so here I was feeling pretty good. [*Laughs.*] So that was for the life cycle of the mite in the cocoon.

Did the NIH grant provide a stipend to you?
Yeah. It was very impressive to me. I don't remember the amount, but it was *more* than enough to be a graduate student.

I was doing a little reading on your earthworm mite. There was something about the chromosomes of the mite that determined the sex of the progeny.
Yeah. You really are up on this, aren't you? Nobody else has ever said that to me or would be interested. [*Laughs.*] But the way it works is, if there is bisexual reproduction, they [the larvae] are going to be all females. And if there is no mating, the [larvae] are going to be haploid and all males. And that's the way of sex determination. That led me to really get interested in genetics—cytogenetics, particularly—because I was studying the chromosomes. I hadn't thought about earthworm mites in a long time.

You finished your Ph.D. at University of Kansas; then what was your next position?
The next position was actually at the University of California–Berkeley.

There is a legend that you had three job offers.
Yeah, I did. [*Laughs.*] It was a lovely time for a student to get out and look for jobs. One was Illinois State University. I took the job at Berkeley, but it didn't pay as much as the one in Illinois. It paid, at the time, a really good salary compared to what I was offered at Berkeley.

What would a really good university salary be in the 1950s?
Ten thousand dollars. Everybody said that your experiences at Berkeley would be so much *richer* long-term, although it's not going to pay as much money as Illinois State University, but long-term, financially, you'll be better off. And it will be much more interesting with the colleagues and you've got prestige and all that. So I took the Berkeley job.

Where does Australia fit into this sequence?
Australia was before I went to Berkeley. It was one of the most exciting and fulfilling years ever. I was from the Southeast, and here I was, going to Australia. This happened while I was finishing my degree at KU. And it was a situation where it was a different world. Animals were different. Plants were different. And people were different; fortunately they spoke English. [*Laughs.*] But I went there with the excuse of studying chromosomes with Michael James Denham White, an Englishman who was transplanted to Australia. He had written several books on animal cytogenetics, and I thought it would be good to associate with him. While there, we got to travel from Melbourne to Adelaide, to Brisbane, to Sydney. It was just wonderful.

And you're collecting ticks in all these locations.
Yeah. Supposedly. [*Laughs.*]

That means that you were also doing something else then?
Yes, yes. I was exploring the people and culture and geography. It was really a wonderful year because I had gone from a KU graduate student, [to] Berkeley, just almost…well, I didn't even go to Berkeley. I was hired, and Bob Usinger, who was at Berkeley, allowed me to take a year's leave of absence to go to Australia and they would hold the position for me.

So Robert Usinger was the department chair.
He was.

He hires you, but allows you to not show up for a year and instead go to Australia?
Yeah, and with a secured position so I didn't have to worry about interviewing [later]. It made a lot of pressure come off. It was a very good year for me. I learned so much about cytogenetics and learned how an international scientist operates.

Usinger must have had a lot of respect for you as a young assistant professor to allow you to take a year sabbatical leave.
That's right. He said, "You'll be much more valuable to us after your year's experience at National Science Foundation expense than ours, but we'll be happy

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to have you come back in a year." Life is good! [*Laughs.*]

It was good for you.
It really was. I made so many valuable contacts and associates there. It was so broadening from an intellectual standpoint and a behavioral standpoint. And it was probably one of the best years I've ever had. It was just great. And when I came back, I had a job.

You mentioned that Australia has unusual animals. What was the most unusual animal on which you found a tick?
I found ticks on snakes. There was a graduate student whose specialty was herpetology, and he would bring in snakes from his collecting. We would examine them and collect the ticks. You know, the herpetology people, they get kind of casual with snakes [*laughs*] and these big stumpy-tailed lizards. He would have them in a cloth sack. These herpetologists would go out with these sacks looped in their belt and [*slaps his hand against his leg*] that was a dumb thing because they [the snakes] could bite right through the bag. But anyway, Peter [the herpetologist] would come in if I wasn't there, open the office door and throw that sack in the office. And I'd come in and see that sack moving and I know there's a snake in there, but I don't know what [species] it is. In Australia, unlike the U.S., most of the snakes there are *poisonous*! I wasn't about to go in there until I got some information. He had tiger snakes.

Tiger snakes are highly venomous.
Yes! And very aggressive. You dump some [snakes] on the concrete, and most of the snakes here [in Georgia] wiggle around and go somewhere else. But there, tiger snakes would come toward you. That was a *bad* sign! [*Laughs.*]

He never threw a bag of tiger snakes into your office, did he?
Yeah! Whatever he caught, he put in there. All kinds of reptiles that we would screen for ticks.

You left Australia and came back to University of California at Berkeley.
Correct.

And had a career there.
Correct. I loved Berkeley.

What was the appeal of Berkeley?
A continuation of my education—academically, of course. But more than that, probably it was the cultural aspect. It was across the bay from San Francisco. It was a good experience because I had spent a year in Australia and I had known a lot of people and it made all kinds of bridges possible. And the climate at Berkeley at that time was just…[well] the attitude, I couldn't ask for a more exciting place. A little too much! [*Laughs.*]

Why was it a little too exciting at times?
Well, I hired one of these students as a technician and she was *incredibly* bright and spoke like five languages. She would study the foreign literature and read it in the original [language]. But socially, she was rebelling. One Monday morning she says, "Dr. Oliver, I've had the most interesting experience, and we're going to have another one." It was really a wild place in Berkeley at that time. And she says, "We're to have a photographer—the *Look* photographer." That's a magazine. "And they're going to interview us at our party."

Yes, I remember* Look *magazine.
And I says, "Well, what are you going to do at the party?" She says, "A lot of people think we go in and dance naked and that sort of stuff." And I said, "Sounds good to me!" [*Laughs.*] But she says, "You don't have to take off your clothes at the party if you don't want to, you are welcome to join us and disrobe in this room over here, or you can just keep your clothes on." And I said, "Then what?" "Well, we sing songs, and play guitars, and dance." I said, "Dancing! That could lead to all kinds of things." She said, "It's up to the individual. And this next Saturday, the *Look* photographer is going to be there." And I said, "Nooooo! I'm *not* going. Can you imagine my mother in Georgia and seeing her son? [*Laughs.*] No, no, no, no, no! I definitely won't be there if the *Look* photographer is there." So it was that kind of environment. It was just unbelievable. I had never seen such free-wheeling [behavior]. You just wouldn't want to be at that party. It was one of the reasons why I came back to the East, because we had two kids. And I didn't want to imprint them with these things as [being] okay. You have to take them out of that environment.

So you left Berkeley and came back to…
University of Georgia as an associate professor. Three of us at Berkeley were hired to come to University of Georgia because they had received a big NSF [National Science Foundation] grant to make good universities great universities. That was the aim. One of us was in botany, and I was in entomology, and we were given nice raises and the option of bringing graduate students we had at Berkeley. And I brought one of my doctoral students—Ziad Al-Ahmadi—from Syria. So he drove my Volkswagen bus across country.

If you lived at Berkeley, you had to have a Volkswagen bus; it was almost a requirement.
That's exactly right [*laughs*]—with stickers all over it.

You had stickers on your bus?
Yeah! Oh, they were anything: political causes, sexual freedom, and flowers, too. It was typical impressions you would have of the Berkeley graduate student in the '60s.

What color was this van?
Green with a little white. I bought it brand new. We had moved from Berkeley to Athens (Georgia) and I had Sue and two boys, and a big German shepherd.

You came back to University of Georgia, but you didn't stay there. Shortly, you went to Georgia Southern University.
I was in Athens one year, and the dean I interviewed with in Athens moved to be president at Georgia Southern. He calls me from Statesboro and says, "It would be good, Jim, if you could come down to Statesboro. There's a new program called the Callaway Foundation where the Callaway family is giving money to establish [distinguished] professors on most of the state colleges." My friends, when I left Berkeley to come to Georgia, thought I'd lost my mind [*laughs*] and now they are going to be further surprised when I go on to a smaller school in the state. So I said, "I'm going to need a lot of things." "Like what?" "Oh, you know, like money, technician, support and that sort of stuff." And he told me to list them and we'll see what we can do. During that year, we talked by phone quite a bit, and finally we agreed I'd be a Callaway Professor here at Georgia Southern, which would be the first Callaway Professor in the state. He said, "It would be a natural because you are a graduate of Georgia Southern." He had a brilliant mind, 'cause it would be

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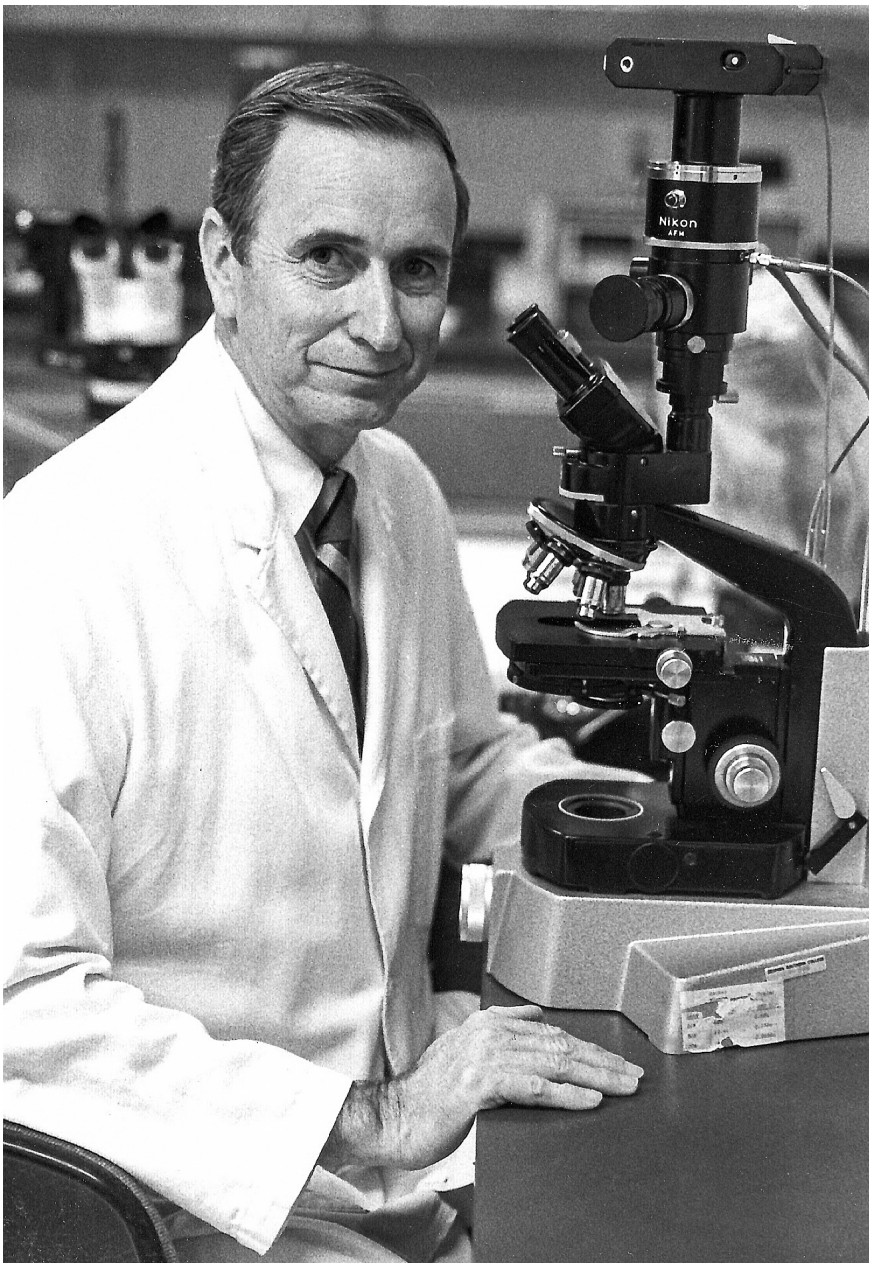

James Oliver in his laboratory, 1982.

good for students to see someone that had been a student there to come back and we can do a lot for each other. And I says, "You're right." So we were only at Athens one year, and that's how I ended up as the first Callaway Professor at Georgia Southern.

This distinguished professorship, did they give you a bump in salary?
Oh, yeah! A big bump. The first year I came down here, I had the assurance of the president that I would be supported, and I applied for three grants: National Science Foundation, NIH, and another was an Air Force thing. I got all three! And all the proposals were overlapping, so I couldn't accept all of them. You just can't imagine how, all of a sudden, I had three national competitive grants. So I didn't have to worry about support, and I stayed with the NIH grant.

And you got a technician and lab support. It sounds like a sweet deal.
It was. And I left the most prestigious place at Berkeley to go to [Georgia]. Harry Hoogstraal—you probably don't know him?

Yes, I know of him. He was a tick person working in Africa.
He worked for the U.S. Navy civil service and lived in Cairo, Egypt. Yeah, [we were] good friends. He was the one that told Sue, "Don't let Jim go to Georgia." In the meantime, I had gone to Cairo for a year with the family, and that was during the Israeli Six-Day War, which was an interesting experience.

You took a year and went to Australia and then a year and went to Egypt.
Jim: Life was sweet.

Sue: It was exceptional. [*Laughs*.] The whole time we were in Egypt, [President Abdel] Nasser would be giving three-hour evening talks about America and how awful we were supporting Israel. It was saber-rattling all the time, but the people in Egypt were fine with us, and I never felt in danger. And all of a sudden, the French withdrew all of their citizens and embassy people. And then the English did it; they're usually the last ones out, [but] the Americans were the last to leave. And the whole time, I'm thinking, "This is nonsense, the Egyptians aren't ready for any kind of military anything." I thought somebody was overreacting, but I have to give credit to the American Embassy; once they decided, all women and children were out of Egypt within two days. And we left our husbands behind, and at the airport, the women just went crazy, crying and screaming, and clutching at their husbands, so here I am, standing with two children—four and eight.

Are these Egyptian women?
Sue: No! American women. They just lost it. One woman started it and the rest just followed along. I wanted to smack some of them. "Get control—there are children here!" They were really scaring the children. We were leaving Jim behind in a very uncertain state and, according to these women, never to be seen again. So the trip home was completely a nightmare. I went and stayed with my parents in New Jersey for two weeks and then went back to Berkeley. Jim didn't know what was going to happen to him. He was there doing research, so the [U.S.] military said, "Why don't you go to Addis Ababa [Ethiopia]"—where there was NAMRU 2 [Naval Medical Research Unit 2]—"and do some work there until this all blows over?" So he closed up everything and got on the plane. About an hour out of Cairo airport, the pilot came on and said, "Ladies and gentlemen, we were the last plane

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out. The Israelis have bombed the airport and [destroyed] all planes on the field."

This was the day that Jim left Cairo?

Sue: Yes! He was up in the air and everyone on the plane realized they would have been at the airport if they had gone any later. They left at night to go to Addis Ababa. He already knew someone in Addis; a roommate he had at Johns Hopkins. He was an Ethiopian national and was director at the Haile Selassie Imperial Lab. And his name was Asseffa Tekla. Jim was very well taken care of in the Tekla home and had a wonderful time, but back here in the States, we had no idea where he was! We thought he was still in Cairo having a horrible time under house arrest. We didn't have a cellphone and we didn't have computers. So he sent me a cablegram and it took three to four weeks to get to me. In the meantime, they realized he wasn't going to get back to Cairo, so they said, "Why don't you go to Uganda?" The Navy was going to have a collection trip and they put him on as an acarologist, so he did some collecting for the Armed Services. Again, being in academics, you have friends everywhere. In Uganda, he stayed at the Rockefeller Institute with John and Evie Nichols, who had been at Berkeley, and he had a very nice time. So after that he went to Kenya and working at ICIPE for a couple for weeks, and they all decided there was no going back to NAMRU [in Cairo] for any foreseeable time, so he came home, back to Berkeley. But it was an exciting time.

So basically you were apart for two months, not knowing exactly his location or condition, while he's hopscotching around East Africa.

Sue: [*Laughs.*] Having a great time with all his colleagues, but honestly, we had no idea where he was until I got the cablegram. I thoroughly enjoyed our times abroad, but there were some hairy situations.

You mentioned you knew Harry Hoogstraal and were good friends. I read that you probably know everybody in the world that works on ticks. That has to be a special fraternity.

Black-legged tick

Jim: It is, and I have been so blessed. A month ago on a Sunday afternoon, I get this phone call, and it's one of my Japanese colleagues and he wants me to come to Japan. I love Japan, and it would be a pleasure to go back for a visit. But I've broken my hip and I'd be a burden.

Let's talk about Lyme disease. In the book* Cure Unknown—Inside the Lyme Epidemic, *author Pamela Weintraub described you as a "world-class entomologist" for piecing together the puzzle that is Southern Lyme disease. Give me the story.

The whole thing is fascinating about Southern Lyme disease; it really is very different, but the clinical [symptoms] are the same. There is a rash, disorientation, and that sort of stuff, but the vectors, that is the important thing. I had a hard time convincing people that we had Lyme disease in the South. A great friend at Harvard had described a variation on *Ixodes scapularis* as a different species.

Ixodes dammini*?*

Yep, and I told him I'm not sure it's a separate species, because if you take the characteristics and go north to south, you'll find a gradation. No, he told me that Harry Hoogstraal thought the one we have here is a separate species. I said, "I disagree with that. I think it's a continuum of the same species that goes up and down the whole coast." But he fell out with me because of that. I had visited with him and had stayed in his home, but all of a sudden, when I disagreed with him, I became an enemy.

I believe you published some research, which showed that the southern* scapularis *would breed with the northern* dammini*, and* scapularis *had taxonomic priority, so* dammini *became a synonym.

That's right, that's right. You can find strains of each that are variable, but if you study the whole range of it and the variations, a lot of these quote "species" are not biologically different.

Is the black-legged tick,* Ixodes scapularis*, the only species known to transmit Lyme disease?

No, not really. It's the major one, but it's still not decided by a lot of physicians. They were impressed by the idea. There are sociological reasons why Lyme disease is reported more in the Northeast than the South: availability of physicians, people differences, tick variation. They still argue with me, and some people at CDC [Centers for Disease Control] keep pushing [against my research].

What other species potentially vector Lyme?

From a very natural standpoint, that's the main one, but from a breeding standpoint, you can get other species to transmit it.

Such as?

The lone star tick is one of the candidates, but it's still a question.

As a tick guy, have you ever contracted a tick-borne pathogen?

I don't know! [*Laughs.*] A lot of tick pathogens are rickettsia and rickettsial diseases. Every time I go to my local physician and I have some strange illness that they don't know what it is, he says it is probably a rickettsia—an idiopathic condition. I've tested negatively for rickettsia, but the symptoms [suggest it] could be.

Have you ever gotten into a challenging situation when you traveled internationally?

I've got a good story about being robbed—mugged twice in the same day! [*Laughs.*]

Where were you when you got mugged?

Jim: In South Africa. I'll let Sue tell it.

Sue: We were in South Africa for a month,

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and Jim was the keynote speaker [at a symposium] in Kruger [National Park]. We arrived in Jo'burg and went to Pretoria. The next day we're out and walking toward a bus stop in front of the parliament buildings. It was about noontime, and everyone goes home on a Saturday. All the stores are closed and all the whites are gone, but the blacks are still out. Then, all of a sudden a guy comes along—*bam!*—and hits Jim and I thought he was running for the bus. And I realized, no he's not, because Jim is fighting and they are rolling around on the ground. Jim was a fighter; he was a boxer. This guy was a young man, and I guess we are in our 70s. So [Jim's] first reaction was *whop! whop!* The boy was stunned. He never expected this skinny old guy to *fight* back! He was after the wallet and easy pickings, [but] had a hard time getting it because Jim was fighting like mad. I'm screaming and kicking and pulling on the boy's hair. But he was much bigger than Jim and got the wallet. And he gets up and Jim says, "Oh no you don't!" And Jim grabs his leg and down they go again. Finally the boy gets up and starts running. Jim says, "I'm getting that wallet!" So there he goes. I'm standing there, looking like a tourist, and I'm thinking this isn't the place for me. [*Laughs*.] So I was running—it's a wide-open racecourse: there's the bad boy, and there's Jim, and there's me in the back chugging along screaming at the top of my lungs. [Then] this black kid comes up and goes straight to Jim and I thought, "Oh no, it's an accomplice!" Jim thought the same thing because he had his fists up, but it was an undercover policeman. He said, "I can catch him. What does he have?" "He has my wallet." He says, "Okay." And off he goes. He could have been in the Olympics. He took off like he had a motor. So then it's bad boy, cop, Jim, and way behind, chugging along, is Sue. We ran a couple of blocks and the cop got him. Then a Jeep-thing police vehicle came up and out steps this big white Afrikaner, with a moustache, and I mean he was *big*. He got that bad boy and he's shaking him and he got the wallet and handed it to Jim. He says, "I want you to come down to the headquarters tomorrow and file a complaint." We said, "We can't, we are going to Kruger." He says, "Well then, we can't do anything." So he grabs this boy and he says something very harsh in Afrikaans and he takes this ham-sized hand and whops him on the side of the head and the kid goes rolling, then he took off and ran. This Afrikaner turns and says, "If I were you, I'd go back to my hotel." "We're on our way!" [*Laughs*.] We're walking along and kinda' thinking we were lucky: we got the money back, we're not hurt, and nobody had a gun or a knife. Then Jim says, "Watch it!" And *another* one hits us! Not ten minutes after we left the cop. Jim is in a clutch with the guy and pounding on him. And again the kid looks *stunned*, like what's this old guy doing? [*Laughs*.] And every time he came around I kicked him and I'm yelling and screaming because there are cars going by—it is a major street. A car came up and two white men got out and that boy saw that and he thought, I'm out numbered, so he gave Jim a major push [and ran]. But nobody got our money, but we were mugged *twice* in less than an hour in day two of a 30-day stay. Oh, the Afrikaner [hosts] were so embarrassed for us.

Lone star tick

A fun story. On a more serious note, what do you consider to be one of your most significant contributions to the science?
Whoa! I don't know. Let's defer that.

Well, how about your legacy?
Let's defer that one too.

One more question then: is there such a thing as a good tick?
Yeah, when it supports my work! [*Laughs*.]

[Oliver asked to provide a written answer to the contribution and legacy questions; his response was provided a month later.] ***What do you consider to be your most significant contributions to science?***
The blending of entomology and parasitology in addressing fundamental questions in biology. More specifically, my work on sex determination and chromosomes in mites and ticks, and the demonstration of the widespread occurrence and importance of haplodiploidy among mites. Also, the occurrence of several types of sex chromosomes, i.e. X-O, X-Y, and multiple sex chromosomes, and their widespread presence in different taxonomic groups were very important. Later in my research, and perhaps more of a practical interest, was the recognition and demonstration of Lyme disease in the southern U.S. It was not only important in the northeastern U.S.; in fact, other researchers have shown it to be important in the western U.S., and now it is known to occur worldwide. Interestingly, Lyme disease is very complex and shown to be caused by several species of spirochetes, and transmitted by several species of ticks. In my lab, we continue to be involved in research dealing with various genetic strains of spirochetes; some are pathogenic while others may not cause Lyme disease.

Acknowledgments

Thanks to Sue Oliver for her contributions to this interview, which was edited for length and clarity. Joel Hutcheson helped in locating photographs of the lone star tick and black-legged tick, which are provided courtesy of Centers for Disease Control and Prevention.

Additional Reading

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Marlin E. Rice is a past President of the Entomological Society of America. Growing up in rural Missouri, he encountered hundreds of ticks in the forests and fields, and never met one he liked.

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CDC blocked reporting for lack of cultures

Lyme and/or Lyme-like Disease in Missouri

Edwin J. Masters, MD and H. Denny Donnell, MD, MPH

Missouri patients who fulfill the strict CDC surveillance definition for Lyme disease have been reported in significant numbers since 1989, although there are no viable Missouri human cultures of *Borrelia burgdorferi*. The Missouri erythema migrans rashes are indistinguishable from those in other areas, and the clinical syndrome appears similar to Lyme disease nationally. The authors suspect atypical *B. burgdorferi*, and/or other *Borrelia* spirochetes of causing this clinical borreliosis syndrome.

Missouri patients who fulfill the strict CDC surveillance definition for Lyme disease have been reported in significant numbers since 1989. The etiology has been unclear and confusing. This enigma was previously addressed in this journal in 1992.[1]

The clinical syndrome is better defined, but the exact etiologies are unproven. We participated with the Centers for Disease Control and Prevention (CDC) on an epidemiologic and diagnostic study of 45 Missouri patients with physician diagnosed erythema migrans (EM) (considered a diagnostic marker for Lyme disease). This study will be published in the August issue of the *Journal of Infectious Diseases*.[2] The CDC conclusion was that the etiology remains idiopathic, but that evidence implicating *Borrelia burgdorferi* is absent. The study design, exclusion of available information, decisions excluding data relevant to the objective evaluation of the problem, and arbitrary authorship were such that we, as the state epidemiologist who initiated the study and the primary clinician who supplied over half of the study patients, took the significant step of declining authorship. We believe that additional information should have been made available.

Visually, Missouri EM rashes are indistinguishable from those associated with Lyme disease elsewhere. Many authors consider erythema migrans pathognomonic for Lyme disease.[3-14] Photographs of Missouri EM rashes have been published in peer reviewed journals[15-17] and have been presented at the last three international conferences on Lyme Borreliosis (Stockholm, 1990;[18] Arlington, VA 1992;[19] Bologna, Italy 1994[20]). Missouri case data show an EM incidence with a summer peak, and rash location on the body, histology, treatment response, incubation time, tick exposure, age, gender, multiple lesions, associated signs and symptoms, and sequelae are all similar to Lyme disease reported nationally. Thus, clinically, the Missouri physician cannot distinguish this rash and syndrome from Lyme disease diagnosed elsewhere. If Lyme disease is a clinical diagnosis, as the world's literature and the CDC state,[21] then this is clinical Lyme disease.

Atypical isolates of *B. burgdorferi* have been cultured from *Ixodes dentatus* ticks in Southeast Missouri.[22] Live motile spirochetes, similar to *Borrelia*, have been visualized in lone star ticks (*Amblyomma americanum*) from the homesites of Missouri EM patients. Further intrigue is added by the growing evidence that some *A. americanum* (lone star ticks) are infected with a very different and as-yet - unidentified spirochete that may be a completely new species of *Borrelia*.[2] One then ponders the question whether this syndrome is caused by a new species of *Borrelia* that is not *B. burgdorferi*; is it, or is it not, Lyme disease if it cannot be distinguished clinically? There are arguments on both sides, depending on whether one makes the diagnosis microbiologically or clinically. For example, the putative etiologic agent for cat-scratch disease has changed from *Afipia felis* to *Bartonella henselae*, and yet the clinical diagnosis remains the same. To use a *Borrelia* analogy, there are numerous *Borrelia* species that cause relapsing fever, both tick borne and louse borne, and yet the diagnosis of "relapsing fever" remained. The issue of a possible different *Borrelia* bacteria in lone star ticks is significant. What is different about Missouri EM patients is that a minority accurately describe the lone star tick as being associated with their clinical Lyme disease. This has also been reported in other states, but the extent to which this may occur is unknown.[23]

Case Report

Case illustration (CDC #6, ID #111): This case illustrates why a borreliosis is suspected in Missouri. A 41-year-old white man presented on July 5, 1991 with an EM rash (Fig 1) which was painless, non-pruritic and with documented expansion. There was no history of recent tick exposure outside Missouri.

Laboratory findings: A biopsy was taken and he was enrolled in a national study. The EKG on July 5, 1991 was normal. An EM rash biopsy photo (Fig 3) shows histology consistent with erythema migrans, and (Fig 2) shows a modified Dieterle stain of the EM biopsy with a dermal spirochete visualized by Dr. Paul Duray of Harvard.

On July 12, 1991 his electrocardiogram showed atrial fibrillation which resolved spontaneously. All subsequent EKG's with over a three year follow up have been normal. Blood Pressure = 112/78; Cholesterol = 165; Glucose = 102; T4 = 7.6; serum ferritin < 200, normal weight, no personal or family history of heart disease and normal echocardiogram. His only medication was amoxicillin 500 mg TID.

Other tests include a positive Lyme ELISA by Dr. R.J. Johnson, of the University of Minnesota, negative RA, negative ANA, negative RPR, and a positive biopsy by Dr. Paul Duray of Harvard (now at the NIH).

Table 1 - Testing by CDC

CDC Case #6	1991 WCS +>1.0 (3SD)	1991 FLA +>1.0 (3SD)	1992 FLA +>1.0 (3SD)	1993 FLA +>1.0 (3SD)	IgM Western Blot	IgG Western Blot
Day 8	2.278	1.394	1.022	0.81 (equiv)	41	25, 28, 30,41, 57, 60, 62
Day 36	2.260	1.354	1.025	0.82 (equiv)	66	15, 25, 28, 34, 41, 56, 62

Discussion

Eight ELISAs were done by the CDC and none was negative (Table 1). The Western blots were indeed negative by Dressler's criteria,[24] but positive by other published criteria.[25-27] We disagree with the CDC and do not believe this case constitutes "absence of evidence" of serological reactivity to *B. burgdorferi* or a related spirochete. Furthermore, not only were positive CDC FLA ELISA results omitted from the JID manuscript,[2] diagnostically significant less intense Western blot bands by the CDC's own research data[28] were ignored. Knowing that atypical *B. burgdorferi* or "related spirochetes" might very likely produce a response such as this, we disagree with the CDC conclusion of "no evidence." We also know that "most patients with early disease have a good IgG response, and some reinfected patients may have only an IgG response."[29]

Considerable information consisting of test results and information, histology results, and complications were all excluded in the CDC analysis. Very few pathologists have published on the histopathology of Lyme disease. Four of these published experts have reviewed Missouri EM cases and all four (Dr. Duray, Harvard; Dr. DeKoning, Netherlands; Dr. S. Granter, Harvard; and Dr. A. MacDonald of Beaumont, TX) agree that both histology consistent with Lyme erythema migrans and spirochetes which appear similar to *Borrelia* are present in Missouri rash biopsies.

The incubation time of the Missouri EM rashes is important because of the etiological implications. On a questionnaire of 20 Missouri patients in the CDC study, the incubation time differed from that contemporaneously recorded in the patient's charts (3 days vs. 5.5 days).[2] Based on that, one might improperly conclude that the chart data were inaccurate. However, the chart data were entered before the CDC study was even begun. Additionally, the differences were due to discrepancies in only four patients where there were long delays of up to a year between the rash and the questionnaire. The chart data were also verifiable by third parties. Furthermore, 10 of these 20 CDC study patients were a subset of 25 consecutive Missouri erythema migrans patients that were included in a national study. They were documented exhaustively long before the CDC study began. The median incubation time for that larger series of 25 patients was seven days, exactly what one would expect. In 1993, the Missouri Department of Health collected data on 30 other consecutive erythema migrans patients from all over the state and the median incubation time was 6 days. The published incubation times usually vary from 2-30 days with a median of 6 or 7 days. Another series of 14 EM rashes following witnessed lone star tick bites (including three of the CDC study patients) had a median incubation time of 7 days.[30] Although the short 3 day time on the CDC questionnaire might support a suggestion for hypersensitivity reaction or other noninfectious cause of these rashes, we believe that data to be inferior, incomplete, and contradicted. Also, the mean duration of the EM rashes following known tick bites in the CDC study was 14 days, arguing against a hypersensitivity reaction as does the median duration of rash expansion of seven days reported by 31 CDC study cases.

There was no correlation between documented Missouri rash size and reported duration. However, with the known impact of treatment on EM rashes and the considerable variation in antibiotic therapy the lack of correlation is not surprising.

We also disagreed with the arbitrary exclusion of available histopathology evaluations of biopsies on EM study patients. These biopsies clearly exclude such entities as granuloma annulare, etc. Obviously, there are probably some instances where a hypersensitivity reaction could be mistaken for an erythema migrans, but that is a completely inadequate explanation for the phenomenon that is being reported in Missouri. Many have had tick bites all their lives and have never had a similar rash. Also many have been followed for several years since their erythema migrans rashes, have had further tick bites, and have had no further rashes. The vast majority of these clinically diagnosed erythema migrans simply cannot be explained as hypersensitivity reactions or toxic phenomenon, based on clinical data, histopathological examinations, clinical history, and follow up.

The absence of a viable human *B. burgdorferi* culture in Missouri is significant. However, absence of proof is not proof of absence and with an organism such as this where increasing heterogeneity is being found, we do not know for sure that atypical variants do not have some atypical growth requirements. This phenomenon exists in other areas and has been reported in Great Britain[31] and also in the northeastern U.S.[32] Also, at least one uncultivable (at least by present methods) organism that may be a *Borrelia* probably exists in lone star ticks.[2] We caution against using the criteria of failure to grow in a highly defined specific medium such as BSK-II as proof of nonexistence. Even

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Table 2.

Results of tests for antibodies to *Borrelia burgdorferi* in serum from Missouri patients with suspected erythema migrans (all patients of Dr. Masters) by enzyme immunoassay (EIA) using whole-cell sonicated (WCS) antigens and flagellar (FLA) antigens and immunoblots performed by the CDC. Bold print indicates strong bands.

CDC Case #	Samp. #	Pt ID#	Days After Rash	1991 WCS +=>1.0 (3SD)	1991 FLA +=>1.0 (3SD)	1992 FLA +=>1.0 (3SD)	1993 FLA	IgM Western blot	IgG Western blot
17	1355	125	37	0.38	0.575	0.192	0.16	39,66,75	ND
11	1358	136a	27	0.552	0.443	0.375	0.23		35, 41, 52, 60, 75
11	1359	136b	100	0.677	0.689	0.204	0.18		15, 25, 37, 41, 45, 60, 62, 75
18	1360	119	16	0.38	0.396	0.257	0.29	41	**29, 41, 45, 57, 62,75**
1	1362	116a	3	1.432	0.519	0.591	0.55	41	**28, 37, 39, 41, 45, 58, 66**
1	1363	116b	34	1.690	0.434	0.678	0.51	ND	**15, 39, 41, 47, 56, 58**
1	1364	116c	97	1.425	0.721	0.638	0.54	66,75	**15, 39, 41, 47, 56, 58**
3	1371	121a	5	0.811	0.434	0.731	0.15		25, 41, 45, 57, 62
3	1372	121b	46	1.116	0.387	0.700	0.21	31, 45, 58	**15, 25, 29, 41, 45, 57, 62**
12	1374	137a	22	0.953	0.575	0.449	0.73	41	25, 34, 41, 56, 66, 75
12	1375	137b	119	0.833	0.415	0.344	0.54		15, 42
19	1380	138	54	0.507	0.179	0.272	0.18	41, 60, 66, 75	41, 62, 68
13	1382	103a	30	1.118	0.472	0.378	0.37	41	30, 41
13	1383	103b	58	1.082	0.557	0.344	0.41	66, 75	15, 29, 41
4	1385	145a	19	0.398	0.274	0.214	0.23	62, 63, 64	21, 34, 37, 41, 60, 62
4	1386	145b	38	0.626	0.358	0.307	0.19	39, 63, 64, 75, 83	15, 34, 41, 60, 62
5	1388	105a	4	0.686	0.538	0.295	0.21		29, 41, 62
5	1389	105b	32	0.809	0.528	0.283	0.27	41	29, 41, 62
14	1397	117a	31	1.846	0.792	0.488	0.39	41, 66	34, 41, 62, 66, 75, 83
14	1398	117b	92	1.836	0.623	0.519	0.31	37, 39, 41, 58, 66	15, 21, 34, 41, 62, 78, 83
2	1404	118a	3	2.694	0.772	0.234	0.22	41	18, 29, 37, 41, 45, 57, 66, 75
2	1405	118b	35	1.931	0.668	0.301	0.28	41	18, 37, 41, 45, 57, 66, 75
2	1406	118c	185	0.900	0.435	0.193	0.25	34	15, 37, 41, 45, 57, 66, 75, 83
6	1407	111a	8	2.278	1.394	1.022	0.81	41	**25, 28, 36, 41, 57, 60, 62**
6	1408	111b	36	2.260	1.354	1.025	0.82	66	**15, 25, 28,** 34, 37, 41, 56, 62
20	1410	131	99	0.364	0.292	0.481	0.13	25, 37, 66	29, 41, 62, 83
7	1411	132a	3	0.318	0.340	0.280	0.3	41, 75	15, 18, 41, 75, 83
7	1412	132b	31	0.440	0.321	0.295	0.21	79	41,75,83
8	1415	140a	3	0.835	0.406	0.314	0.35	25, 41, 83	15, 28, 29, 36, 39, 41, 62
8	1416	140b	94	1.096	0.415	0.197	0.18	25, 37, 60, 66, 83	15, 28, 29, 37, 41, 60, 62
15	1418	141a	1	1.207	0.785	0.386	0.23	41	28, 30, 35, 41, 45, 62
15	1419	141b	24	0.587	0.425	0.380	ND	41	30, 35, 36, 41, 45, 62
15	1420	141c	176	0.716	0.443	0.324	0.27	41, 58, 60, 66, 83	15, 29, 30, 41, 62
21	1424	133	94	1.606	1.087	0.693	0.64	83	41
9	1425	113a	3	0.452	0.106	0.130	0.13	41	15, 25, 34, 41, 57
9	1426	113b	33	0.782	0.821	0.546	0.29	41	15, 25, 28, 34, 41, 62
16	1428	120a	25	1.338	0.577	0.507	0.79	25, 31, 39, 41	15, 25, 41, 55, 62, 75
16	1429	120b	97	0.700	0.481	0.423	0.32	15, 25, 31, 37, 39, 41	28, 41, 62, 66, 75, 83
10	1431	114a	8	2.185	0.358	0.394	0.39	41	15, 41, 60
10	1432	114b	39	2.636	0.472	0.208	0.25	28, 37, 39, 41	15, 25, 41, 60
22	1433	127	76	0.552	0.679	0.261	0.10	ND	ND

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Dr. Kelly, when originally cultivating relapsing fever spirochetes, had to use different media for different species.[21] The syphilis spirochete has still not been cultivated in a cell free media.[22]

The CDC concluded that in the Missouri study of 22 patients tested there was no serologic evidence of a *B. burgdorferi* or related spirochetal infection.[3] All 22 were patients of Dr. Masters and the results are presented in Table 2. We disagreed with the CDC's negative conclusion and present here data that was excluded from the CDC manuscript. There were 45 patients in the CDC study and 40 of these patients had no less than 57 different positive Lyme serologies performed by 7 different laboratories.

Even though the CDC itself has researched and presented on the diagnostic utility of using faint or less intense Western blot bands for diagnosing Lyme disease,[18] as have other authors,[23] these were specifically excluded in this Missouri study. We know that atypical *B. burgdorferi* exist in Missouri[22] and that Zoller et al published that strain dependent differences of Western blot band intensities exist.[23] Additionally, there are numerous publications which have included faint bands. Engstrom et al. showed that most less intense bands should be counted.[25] Obviously, band intensity is not only a subjective interpretation by the laboratory, it can also be a function of technique and storage. The Missouri Western blots were performed by the CDC on stored sera that had gone through multiple freeze/thaw cycles. Also, in the Engstrom paper,[25] they could not match a number of their protein bands with those described by Dressler and thus there is published concern about the arbitrary use of such a rigid Western blot interpretation. We also have questions about the arbitrary decisions on IgM band specificity, as well as exclusion of OspA (31kDa) and OspB (34kDa) from the diagnostic criteria, which is contrary to all previously published literature.[24]

The gravamen of the CDC analysis is their assumption that their FLA ELISA is accurate in its negative results and their WCS ELISA is wrong in its positive results in detecting *B. burgdorferi* or a related spirochete. If one were biased toward a negative conclusion as we believe the CDC authors were, then the sensitivity of relatively comparable tests would take precedence. The CDC admits the ELISA test with Whole Cell Sonicate (WCS) is more sensitive.[2,12,15]

We believe that the differences should be examined and compared not only with the positive, but also with the negative control population. Of the 22 Missouri EM patients tested serologically by the CDC, 11 of the 22 had discordant results by having positive WCS ELISAs, using a three standard deviation cutoff for positive. The CDC did not mention that 38 non-Lyme Missouri controls were also tested by the WCS ELISA method and that only one of the 38 tested positive, as compared to 50% of the Missouri EM rash patients. The odds of this result occurring by chance exceed 25,000,000 to one. Missouri controls were not tested with the CDC FLA ELISA. Furthermore, if one takes the 21 Missouri patients who had both IgM and IgG Western blots and compares them to published studies in which faint bands were included, there are some interesting results. Using the same criteria, the Western blots on these 21 Missouri patients performed by the CDC were compared by us to the Ma study controls.[26] (Table 3) Of the major published *Borrelia* associated Western blot bands such as 20kDa, 31kDa, 34kDa, 39kDa, 66kDa, and 83kDa, the p values in the Missouri EM patients were significantly higher than the Ma study controls ($p < .002$) in every instance. Fawcet et al published a series of 200 non-Lyme controls in 1992 and of their Western blots, only one patient of 200 controls had four IgG bands.[26] As shown in Table 2, the vast majority of these Missouri EM patients had four or more IgG bands. These Missouri EM patients have Lyme serologies meaningfully different from published negative control populations.

Table 3. IgM & IgG Lyme Disease Western blot Bands

A comparison of 21 Missouri EM patients' Borrelia associated bands when tested by the CDC with published normal non-Lyme controls.

kDa	Missouri EM #	21 pts. %	Ma Controls* #	320 pts. %	p
20	2	9.5	2	0.6	< .001
31	3	14	7	2.2	< .002
34	6	28	8	2.5	< .001
39	7	33	4	1.3	< .001
41	21	100	138	43	< .001
66	11	52	42	13	< .001
83	8	38	4	1.3	< .001

* Ma et al. Serodiagnosis of Lyme borreliosis by Western Immunoblot: Reactivity of Various Significant Antibodies against *Borrelia burgdorferi*. *Journal of Clinical Microbiology*, February 1992, p. 370-6.

Analysis of the 11 CDC study patients with positive WCS ELISAs and negative or equivocal flagellar (FLA) ELISAs is also significant. The CDC had previously stated their WCS and FLA ELISAs were highly correlated with an $r > .90$.[24] We believe this needs to be explained. All 11 patients had a negative rapid plasma reagin (RPR), rheumatoid arthritis (RA), and antinuclear antibody (ANA) to help rule out cross-reactivity. All tested negative for antibodies to *Francisella tularensis*, *Rickettsia typhi*, *Rickettsia rickettsii*, arboviruses, and *Ehrlichia chafeensis*. One out of 10 patients tested positive to *Q fever (Coxiella burnetii)*. Two patients also tested positive to a CDC 1991 Lyme FLA ELISA. As mentioned, when compared with published controls patients[26] the p value for Western blot *Borrelia* associated bands (20kDa, 31kDa, 34kDa, 39kDa, and 83kDa) was .002 or less. Nine of these 11 patients had four or more bands on at least one Western blot performed by the CDC and eight of the eleven had five or more bands. The CDC itself has presented research that indicates that the presence of five IgG bands, even faint, have a high correlation with Lyme disease. [18] Eight of these 11 patients were also part of a national prospective study. Lyme serologies were done by Dr. R.J. Johnson of the University of Minnesota and 2 Standard Deviations (SD) was considered borderline and 3 SD positive. All eight (100%) of this subgroup had one or more Lyme ELISAs at least two SD from normal with many having strongly positive tests.

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Additionally, the CDC indicated the presence of an 83kDa, 39kDa, 21kDa, or 18kDa band was also highly specific for Lyme arthritis, even when faint.[28] As one can see from Table 2 many of these bands were present in the Missouri EM patients tested by the CDC. Engstrom et al showed that low intensity bands can have diagnostic utility and need not be excluded.[25] Bunikis et al showed that variable reactivity against different Lyme disease *Borrelia* spp. exists and concluded that differences in antigen compositions among *Borrelia* spp. may result in variable immune responses.[37] Knowing that different *Borrelia* spp. may exist and that atypical *B. burgdorferi* definitely exist in Missouri,[21] we believe that all of the available data should be evaluated before negative conclusions are made and published.

We know from Oliver and Kollar's work that *B. burgdorferi* are in Missouri *I. dentatus* ticks that preferentially feed on rabbits (but also occasionally on humans).[22] Bridge vectors should be researched. In Ryder et al's tick transmission study using geographically unmatched spirochetes in *Amblyomma americanum*, they showed a transstadial transmission rate of 1:60 (1.7%) that left open the question of whether lone star ticks could be involved in occasional human cases.[38] One cottontail rabbit in Southeast Missouri at the farm of an EM patient was examined and had *I. dentatus* ticks on it as well as over 1000 lone star larvae.[39] Even at a 1.7% rate, that single rabbit could theoretically be the source of infection for 17 lone star nymphs. Other researchers have seen spirochetes appearing similar to *Borrelia* that stained variably with H5332 monoclonal antibodies for *B. burgdorferi* in this area.[39,40-42] The CDC paper dismisses the positive IFA and PCR results of Feir et al[32] by stating that *B. burgdorferi* failed to be amplified using a second primer pair.[2] Three ticks were tested using the two primers and only one failed to amplify *B. burgdorferi* DNA with the second primer. The ability to amplify *B. burgdorferi* DNA using one set of primers while failing using a different set of primers is not uncommon and can possibly be attributed to strain variation. For example, Oliver et al (1994) found in one *B. burgdorferi* isolate from Missouri, that FLA DNA was amplified while OspA DNA was not using a second primer pair.[22] In the Feir et al study, PCR positivity was significantly associated with IFA positivity, and there were no PCR positive tests from IFA negative ticks from areas believed to be free of Lyme disease or from laboratory reared ticks.[32]

Conclusion

In summary, we have presented evidence documenting Missouri patients that have clinical presentations that meet diagnostic and surveillance criteria for Lyme disease and that cannot be easily explained in the absence of a borreliosis. Previously, some believed that because Missouri did not have the *Ixodes dammini* deer tick, Lyme disease was not possible. We now know that *I. dammini* is not a valid separate tick species but is the same as *I. scapularis*, which is common in Missouri.[43] Missouri EM patients often have histological and serological results that are clearly abnormal and are consistent with a borreliosis.

We also believe that this illness can have marked sequelae. In the CDC study of 45 patients treated early,[2] there were still two cases of associated arthritis with visibly swollen joints and one carditis. Also, there were at least two cases of marked non-specific symptoms such as fatigue, impaired cognition, myalgias, etc. Three of these patients, including the patient with carditis at three weeks, one with arthritis at 2 1/2 months, and another with arthritis at 4 1/2 months were not reported by the CDC because of the study design.

We previously published on all 672 Missouri cases reported from 1989-1992 that met the CDC Lyme surveillance definition and compared the signs and symptoms to Lyme disease reported elsewhere. We concluded that Lyme disease reported in Missouri was similar in terms of signs and symptoms to Lyme disease reported nationally.[15] We agree with the CDC that physicians should maintain a high index of suspicion, not only for this syndrome, but for other Missouri tick borne illnesses such as rickettsiosis, tularemia, babesiosis, and ehrlichiosis. We also believe that there are insufficient available data to exclude clinical borreliosis, possibly caused by more than one variety of spirochete. At this time there is no alternative plausible diagnosis and we encourage physicians to comply with the Missouri law requiring reporting of Lyme disease.[44] Even though the exact etiology or etiologies of this clinical syndrome remain unproven, patients who meet the clinical criteria for Lyme disease should be reported. The Missouri Department of Health recognizes the etiological controversy and in order to further understand this illness and resolve the issue of Lyme vs. Lyme-like disease, data are needed and patients need to be evaluated. We have both identified and unidentified spirochetes in Missouri ticks that are biting our patients who then become ill with signs and symptoms that are extremely difficult to explain in the absence of a borreliosis - whether it is caused by *B. burgdorferi*, an atypical *B. burgdorferi*, or some other infectious agent or spirochete. Further research is needed.

Figures on pages 350 and 351

Figure 1 Case Illustration. CDC Case #6, I.D.#111. MO EM rash, right foot, transient atrial fibrillation. **Figure 2** Modified Dieterle stain of EM biopsy in figure 1 showing a dermal spirochete. Photo courtesy of Dr. P. Duray, M.D., Harvard. **Figure 3** Histology of EM biopsy, case in figure 1 & 2, showing a dense lymphoplasmacellular infiltrate consistent with erythema migrans (Photo courtesy of P. Duray, M.D., Harvard). **Figure 4** Missouri EM, Bollinger County; (insert: Steiner-Steiner silver stain showing a dermal spirochete in his EM biopsy). **Figure 5** "Target" lesion characteristic of EM, Scott County. **Figure 6** MO EM after lone star tick bite. **Figure 7** Histology of EM in figure 6. Photo courtesy of P. Duray, M.D., Harvard. Also, on modified Dieterle stain of this biopsy, a dermal spirochete was visualized. **Figure 8** Lone star tick midgut smear showing spirochetes. **Figure 9** DS, CDC Case #21, ID#133 - positive CDC FLA ELISA in 1991, and positive IgM and IgG ELISA by Dr. R.J. Johnson. **Figure 10** Modified Dieterle stain showing a dermal spirochete in CDC case #21, figure 9. Photo courtesy of P. Duray, M.D., Harvard. **Figure 11** CDC Case #1, ID #116 (also seroconverted to Q fever) and seroconverted to Lyme with IgG ELISA by Dr. R.J. Johnson. **Figure 12** K.K. CDC study case. Enlarging rash noticed three weeks after tick bite while turkey hunting in Phelps County. PCR was positive for Lyme disease; **Figure 13** PH CDC Case#5, ID #105. **Figure 14** FN CDC Case #7, ID #132. **Figure 15** BW CDC Case #22, ID#127. **Figure 16** LH CDC Case #4, ID#145. **Figure 17** CP CDC Case #8. ID#140, CDC WCS ELISA seroconversion. **Figure 18** LA CDC Case #18, ID#119. **Figure 19** BT CDC Case #9, ID#113. **Figure 20** CC CDC Case #3, ID#121 (seroconverted on WCS ELISA). **Figure 21** LL CDC Case #2, ID#118 (Western blot seroconversion at North American Laboratories). Also developed a migratory oligoarticular arthritis. **Figures 5, 12, and 15** reprinted with permission of *Postgraduate Medicine*. **Figure 21** reprinted with permission of *Journal of Spirochetal and Tick-borne Diseases*.

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Bacteriophages and Ultrastructural Alterations of *Borrelia Burgdorferi* Induced by Ciprofloxacin

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In a former study, a lysogenic isolate of *Borrelia burgdorferi* harboring two different types of tailed A-1 and B-1 bacteriophages inducible by subinhibitory ciprofloxacin concentrations was described. In the present study, two further *Borrelia burgdorferi* isolates obtained by culture from a nymphal *Ixodes ricinus* tick and from human skin were exposed to increasing concentrations (0.125 to 8 μg/mL) of ciprofloxacin.
The *in vitro* minimal inhibitory concentration (MIC) was determined to be 1 μg/mL by a broth dilution method. In both isolates, belonging to the genospecies *Borrelia burgdorferi, sensu stricto* A-1 bacteriophages were discovered exclusively at subinhibitory concentrations of ciprofloxacin (0.125 to 0.5 μg/mL). After exposure of the isolates to ciprofloxacin concentrations coinciding with or exceeding the MIC, the following alterations of the borrelial ultrastructure became visible: (1) at a ciprofloxacin concentration of 1 μg/mL electron-lucent swollen areas within the protoplasmic cylinder complex of otherwise intact cells as well as very short borrelial cell fragments, (2) at a ciprofloxacin concentration of 2 μg/mL numerous small-membrane defects of the peptidoglycan layer, (3) at ciprofloxacin concentrations of 4 and 8 μg/mL disruption of the protoplasmic cylinder complex into many small particles. These ultrastructural alterations caused by high ciprofloxacin concentrations proved to be clearly different from the features of phage-induced cell lysis found at subinhibitory ciprofloxacin concentrations.

Key words: Borrelia burgdorferi, Ciprofloxacin, Ultrastructure, Bacteriophages, Electronmicroscopy

INTRODUCTION

Recently, we reported on the discovery of two different types of bacteriophages induced by subinhibitory concentrations of ciprofloxacin in a *Borrelia burgdorferi* skin isolate and described the typical phage-induced alterations of the borrelial morphology (1).

Ciprofloxacin is a fluorinated, piperazin-substituted quinolone related to nalidixic acid. By inhibiting the bacterial DNA-gyrase, this drug has a high *in vitro* activity against many gram-positive and gram-negative bacteria (2). In several reports, the ultrastructural alterations of ciprofloxacin treated gram-negative and gram-positive bacteria were described comprehensively (2–4). To our knowledge, there are only two studies dealing with the *in vitro* susceptibility of *Borrelia burgdorferi* to ciprofloxacin. Preac-Mursic et al. reported in 1987 that ciprofloxacin showed only low activity against *Borrelia burgdorferi* (5). Similar results were reported by Levin et al. (6) in 1993. So far, we are not aware of reports concerning ultrastructural alterations of spirochetes caused by ciprofloxacin.

In the present study, we determined the *in vitro* minimum inhibitory concentration (MIC) of ciprofloxacin for two *Borrelia burgdorferi* isolates and examined the morphological alterations of the borrelial cells after treatment with ciprofloxacin concentrations ranging from the MIC of 1 to 8 μg/mL.

Moreover, we examined borrelial cells exposed to subinhibitory concentrations of ciprofloxacin in order to look for the presence of further lysogenic isolates.

MATERIALS AND METHODS

Borrelia burgdorferi isolates

The *Borrelia burgdorferi* skin isolate was obtained by biopsy from an erythema migrans lesion located at the left mamma of a 63-year-old woman. The tick isolate was cultivated from a nymphal tick removed from a patient visiting our out-patient clinic.

Isolation and subcultivation of the borreliae were accomplished in BSK II-medium (7) modified by adding 0.15% agarose (Serva, Fine Biochemicals Inc., Paramus, New Jersey, No. 11397) (8). The two isolates were classified by nondenaturing polyacrylamide gel electrophoresis of RNA complementary to amplified *Borrelia burgdorferi*-specific gene segments (9, 10). Both isolates were found to belong to the genospecies *Borrelia burgdorferi* sensu stricto, according to the *Borrelia burgdorferi* subspecies classification delineated by Baranton et al. (11).

Evaluation of MICs of ciprofloxacin

In vitro susceptibility to ciprofloxacin (Bayer, Leverkusen, No. 521532) was determined via the broth dilution method (5). Here, 100 μL of an actively growing culture (log-phase) containing 10^7 cells/mL were added to tubes with 9.9 mL BSK II-medium, resulting in a final concentration of 10^5 cells/mL. Ciprofloxacin concentrations ranged from 0.125 to 8 μg/mL. Control tubes without antibiotics were inoculated with 100 μL of the log-phase culture. Each concentration was prepared in triplicate. Cultures were examined for the presence of spirochetes by dark-field microscopy after 5 days of incubation at 33°C. The MIC was defined as the lowest concentration of ciprofloxacin completely inhibiting growth, i.e., at which the spirochete count was 10^5 cells/mL or less.

The number of spirochetes was determined by using a Petroff Hausser counting chamber.

Preparation for electron microscopy

Each tube was centrifuged at 4000 × *g* for 20 minutes at 33°C. The resulting pellets were suspended in SMC [0.03% sucrose in redestilled water with 0.01 M $CaCl_2$ and 0.01 M $MgCl_2$ added (12)]. Two drops of each suspension were placed on grids for electron microscopy. In some experiments, the samples were negatively stained with 2% phosphotungstic acid for 30 seconds. In other experiments, the samples were first fixed with 1.5% glutaraldehyde (pH 7.2, in 0.1 M PO_4-buffer) and then negatively stained with 1% phosphotungstic acid for 30 seconds.

We decided to examine fixed and unfixed borrelial cells of each ciprofloxacin concentration, as the specific ciprofloxacin-induced cell alterations were better visible in the fixed samples; the bacteriophages, however, were better visible in the unfixed samples.

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FIG. 1. Untreated *Borrelia burgdorferi with a (a) smooth-structured outer cell membrane without blebs and (b) unchanged peptidoglycan layer. Phosphotungstate stain, × 68,000, fixed. Bar = 0.1 μm.*

FIG. 2. (a) Unassembled tails and (b) heads of A-1 bacteriophages inside of a *Borrelia burgdorferi cell. Phosphotungstate stain, × 330,000, unfixed. Bar = 30 nm.*

RESULTS

Ciprofloxacin susceptibility

The mean MIC of both isolates was 1 μmL.

Ultrastructure of untreated Borrelia burgdorferi cells

The untreated spirochete in Fig. 1 confirms the often-described structural characteristics of borrelial cells (12–15).

No phages were observed in borreliae grown in the untreated control cultures.

A-1 bacteriophages induced by subinhibitory ciprofloxacin concentrations

While the majority of the cells presented a regular shape, approximately 20% of the borrelial cells of both isolates showed severe abnormalities of ultrastructure when exposed to subinhibitory ciprofloxacin concentrations, ranging from 0.125 to 0.5 μg/mL. In the phage-carrying and morphological-altered borreliae, the outer envelope appeared to be undamaged, while the protoplasmic cylinder showed at least three different stages of destruction (1):

(1) numerous irregular constrictions of the normally smooth-structured peptidoglycan layer,
(2) disruption of the protoplasmic cylinder into several segments within a largely intact outer envelope, and
(3) small plasmolyzed protoplasma cylinder debris particles within an enlarged and irregularly shaped outer envelope.

In both isolates, plasmolyzed cells were filled with clusters of numerous unassembled heads and tails of bacteriophages (Fig. 2) showing an A-1 morphology (1, 16–18). According to the classification of Ackermann (17), this type consists of an isometric head (30 nm), a thin collar, and a long contractile tail (length 50 to 64 nm, width 13 to 19 nm) with a baseplate. In contrast to our former study (1), only unassembled heads and tails of A-1 bacteriophages could be observed within the borrelial cells. We detected no phages in borrelial cells exposed to ciprofloxacin concentrations equal to or higher than the MIC or in the untreated controls.

ULTRASTRUCTURE OF BORRELIA BURGDORFERI EXPOSED TO CIPROFLOXACIN CONCENTRATIONS ≥ 1 μg/mL

As previously described, nearly 20% of the cells showed severe *phage-induced* morphological alterations at subinhibitory ciprofloxacin concentrations. In the remaining borreliae, which presumably were not infected by temperate phages, no ultrastructural changes were seen when exposed to ciprofloxacin concentrations from 0.125 to 0.5 μg/mL.

The majority of borrelial cells exposed to a ciprofloxacin concentration of 1 μg/mL showed irregular constrictions of the peptidoglycan layer, which were located near the end of the cell (Fig. 3a). Obviously, as a result of these irregularly located constrictions, abnormally short distinct fragments of borrelial cells (Fig. 3b) became visible. The size of these fragments ranged from 0.6 to 0.8 μm. Moreover, the protoplasmic cylinder complex of *Borrelia burgdorferi* cells exposed to 1.0 μg/mL of ciprofloxacin showed electron-lucent swellings (Fig. 4a).

At a ciprofloxacin concentration of 2 μg/mL, approximately 75% of the treated cells revealed numerous defects of the peptidoglycan layer (Fig. 5a). The diameters of the protoplasmic cylinder complex varied from 0.09 to 0.20 μm (Fig. 5 arrows). Finally, at 4 and 8 μg/mL (Fig. 6a), in almost all cells the protoplasmic cylinder complex and the outer envelope were disrupted into many small plasmolyzed particles.

At ciprofloxacin concentrations from 0.125 to 2 μg/mL, spherical structures were seen (Fig. 7). Coiled up spirochetes were lying within these spheres.

No bacteriophages became visible in borrelial cells treated

FIG. 3. *Ultrastructural alterations in Borrelia burgdorferi after being exposed to a ciprofloxacin concentration of 1 μg/mL. (a) Irregular constriction of the peptidoglycan layer at the cell periphery. (b) Abnormal short fragments of borrelial cells showing a length of 0.9 μm. Phosphotungstate stain, × 97,000, fixed. Bar = 0.1 μm.*

FIG. 4. Ultrastructural alterations in Borrelia burgdorferi after being exposed to a ciprofloxacin concentration of 1 μg/mL. (a) Electron-lucent swelling of the protoplasmic cylinder complex. Phosphotungstate stain, × 97,000, fixed. Bar = 0.1 μm.

FIG. 5. Ultrastructural alterations in Borrelia burgdorferi after being exposed to a ciprofloxacin concentration of 2 μg/mL. (a) Numerous defects of the peptidoglycan layer. Note the different diameters of the protoplasmic cylinder complex (arrows). Phosphotungstate stain, × 97,000, fixed. Bar = 0.1 μm.

FIG. 6. Ultrastructural alterations in Borrelia burgdorferi after being exposed to a ciprofloxacin concentration of 8 μg/mL. (a) Debris of the disrupted protoplasmic cylinder complex partially enclosed by fragments of the outer envelope. Phosphotungstate stain, ×90,000, unfixed. Bar = 0.2 μm.

FIG. 7. Ultrastructural alterations in Borrelia burgdorferi after being exposed to a ciprofloxacin concentration of 0.125 μg/mL. Coiled-up spirochete forming a spherical structure (spheroplast). Phosphotungstate stain, × 37,000, unfixed. Bar = 1 μm.

with ciprofloxacin concentrations of 1 μg/mL (MIC) and more.

DISCUSSION

The MIC of 1 μg/mL for our *Borrelia burgdorferi* strains was comparable to that found by investigators of other studies (5, 6). Our data confirm that *Borrelia burgdorferi* shows only moderate susceptibility to ciprofloxacin.

The ultrastructural morphology of our untreated *Borrelia burgdorferi* isolates (Fig. 1) corresponded with former morphological descriptions by Barbour and Hayes (13), Hovind-Hougen and coworkers (12, 14), and Hayes and Burgdorfer (15). Also, the measurements for length and diameter as well as the numbers of flagella were characteristic for Borrelia species (12–15).

Both *Borrelia burgdorferi* isolates examined in this study contained temperate bacteriophages showing an A-1 morphology that were inducible exclusively by subinhibitory ciprofloxacin concentrations (Fig. 2). These phage-carrying *Borrelia burgdorferi* cells showed severe ultrastructural alterations of their morphology (1), which completely differed from the ciprofloxacin effects on borreliae observed at concentrations of 1 to 8 μg/mL. Induction of prophages occurred only at subinhibitory ciprofloxacin concentrations, presumably as production and release of bacteriophages depend on an undisturbed metabolism of the host organism. Including our former study (1), we examined two erythema migrans isolates and one tick isolate for the presence of bacteriophages. All lysogenic borreliae contained A-1 bacteriophages, the first skin isolate in addition a B-1 bacteriophage (1).

Besides these phage-induced morphological alterations of borrelial cells, other severe ciprofloxacin-induced ultrastructural changes could be observed at concentrations of 1 μg/mL (MIC) and more. The normal cell division was considerably disturbed at a ciprofloxacin concentration of 1 μg/mL (MIC). Multiplication of *Borrelia burgdorferi* occurs by binary transverse fission (13). Usually, cell division is started by constriction of the peptidoglycan layer in the middle of a long cell (13). Obviously, as a result of the irregular constriction of the peptidoglycan layer in the periphery of abnormal elongated borrelial cells, very short cell fragments became visible (Fig. 3). The damaging effect of ciprofloxacin first led to swellings (Fig. 4a), after that to membrane defects (Fig. 5a), and finally to the disruption of the protoplasmic cylinder complex (Fig. 6).

At ciprofloxacin concentrations ranging from 0.125 to 2 μg/mL, large spherical forms filled with remnants of the protoplasmic cylinder complex, as described before (Fig. 7), were observed (13, 15), but the significance and function of such structures are still unknown. In comparison with the results of Voigt and Zeiler (2), Elliott et al. (3), and Rodgers et al. (4), who demonstrated that ciprofloxacin primarily affected areas located in the cell wall of gram-negative and gram-positive bacteria, we found severe morphological alterations concerning mainly the protoplasmic cylinder complex of *Borrelia burgdorferi*.

In contrast to penicillin-treated borreliae, which showed morphological alterations even at subinhibitory concentrations (Schaller M, Neubert U. Morphology of *Borrelia burgdorferi* exposed to benzylpenicillin. Infection, in press), in nonlysogenic borreliae, no ciprofloxacin-induced changes were visible at concentrations below the MIC.

This may be a further explanation why ciprofloxacin does not show the same *in vivo* efficacy (Meisel C. personal communication) in comparison to the β-lactam antibiotics preferentially used in treatment of early Lyme-borreliosis (5, 6).

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Identification of an Uncultivable *Borrelia* Species in the Hard Tick *Amblyomma americanum*: Possible Agent of a Lyme Disease–like Illness

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Bites from the hard tick *Amblyomma americanum* are associated with a Lyme disease–like illness in the southern United States. To identify possible etiologic agents for this disorder, *A. americanum* ticks were collected in Missouri, Texas, New Jersey, and New York and examined microscopically. Uncultivable spirochetes were present in ~2% of the ticks. *Borrelia* genus–specific oligonucleotides for the flagellin and 16S rRNA genes were used for amplification of DNA. Products were obtained from ticks containing spirochetes by microscopy but not from spirochete-negative ticks. Sequences of partial genes from spirochetes in Texas and New Jersey ticks differed by only 2 of 641 nucleotides for flagellin and 2 of 1336 nucleotides for 16S rRNA. Phylogenetic analysis showed that the spirochete was a *Borrelia* species distinct from previously characterized members of this genus, including *Borrelia burgdorferi*. Gene amplification could be used to detect these spirochetes in ticks and possible mammalian hosts.

A puzzling phenomenon about Lyme disease has been the reports of this tickborne infection from areas where transmission of the etiologic agent, *Borrelia burgdorferi*, to humans has not been documented [1, 2]. This situation has been reported from Georgia and Missouri but may occur in other parts of the United States [3–5]. The primary manifestation of this Lyme disease–like illness is a localized, expanding circular skin rash accompanied by constitutional symptoms [5, 6]. The skin rash is identical or very similar to erythema migrans, the clinical hallmark of Lyme disease [5, 6]. Many of the patients with this disorder have had negative serologic assays for antibodies to *B. burgdorferi*, a finding that has fueled a controversy about so-called "seronegative Lyme disease" [2].

Although both *B. burgdorferi* and the hard tick *Ixodes scapularis*, its most common vector for human transmission, have been identified in some areas of the south-central and southeastern United States [7, 8], the more commonly reported exposure for patients with a Lyme disease–like disorder in that region has been to another hard tick: *Amblyomma americanum*, the Lone Star tick [9–11]. Moreover, in a retrospective case-control study of patients with suspected Lyme disease in Missouri, the investigators could not implicate *B. burgdorferi* as the etiologic agent [5]. There was no evidence that *Francisella tularensis*, *Ehrlichia chaffeensis*, or arboviruses caused the disease either [5].

The incompetence of *A. americanum* as a vector of *B. burgdorferi* has been documented [8, 11–14]. Nevertheless, spirochetes were seen in 1%–9% of ticks in collections of *A. americanum* from New Jersey, North Carolina, Oklahoma, Indiana, Alabama, Missouri, and Texas [10, 15–19]. For example, 1%–2% of *A. americanum* ticks in Texas contained a spirochete that was bound by polyclonal antisera to *B. burgdorferi* [19]. Attempts to axenically cultivate the *A. americanum* spirochetes in media that support the growth of *B. burgdorferi* and several other *Borrelia* species, such as the relapsing fever agent *Borrelia hermsii* [20], have been unsuccessful [19, 21, 22] (unpublished data).

Two hypotheses can be postulated from these observations: First, the spirochete in *A. americanum* is not *B. burgdorferi* [22], and second, in some areas of the United States, there is an infection that mimics features of Lyme disease but is caused by another tickborne agent [5]. To test the first hypothesis and to provide the means to test the second, we characterized the uncultivable *A. americanum* spirochete by amplifying from these organisms selected DNA sequences with high information content for taxonomic purposes.

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Materials and Methods

Tick examinations. *A. americanum* ticks from New Jersey, New York, Missouri, and Texas were collected from the field by flagging [23]. Some ticks from Texas had been removed from human hosts and submitted for examination [19]. Ticks were individually dissected with sterile instruments, and portions of their midguts were examined by direct immunofluorescence with fluorescein-labeled anti–*B. burgdorferi* polyclonal antisera [19, 23].

DNA procedures. The remainder of each tick was subjected to one of two procedures for DNA extraction. Ticks from New York and New Jersey were individually placed in sterile plastic bags, then frozen and crushed with a hammer. To the homogenate was added 0.5 mL of 10 m*M* TRIS, pH 8.0, 1 m*M* EDTA, 0.01% yeast tRNA, and 1% SDS and then 0.5 mL of phenol. The aqueous phase was extracted with ether. Ticks from Texas were placed in sterile microfuge tubes, to which was added 0.2 mL of 10 m*M* TRIS, pH 8.0, 50 m*M* EDTA, and 2% SDS. The suspension was heated to 64°C for 20 min and extracted once with phenol and twice with chloroform. By either method, DNA was precipitated with ethanol and resuspended in 10 m*M* TRIS, pH 8.0, and 1 m*M* EDTA.

DNA was separated in a 0.9% GTG agarose gel (FMC BioProducts, Rockland, ME) in TRIS-borate-EDTA buffer or in a 4% NuSieve gel (FMC) with TRIS-acetate-EDTA as described [24]. The latter conditions were used for DNA digested with *AluI*. After electrophoresis, DNA was transferred to 0.22-µm Nytran membranes (Schleicher & Schuell, Keene, NH) and probed with DNA labeled with [^{32}P]dATP using a nick translation kit (Life Technologies GIBCO BRL, Gaithersburg, MD). Hybridization and washing conditions were as described [24]. The final wash was at 64°C in 15 m*M* NaCl, 1.5 m*M* sodium citrate, 0.1% SDS, and 1 m*M* EDTA. Radiographic film was exposed with an intensifying screen.

Retrieved sequences. Complete or partial flagellin gene sequences of the following species (with data base accession numbers) were used: *B. burgdorferi* (X69611 and P11089), *Borrelia turicatae* (M67462), *Borrelia parkeri* (M67461), *Borrelia anserina* (X75201), *B. hermsii* (A44894 and M67460), *Borrelia crocidurae* (X75204), *Borrelia afzelii* (L29238), *Borrelia garinii* (X75203), and *Treponema pallidum* (A41489). Complete or partial 16S rRNA gene sequences of the following microorganisms were used: *B. burgdorferi* strain B31 (U03396 and X57404), *B. hermsii* (M60968 and L10136), *B. anserina* (M72397 and M64312), *Borrelia miyamotoi* sp. nov. from *Ixodes persulcatus* in Japan (D45192), the "Florida canine borrelia" (L37837; [25]), and *T. pallidum* (M88726).

Polymerase chain reaction (PCR). Primers were based on identical sequences in flagellin and 16S rRNA genes of *Borrelia* species. The positions listed in parentheses refer to *B. burgdorferi* flagellin (Fla) and 16S rRNA (16Rna) genes: FlaLL, 5'-ACATATTCAGATGCAGACAGAGGT-3' (301–324); FlaLS, 5'-AACAGCTGAAGAGCTTGGAATG-3' (438–459); FlaRS, 3'-CGATAATCTTACTATTCACTAGTTTC-5' (766–791); FlaRL, 3'-TGTTAGACGTTACCGATACTAACG-5' (942–965); 16RnaL, 5'-CTGGCAGTGCGTCTTAAGCA-3' (36–55); 16RnaR, 3'-CATATAGTCTTACTATGCCACTTATG-5' (1346–1368). PCR reactions in volumes of 100 µL containing 2.5 U of Taq DNA polymerase (Boehringer-Mannheim, Indianapolis), 50 pmol of each primer, 200 µ*M* each dNTP, 10 m*M* TRIS (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, and 0.001% gelatin were done in a thermal cycler (Perkin-Elmer Cetus, Foster City, CA). The reaction program was first 95°C for 3 min and then 40 cycles of 95°C for 1 min, 55°C for 1 min, and 75°C for 1 min. PCR products were cloned into vector pCRII using the TA Cloning System and *Escherichia coli* strain INVαF' (Invitrogen, Portland, OR). Sequences of both strands from at least two clones of each PCR product were determined from double-stranded DNA using Sequenase version 2.0 (United States Biochemicals, Cleveland) and custom-synthesized primers.

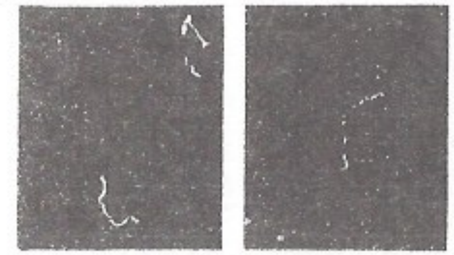

Figure 1. Photomicrographs of *Borrelia turicatae* (left) and spirochetes (right) in the crushed midgut of an *A. americanum* tick. Slides were incubated with 1:10 dilution of fluorescein isothiocyanate–conjugated rabbit antibodies to *Borrelia burgdorferi* and examined by fluorescent microscopy. Neither *B. turicatae* nor spirochete from *A. americanum* was detected with 1:100 dilution of antiserum. Magnification, ×400.

Sequence analysis. Nucleotide and deduced amino acid sequences were aligned by the PileUp (multiple) or GAP (pairwise) algorithms (Genetics Computer Group, Madison, WI). Aligned sequences were analyzed with the PHYLIP program package, version 3.5 [26]. Distance matrices were calculated with the Jukes-Cantor option of the DNADIST program. Multiple data sets were generated with SEQBOOT, unrooted trees were constructed using the NEIGHBOR program with the neighbor-joining option, and a consensus tree was generated with CONSENSE. Data sets of aligned amino acid sequences were analyzed with PROTDIST using the Dayhoff PAM matrix for substitutions. Tree topology was also examined by subjecting bootstrapped data sets of nucleotide sequences to parsimony analysis with the DNAPARS algorithm. The consensus tree files from distance matrix and parsimony analyses were expressed in the New Hampshire standard format [26]. The sequences reported here have been deposited in the GenBank data base (accession nos. U23211, U26704, and U26705).

Results

Tick examinations. *A. americanum* ticks were collected from field locations in Missouri, New Jersey, New York, North Carolina, and Texas and examined with anti–*B. burgdorferi* polyclonal antisera in concentrations that would cross-react with other *Borrelia* species [19, 23]. Representative reactions of the fluorescent antibody conjugate with the microorganism found in *A. americanum* and with *B. turicatae*, a relapsing fever agent, are shown in figure 1. About 2% of the ticks, both nymphs and adults, in Missouri, New Jersey, New York, and North Carolina contained immunoreactive spirochetes ranging

Table 1. Presence of immunofluorescence-reactive spirochetes in *A. americanum* nymphal and adult ticks.

Tick origin	No. positive (no. of adults)	No. examined (no. of adults)
Monmouth County, New Jersey	3 (2)	110 (50)
Suffolk County, New York	10 (9)	375 (318)
Currituck County, North Carolina	1 (0)	95 (26)
Southeast Missouri*	6 (0)	295 (29)
Total	20 (11)	875 (423)

NOTE. Spirochetes were reacted with 1:10 dilution of fluorescein-conjugated antiserum to *Borrelia burgdorferi* [23]. Total % positivity was 2.3% (range, 1.1%–2.7%).

* Bollinger, Pulaski, and Stoddard counties

from 10 to 20 μm in length (table 1). The results in Texas were similar and confirmed the findings of Rawlings and Tellow [19].

PCR. As an alternative strategy for definition of the microorganism, we applied PCR and amplification of conserved genes using primers designed on the basis of sequences of possibly related organisms [21]. For this study, we assumed that the organism was a member of the genus *Borrelia*. *A. americanum* ticks were examined first by immunofluorescence. DNA from ticks positive and negative for immunoreactive spirochetes was extracted in Fort Collins (New Jersey and New York specimens) or San Antonio (Texas specimens) using different extraction methods. The investigator responsible for PCR studies and their interpretation was blind to the findings of the tick examinations.

The first set of primers (FlaLS and FlaRS) used was expected to amplify a ~350-bp fragment of the flagellin gene of *Borrelia* species. The primers differed in sequence at two or more positions from homologous sequences of other spirochetes and bacteria. Subsequent *Alu*I restriction enzyme digestion of the PCR products would then yield characteristic restriction fragments for different species of *Borrelia*.

PCR products of DNA from *B. burgdorferi*, from two North American relapsing fever agents, *B. hermsii* and *B. turicatae*, and from immunofluorescence-positive ticks from Texas and New Jersey were all 350 bp (not shown). Figure 2 (left) shows *Alu*I restriction fragment polymorphisms of these PCR products. The patterns of restriction fragments of the 2 *Amblyomma* tick samples both differed from the digested products from *B. burgdorferi*, *B. hermsii*, and *B. turicatae* in the size range of 60–150 bp shown in the figure.

To assess the specificity of the PCR reaction, we examined extracts from 21 *A. americanum* ticks from New York. For this experiment, the PCR products with primers FlaLS and FlaRS were subjected to Southern blot analysis with cloned DNA from the Texas tick as a probe (figure 2, right). Eight of the 10 extracts from ticks positive for *Borrelia* species by microscopic examination had products that detectably hybridized with the probe; none of the 11 *Borrelia*-negative ticks did ($P < .001$ by two-tailed Fisher's exact test). We also examined 15 Texas *A. americanum* ticks by immunofluorescence and by PCR with primers FlaLL and FlaRL. The 8 ticks positive by immunofluorescence were the only specimens to have the expected 664-bp PCR product ($P < .001$ by two-tailed Fisher's exact test; data not shown).

Analysis of flagellins. With evidence that the spirochete in *A. americanum* was a new *Borrelia* species, we next used sets of primers that would amplify either a larger region of the flagellin gene (FlaLL and FlaRL) or most of the 16S rRNA genes (16RnaL and 16RnaR). PCR products from organisms in ticks from Texas and New Jersey were sequenced over both strands and as different recombinant clones. The partial flagellin gene sequences were each 641 nucleotides, and the partial 16S rRNA gene sequences were each 1336 nucleotides. The 2 strains differed by only 2 nucleotides at each locus. This represented identities of 99.7% and 99.9% for the flagellin and rRNA sequences, respectively.

The deduced amino acid sequences for flagellin proteins of the 2 microorganisms found in *A. americanum* were identical over 213 residues; the nucleotide differences between strains were synonymous. Figure 3 shows the alignment of part of the deduced flagellin sequences of the spirochetes found in *A. americanum* in Texas and New Jersey with the comparable variable regions of the flagellin proteins of 8 *Borrelia* species and *Treponema pallidum*, the spirochete that causes syphilis. The amino acid positions are numbered according to the full-length *B. burgdorferi* flagellin protein. The flagellin proteins of microorganisms found in *A. americanum* differed from other borrelial flagellins at several positions and, uniquely among the *Borrelia* species, lacked most of a proline-alanine-rich region beginning around residue 220. The spirochetes found in *A. americanum* resembled *B. turicatae*, *B. hermsii*, *B. parkeri*, *B. crocidurae*, and *B. anserina* in being without the QAA at residues 204–206 of the Lyme disease agents *B. burgdorferi*, *B. garinii*, and *B. afzelii*.

Analysis of 16S rRNA genes. Further phylogenetic classification was provided by comparison of 16S rRNA gene sequences (figures 4 and 5). The sequence of the spirochete found in *A. americanum* from Texas had the following identities with selected other spirochete 16S rRNA genes: *T. pallidum*, 79.6%; *B. burgdorferi*, 96.0%; *B. anserina*, 97.5%; *B. hermsii*, 97.8%; *B. miyamotoae* sp. nov., 98.3%; and the "Florida canine borrelia," 98.4%. By distance matrix and parsimony analyses of the aligned sequences (figure 4), the spirochete found in *A. americanum* clustered with a group containing the relapsing fever species *B. hermsii*, *B. anserina*, the unnamed organism recovered from the blood of 2 dogs in Florida [25], and *B. miyamotoae* sp. nov. (accession no. D45192).

Additional parsimony analysis was restricted to base positions that were polymorphic in at least 2 of 6 species (figure

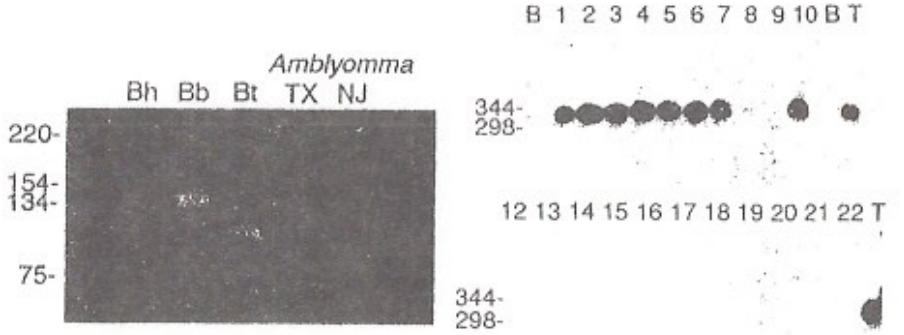

Figure 2. Polymerase chain reaction (PCR) products with *Borrelia* genus–specific primers for flagellin genes. Left, Electrophoresis of *Alu*I fragments of PCR products with primer pairs FlaSL and FlaSR. Sources of DNA were *Borrelia hermsii* HS1 (Bh), *Borrelia burgdorferi* B31 (Bb), *Borrelia turicatae* "Ozona" (Bt), and extracts from *A. americanum* ticks from Texas (TX) and New Jersey (NJ). Molecular weight standards (bp) are in side lanes. Restriction fragments <60 bp are not shown. Right, Southern blot analysis of PCR products of DNA from *A. americanum* ticks from New York. Ticks 1–10 were positive by direct fluorescence assay with conjugated rabbit antibody to *B. burgdorferi*, ticks 12–22 were negative. T, same as TX at left; B, negative PCR control. Extracted DNA was subjected to PCR with primer pairs FlaSL and FlaSR. Blots were probed with PCR product from tick T.

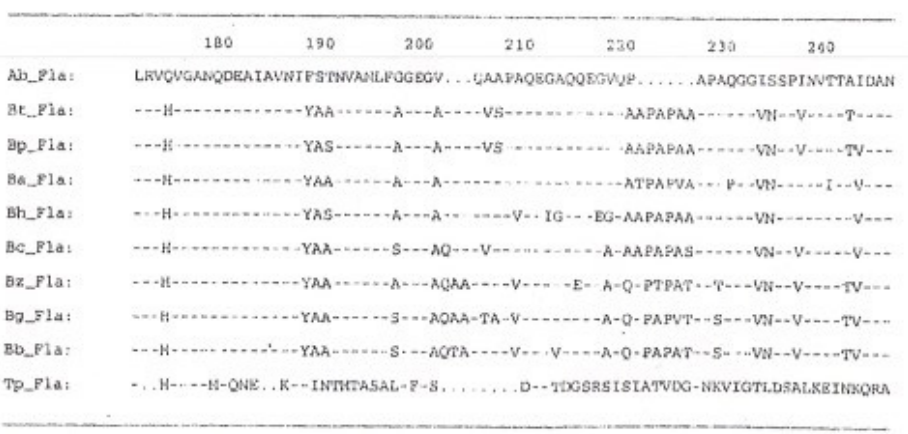

Figure 3. Alignment of variable regions of flagellin proteins (Fla) of borrelia from *A. americanum* (Ab), *Borrelia turicatae* (Bt), *Borrelia parkeri* (Bp), *Borrelia anserina* (Ba), *Borrelia hermsii* (Bh), *Borrelia crocidurae* (Bc), *Borrelia afzelii* (Bz), *Borrelia garinii* (Bg), *Borrelia burgdorferi* (Bb), and *Treponema pallidum* (Tp). Numbers correspond to amino acid positions of *B. burgdorferi* flagellin protein.

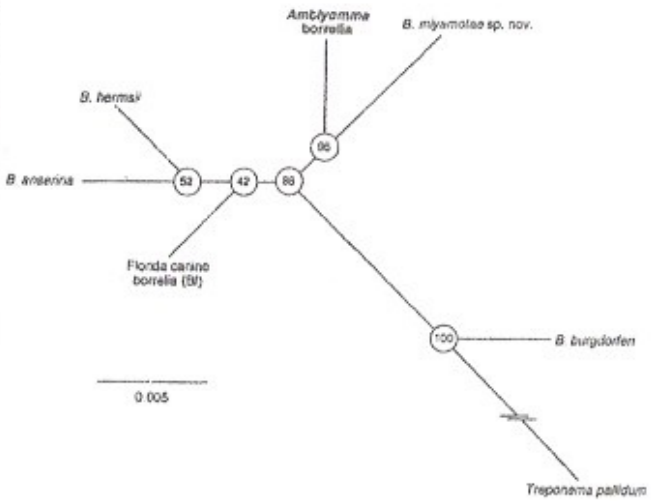

Figure 4. Unrooted distance matrix phylogenetic tree of *Borrelia* species with *Treponema pallidum* as outgroup. 16S rRNA sequences corresponding to base positions 36–1371 of *Borrelia burgdorferi* 16S rRNA gene were aligned and analyzed with PHYLIP program package. Exhibited tree in New Hampshire standard format is: (((Florida canine borrelia: 100, (*Borrelia anserina:* 100, *Borrelia hermsii:* 100): 52): 42, (borrelia from *A. americanum:* 100, *Borrelia miyamotoe* sp. nov.: 100): 96): 88, *T. pallidum:* 100, *B. burgdorferi:* 100). Circled numbers indicate number of times (in 100) that particular node was supported by bootstrap analysis. Approximate evolutionary distances are measured along line segments; bar represents distance by Jukes-Cantor criteria of 0.005. Similar tree (not shown) was obtained by parsimony analysis of 100 bootstrapped datum sets: (((((borrelia from *A. americanum:* 100, *B. miyamotoe:* 100): 94, *B. hermsii:* 100): 34, Florida canine borrelia: 100): 25, *B. anserina* 100): 81, *B. burgdorferi:* 100): 100, *T. pallidum:* 100).

5). Among the 6 sequences represented in figure 5, there were 49 aligned positions at which only 1 of the 6 species differed; 27 (53%) of these differences were in *B. burgdorferi*. The following tree was produced with 100 bootstrapped data sets of these positions: (((borrelia from *A. americanum:* 100, *B. miyamotoe:* 100): 94.4, (Florida canine borrelia: 100, (*B. hermsii:* 100, *B. anserina.* 100): 38): 64): 100, *B. burgdorferi:* 100). Again, the borrelia from *A. americanum* clustered with the non–Lyme disease *Borrelia* species; it was most closely related to *B. miyamotoe* sp. nov. and the Florida canine borrelia.

Base:	77	126	170	181	253	260	303	471	473	558	590	600	999	1110	1179	1251
Ab_rna:	T	C	A	T	A	G	T	G	T	C	T	T	A	G	A	T
Bm_rna:	T	C	A	A	G	G	T	A	C	C	C	T	A	G	A	T
Bf_rna:	C	T	G	A	A	G	A	G	T	T	T	C	G	A	G	T
Bh_rna:	C	T	G	A	A	A	A	A	T	T	C	C	G	A	A	T
Ba_rna:	C	T	G	A	G	A	A	A	C	T	C	C	G	A	G	A
Bb_rna:	C	T	G	T	A	A	A	A	T	T	T	C	A	G	A	A

Figure 5. Signature base positions of 16S rRNA genes (rna) of *Borrelia* species from *A. americanum* (Ab), *Borrelia miyamotoe* sp. nov. (Bm), Florida canine borrelia (Bf), *Borrelia hermsii* (Bh), *Borrelia anserina* (Ba), and *Borrelia burgdorferi* (Bb). Base positions correspond to positions of 16S rRNA gene of *B. burgdorferi*.

Discussion

In this study, ~2% of *A. americanum* ticks from Missouri, New Jersey, New York, North Carolina, and Texas contained

spirochetes by immunofluorescence. The anti–*B. burgdorferi* polyclonal antiserum was used at a concentration at which *B. turicatae*, a relapsing fever species, was also bound. Thus, it is likely that reactions with the antisera were no more than genus-specific. Other investigators identified spirochetes in *A. americanum* ticks in Alabama, New Jersey, and Texas with polyclonal antisera to *B. burgdorferi* [10, 15, 19].

A genus level of specificity was also used for the PCR analysis. By this approach, spirochetes were detected in the ticks from New Jersey, New York, and Texas that were examined. Spirochete-bearing ticks from Texas and New Jersey were the source for further PCR reactions in which flagellin and 16S rRNA genes were amplified. The spirochetes in these ticks were neither the same nor closely related to species that are known to cause Lyme disease. Phylogenetic analysis of the deduced flagellin sequence and the 16S rRNA gene sequences indicate that the microorganism clusters instead with relapsing fever *Borrelia* species, *B. anserina*, and two incompletely characterized organisms, the Florida canine borrelia and *B. miyamotoe* sp. nov. from *I. persulcatus* ticks in Japan.

Justification for classification of the borrelia found in *A. americanum* as a separate species are, first, the sequence differences from other *Borrelia* species in both 16S rRNA and flagellin and, second, biologic differences between the organisms. The newly discovered spirochete is associated with hard ticks and not soft ticks exclusively. The spirochete does not grow in media that support the multiplication of several other species of *Borrelia* [20, 25] (unpublished data). On the basis of these differences from other *Borrelia* species, we provisionally name the *A. americanum* spirochete *B. lonestari* sp. nov. We recognize that the borrelia from *A. americanum* may be similar if not identical to *Borrelia theileri*, the agent of bovine and equine borreliosis. This uncultivable, uncharacterized spirochete is transmitted by hard ticks of the genera *Boophilus* and *Rhipicephalus* (reviewed in [28]). A spirochete consistent with *B. theileri* in its infectivity for calves and transmission by *Boophilus microplus* has been identified in Texas [29].

If the spirochetes in *A. americanum* ticks characterized in this study were not *B. burgdorferi*, what were the *A. americanum* spirochetes noted by other investigators [15, 18, 30, 31] and called *B. burgdorferi*? Schulze et al. [15] used a polyclonal antiserum; the organism was not isolated for identification [21]. Fier et al. [18] reported spirochetes that hybridized with a probe for 16S rRNA sequence representing *B. burgdorferi*, but the relevant sequence of the *A. americanum* spirochetes was not determined. In two other studies, the spirochetes in *A. americanum* were bound by a monoclonal antibody to the OspA protein of *B. burgdorferi*, but the specificities of the reactions with this antibody were not presented [22, 30]. The study by Teltow et al. [31] is the only one to report in vitro cultivation of *B. burgdorferi* or any spirochete from *A. americanum*. However, these isolations are paradoxical; in a subsequent study, cultivation of any spirochetes from *A. americanum* of Texas was not successful [19]. Accepting the evidence that *A. americanum* is not competent to transmit *B. burgdorferi* [8, 11–14], we propose that the majority if not all of the spirochetes previously noted in *A. americanum* ticks were *B. lonestari* sp. nov. and not *B. burgdorferi*.

Whether *B. lonestari* sp. nov. is the etiologic agent of the aforementioned Lyme disease–like illness reported from several areas of the United States remains to be determined; circumstantial evidence suggests that it is. Reports from several locations in the southeastern and south-central regions of the United States indicate that seasonal rash illnesses, which are apparently ameliorated by antibiotics, are associated with bites by the Lone Star tick [5, 6]. *A. americanum* is a common biting tick for humans in these areas [32]. Its usual hosts are white-tailed deer, medium-sized mammals, and ground-feeding birds; rodents are only rarely infested by *A. americanum* [33]. The tick's distribution in the United States extends east from central Texas to Florida and as far north as Rhode Island [32]. In a recent survey of ticks in central and eastern Texas, *A. americanum* outnumbered *I. scapularis*, the usual vector of Lyme disease, by 100-fold [19]. Although *A. americanum* is a vector as well of the obligate intracellular pathogen *E. chaffeensis* [34], investigations to date have not implicated this agent as the cause of Lyme disease–like illness in Missouri [5].

Confirmation of the role of *B. lonestari* sp. nov. in these illnesses may be hastened by the availability of the spirochete components identified here. The flagellin protein is sufficiently different from that of other *Borrelia* species that a serodiagnostic assay based on recombinant flagellin antigen is conceivable. The DNA sequences of the flagellin gene and the rRNA gene provide a means by which PCR and other nucleic acid–based technologies can be used to identify the organism in infected persons and in suspected animal reservoirs, for example, deer, rabbits, and ground-feeding birds. Identification of a mammalian or avian reservoir in turn may provide insights for more successfully formulating media to cultivate the spirochete.

Acknowledgments

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Seronegative Chronic Relapsing Neuroborreliosis

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Key Words
Lyme disease, seronegative
Immune complex
Herxheimer

Abstract
We report an unusual patient with evidence of *Borrelia burgdorferi* infection who experienced repeated neurologic relapses despite aggressive antibiotic therapy. Each course of therapy was associated with a Jarisch-Herxheimer-like reaction. Although the patient never had detectable free antibodies to *B. burgdorferi* in serum or spinal fluid, the CSF was positive on multiple occasions for complexed anti-*B. burgdorferi* antibodies, *B. burgdorferi* nucleic acids and free antigen.

Introduction

The nervous system manifestations of Lyme disease have usually been described as responsive to appropriate antibiotic therapy, and without relapses. We describe a patient with Lyme disease manifested by episodic peripheral and cranial motor and sensory neuropathies, and central nervous system deficits. Progressive neurologic symptoms and signs led to six courses of intravenous antibiotic therapy in the last 5 years. Each time, initiation of therapy promoted a Jarisch-Herxheimer (J-H) reaction with fever, acute encephalopathy and abrupt neurologic deterioration. Although the patient's serum was consistently negative for free antibody to *Borrelia burgdorferi* (Bb), there was laboratory evidence of active infection in the cerebrospinal fluid (CSF) even after intense prolonged antibiotic therapy. The patient had lived in New York City for the last 40 years and her only known exposure to Bb was on a camping trip through the countryside in France and Switzerland in the summer 1981.

Methods

The Bb blastogenesis test was performed by Dr. David Volkman [1]. Intrathecal Bb antibody synthesis was demonstrated in Dr. John Halperin's laboratory [2]. PCR analyses were done in the laboratories of Halperin [3], Luft [4], and Marconi [5]. Dissociation of CSF immune complexes and Western blots for antibodies to Bb [6] and Bb antigen identification by ELISA and Western blot [7] were done by Dr. P.K. Coyle. HLA typing was performed by Dr. Edwin Dwyer [8].

The following tests were performed by MetPath Laboratories (Teterboro, N.J.): C1qBA, Raji assay, HTLV-I antibody, IgG subclasses and T lymphocyte profile. The other tests were done in the hospital's routine laboratory.

Case History

In January 1989, a previously healthy 58-year-old woman presented with 3 months of right shoulder girdle weakness, atrophy and radicular pain. Electromyographic studies demonstrated right C4 and C5 radicular dysfunction, with C4–C8 paraspinal denervation. Routine laboratory tests were normal or negative, including that for Lyme antibodies. In February 1989, mild distal weakness of the right hand and atrophy of the right hypothenar eminence were noted. Right hand weakness increased and right iliopsoas weakness developed, with right-sided hyperreflexia. Sternocleidomastoid weakness and a positive Romberg sign appeared. MRI studies of the head, cervical cord and brachial plexus were normal, as was a cervical myelogram.

Despite the negative serology for Lyme disease, in April 1989 a Bb blastogenesis test was strongly positive with a stimulation index of 50 [1]. CSF obtained as an outpatient showed only a slightly elevated protein (table 1). However, stored spinal fluid was subsequently shown to be positive for Bb-specific antigens by ELISA and Western blot (table 1) [7]. A decision was made to treat for possible seronegative Bb infection, and the patient was hospitalized for intravenous antibiotics. Twelve hours after initiating ceftriaxone the patient became confused, and then stuporous, with a temperature of 39.2°C. The time course was consistent with a J-H reaction and those symptoms resolved spontaneously in 48 h. She improved and was discharged to complete 3 weeks of intravenous ceftriaxone. Three months later she developed right anterior uveitis followed by two episodes of bilateral keratitis. In August 1989 she developed right pulsatile tinnitus and loss of taste with dysgeusia.

In December marked left tongue atrophy was noted. The patient was therefore hospitalized for a second course of intravenous ceftriaxone. Another J-H reaction occurred 24 h after starting antibiotics and then spontaneously cleared. The patient was treated for 8 weeks, with gradual improvement and was able to return to work. Spinal fluid from this admission that had been stored at –70°C was in retrospect shown to contain Bb nucleic acids by PCR [5] and Bb antigen by ELISA and Western blot [7].

In May 1990, she experienced severe right retro-orbital pain with a tender, engorged right temporal artery. Visual evoked potentials demonstrated right monocular delay. Two months later she developed a progressive right hemiparesis. CSF obtained at this time showed intrathecal synthesis of IgA antibody to Bb [2], and was positive by PCR for Bb nucleic acids in 2 different laboratories (table 1) [3, 5]. During the third hospitalization in July 1990 she was retreated with ceftriaxone. Twenty-one hours after antibiotic was started she developed blurred vision in her right eye followed by stupor. This was again considered to be a J-H reaction and she was treated with 1 g solumedrol i.v., followed by 250 mg every 6 h. Visual acuity returned to normal within 4 days. Following 2 weeks of intravenous ceftriaxone, doxycycline 200 mg p.o. b.i.d. was continued for 19 weeks. The patient's right hemiparesis continued to improve and no new neurologic symptoms developed while on doxycycline.

Within 2 weeks of stopping doxycycline the patient developed vertigo. Two months later, she experienced bilateral facial paresthesias and numbness of the mucous membranes of her mouth and gingiva. On examination there was absent sensation of the buccal and gingival mucous membranes, diminished sensation in the fifth cranial nerve V1–3 distribution and depressed corneal reflexes. Repeat lumbar puncture at this time revealed a protein of 75 mg/dl, and Bb-specific OspA antigen by ELISA and Western blot (table 1) [7]. The patient was hospitalized a fourth time and given intravenous cefotaxime 2 g every 8 h. Within 24 h of starting antibiotics, she developed multifocal myoclonic jerks and became unresponsive, with a dense right hemiparesis. This J-H reaction occurred despite premedication with 80 mg prednisone followed by 20 mg every 6 h. The patient was given 1 g of solumedrol i.v. followed by 250 mg every 6 h. Twelve hours later, 30 h after starting antibiotics, the patient was able to speak and the hemiparesis had improved. On day 21 agranulocytosis was noted so cefotaxime was discontinued, and 200 mg p.o. b.i.d. of doxycycline was started. While receiving this second course of doxycycline, the patient developed vertigo, experienced increasing hypesthesia of her face and burning paresthesias and numbness of her legs to mid-thighs.

Table 1. Supportive laboratory findings for Bb infection in the CSF

Dates	Hospital admissions	Protein mg/dl	Cells per mm³	PCR	Intrathecal antibody	Bb antigens	Bb specific IC
03-31-89	Office	56	3			OspA	
04-05-89	Office						
04-21-89	Pre-Rx 1	50	0				
09-08-89	Office						
12-28-89	Pre-Rx 2	40	0	+[a]		OspA	
01-16-90	Office						
06-04-90	Office						
07-16-90	Pre-Rx 3	38	0	+[a,b]	IgA 1:14[b]	OspA	IgG
07-18-90	36 h post-Rx 3	42	0	+[b]		OspA	IgG
08-13-91	Pre-RX 4	75	0			OspA	
09-10-91	Pre-Rx 5						
04-16-92	Office	52	1			OspA	
08-03-92	Pre-Rx 6	56	1	+[a,c]		OspA	IgG

Many of these studies were obtained retrospectively on CSF that had been stored at –70°C.
PCR performed in laboratories of Dr. R. Marconi[a], Dr. J. Halperin[b] and Dr. B.J. Luft[c].
Bb antigens (ELISA and/or Western blot) and specific immune complex (IC) dissociation studies by Dr. P.K. Coyle.

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According to Dr. Coyle she was blocked from publishing in US journals. So they went to a European journal. KVF 5/6/2020

Coyle

She was hospitalized for the 5th time in September 1991. To prevent another severe J-H reaction, she was premedicated with Motrin and 1 g of solumedrol in the 24 h prior to being given 2 g of i.v. ceftriaxone. There was only a mild J-H reaction with confusion and dysarthria. She was discharged on the 4th day and continued on 2 g daily of ceftriaxone for 2 weeks followed by 3 g of pulse ceftriaxone for 2 days of each weeks for 9 weeks. Episodes of fever, chills and arthralgias after the third pulse of antibiotic were considered to be serum sickness-like reactions and were initially controlled by 800 mg p.o. of Motrin. Progressive chills, fever and arthralgias necessitated discontinuation after 9 cycles in early December (fig. 1).

In the spring of 1992 the patient developed trigeminal sensory neuropathy, progressive diffuse paresthesias and leg numbness. Neurologic examination suggested mononeuritis multiplex. Spinal fluid was reexamined and the findings were consistent with persistent Bb infection (table 1). The patient declined admission until August, when she experienced exertional dyspnea. An EKG was normal and there was normal excursion of the right diaphragm. Right chest wall motion was limited due to an intercostal neuropathy. The patient declined EMG testing. She was again premedicated with 250 mg i.v. solumedrol and 400 mg p.o. ibuprofen every 6 h for 24 h before beginning antibiotics. She had only a mild J-H reaction and was discharged to continue 2 weeks of 2 g i.v. ceftriaxone, followed by 500 mg b.i.d. of oral clarithromycin. The latter was begun in August 1992 and the patient has had gradual regression of the sensory symptoms. No further new symptoms or deficits have occurred in the ensuing 22 months.

Negative tests included antibodies to HIV, HTLV-1, FANA and VDRL. Serum complement (C3, C4), ESR, quantitative immunoglobulins, IgG subclasses and the T-lymphocyte profile (CD4, CD8, CD4/CD8 ratio) were normal. Elevated C1qBA titers for immune complexes paralleled her clinical course (fig. 1). The Raji assay for immune complexes was negative.

Complete HLA typing was A2, A24, B35, B39 Bw6, Cw4, Dr2, DQw1 [8]. Paired serum and spinal fluid specimens taken at the time of each lumbar puncture were stored at –70°C. The spinal fluid PCR from 12/28/89 and the antigen capture ELISA and Western blots from 3/20/89, 12/28/89, 7/16/90, 7/18/90 and 6/13/90 were done retrospectively in the spring of 1993 (table 1).

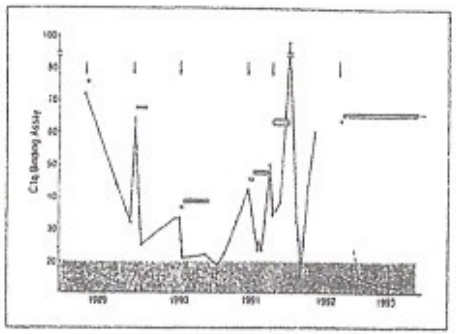

Fig. 1. Serum immune complex levels by C1q binding assay. Normal values are less than 20 (shaded). Arrows indicate hospitalizations. Narrow horizontal rectangles indicate antibiotics, as follows: solid bar – ceftriaxone; solid bar with open circle – cefotaxime; vertical shading – doxycycline; horizontal shading – ceftriaxone 'pulses', with serum sickness reaction; diagonal shading – clarithromycin.

Discussion

We describe a previously healthy woman with a 5-year history of a relapsing and remitting neurologic disorder involving the central nervous system (CNS) and multiple peripheral and cranial nerves. We believe this to be an unusual case of seronegative Lyme disease, though she did not have a recognized tick bite or erythema migrans. The patient's clinical involvement is compatible with that reported for neuroborreliosis. During her 5-year course, despite extensive evaluations, there has been no evidence of another disease such as multiple sclerosis, sarcoidosis, collagen-vascular disease or an occult infection. Severe J-H reactions, with acute encephalopathy, fever and worsening motor deficits, occurred repeatedly within 24 h of starting antibiotics [9]. During the last four admissions these symptoms were attenuated by premedication with Motrin and high dose intravenous glucocorticosteroids. Each course of antibiotics produced a therapeutic response, with temporary arrest of neurologic progression. During two prolonged courses of antibiotics no new manifestations developed. The patient has now been on p.o. clarithromycin for 22 months, and no new symptoms or deficits have occurred during this time.

Several experimental laboratory tests support infection with Bb (table 1). Before therapy in 1989 the patient had a strongly positive lymphoproliferative response to Bb (stimulation index 50) [2], a value more than 10 SD above the mean. When the stimulation index is more than 3 SD above the mean, positive results have had a 95% specificity [10]. When serum and spinal fluid immune complexes were dissociated, they contained antibodies that reacted on Western blot with OspA, B, and flagellar antigens from Bb [6]. Spinal fluid PCR assays, performed in three different laboratories, have been positive for Bb nucleic acids [3–5]. Intrathecal synthesis of IgA antibodies to Bb was demonstrated in the CSF in July 1990 [2]. Bb-specific OspA antigen was demonstrated by antigen capture ELISA and Western blot in CSF specimens that had been obtained and stored at –70°C prior to 5 hospitalizations [7], including the last admission in August 1992.

This patient's serum and CSF antibody negativity was attributed to immune complex formation. After dissociating the complexes, antibody to Bb antigens could be demonstrated [6]. CSF Bb antigen was demonstrated repeatedly in 7 spinal fluids [7]; such antigen excess could bind available antibody. Serum I-C levels roughly paralleled her clinical course, peaking before each course of antibiotics (fig. 1) and declining rapidly after antibiotic therapy. The striking clinical improvement after antibiotic therapy, coincident with a precipitous drop in the C1qBA titer and normal ESR, suggest that these changes were a result of therapy and unlikely related to a primary immune complex disease. Furthermore, the C1qBA has been within the normal range for the past 17 months while the patient has has been receiving clarithromycin. Previous studies have shown that persistently elevated serum I-C levels after Bb infection are associated with a higher incidence of cardiac and neurologic involvement [11]. The findings in this patient and others suggest that circulating I-C may confound efforts at serologic diagnosis using free antibody-based tests [6]. The use of experimental studies such as PCR, antigen detection, intrathecal antibody synthesis and fractionation of I-C may provide evidence for Bb infection in such seronegative cases. Before undertaking repeated empiric courses of antibiotic therapy, we attempted to establish the diagnosis by the use of these assays.

There is anecdotal evidence that pulse cefotaxime has succeeded in eradicating Bb in chronic relapsing Lyme disease [12] but cefotaxime could not be given to this patient because agranulocytosis followed its previous use on the 4th hospitalization. Therefore, after the fourth relapse, the patient was treated with 9 weekly pulses of ceftriaxone. However, pulse therapy had to be stopped when the symptoms of serum sickness were no longer controlled by premedication with 800 mg Motrin. The serum sickness reaction was accompanied by an abrupt rise in the C1q binding assay to 98.3 µg/l (normal less than 20), that rapidly returned to normal when the antibiotic was discontinued (fig. 1). Nine pulses of ceftriaxone did not eradicate Bb.

This is a very unusual example of Bb infection. It is not known why this previously healthy patient was not cured by successive courses of intense and prolonged antibiotic therapy. In a limited study of patients with Bb encephalomyelitis, all were HLA Bw6 positive; the 6 who were also DQw1 positive had no response to treatment or had relapsing disease [2]. Our patient was typed as HLA Bw6 and functionally homozygous for DR2, DQw1 [8]. It is possible that homozygosity (or hemizygosity with a null allele) for this HLA genetic type might predispose to a chronic relapsing course. Despite evidence for CNS infection with an elevated spinal protein level, this patient never had a CSF pleocytosis. Thirty seven percent of patients with subacute encephalopathy reported by Logigian had no spinal fluid pleocytosis [13]. Benach et al. [14] showed that I-C may block the Fc receptors on neutrophiles and monocytes, and prevent phagocytosis of spirochetes. This patient has had increased serum I-C levels throughout her illness (fig 1) and may therefore have been unable to contain and kill or prevent the dissemination of the Bb spirochete.

The PCR analysis of her CSF in August 1992 gave significant amplification products only with the specific primer sets for genomic group 3 (previously referred to as genomic group VS 461), which has heretofore been identified only in Europe and Asia [5]. This patient may have acquired Bb during a 3-week camping trip through the countryside of France and Switzerland in 1981. If so, there was a latent period of 7 years before the onset of neurologic symptoms, which is more typical of neuroborreliosis described in Europe than in the United States [15, 16]. Among eight European countries, the incidence of erythema migrans was lowest in Switzerland, whereas neurologic manifestations were reported there in 62% of patients with Lyme disease [17].

Before her 6th hospital admission this patient had received four courses of ceftriaxone, one of cefotaxime and two of doxycycline (of 19 and 8 weeks). Increasing right hemiparesis and dyspnea with right intercostal muscle weakness prompted her 6th admission to the hospital. Following intravenous ceftriaxone for 2 weeks, it was decided to place the patient on long-term therapy with clarithromycin. Although there is no information on the penetration of clarithromycin into the CNS, it achieves high concentrations within macrophages [18] a known sanctuary for the Bb spirochete [19]. The clinical response to clarithromycin in this patient has now been sustained for over 22 months.

Intracellular pathogens are notoriously difficult to treat and cure [20]. In experimental animals, Bb is relatively insensitive to humoral antibodies and is eradicated in vitro only by prolonged incubation with relative high doses of antibiotics [21, 22]. Survival of Bb in humans, despite aggressive antibiotic therapy has been previously reported [2, 22]. We believe this to be an example of a patient with chronic relapsing Bb infection. It is important to evaluate unusual patients like this thoroughly in order to determine the effectiveness of prolonged oral antibiotics as a therapeutic option.

Coyle

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Book Review

Maurizio Versino, Daniela Zambarbieri (eds)

Ottorino Rossi Award Conference

International Workshop on Eye Movements

Pavia, June 13–14, 1994

The book was published in June 1994 for the Ottorino Rossi Award Conference, a neurological symposium held in Pavia every year. This year the topic was the neurology and neurophysiology of eye movements, and Prof. David Zee from the Johns Hopkins University in Baltimore was awarded for his fundamental contributions in this field.

Once the reader has found the table of contents, which unusually is placed before the author index at the end of the book he will find many contributors acknowledged worldwide for their publications on eye movements.

The book includes several sections: the main lecture by Prof. Zee deals with disorders of adaptive control of eye movements and clinical implications. The Invited Lectures are review articles (with some original experimental data) on the saccadic and the vestibular system. The Short Communication section includes original experimental data from Italian researchers. A small Abstract section consists of 5 very short, but self-consistent papers.

This is a very up-to-date introductory book on the saccadic and vestibular eye movement systems.

Finally, the book is available for free upon request from one of the editors at the following address: Fondazione Istituto Neurologico C. Mondino, Via Palestro 3, I–27100 Pavia (Italy).

Otmar Meienberg, Binningen

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First Isolation of *Borrelia burgdorferi* from an Iris Biopsy

V. Preac-Mursic, M.D., H. W. Pfister, M.D., H. Spiegel, M.D., R. Burk, M.D., B. Wilske, M.D., S. Reinhardt, M.D., and R. Böhmer, M.D.

The persistence of *Borrelia burgdorferi* in six patients is described. *Borrelia burgdorferi* has been cultivated from iris biopsy, skin biopsy, and cerebrospinal fluid also after antibiotic therapy for Lyme borreliosis. *Lyme Serology:* IgG antibodies to *B. burgdorferi* were positive, IgM negative in four patients; in two patients both IgM and IgG were negative. Antibiotic therapy may abrogate the antibody response to the infection as shown by our results. Patients may have subclinical or clinical disease without diagnostic antibody titers. Persistence of *B. burgdorferi* cannot be excluded when the serum is negative for antibodies against it.

Key Words: Lyme borreliosis—*Erythema migrans*—Antibiotic therapy

Lyme borreliosis, the most widespread disease transmitted by ticks, and caused by the spirochetal organism *Borrelia burgdorferi* (1) is characterized by various clinical stages, including dermatologic, neurologic, cardiac, rheumatologic, and ocular manifestations (2). Overlapping symptomatology of these stages is possible. Disease usually begins with the characteristic localized skin lesion *Erythema migrans* at the site of the tick bite, whereas the later phases—weeks, months to years after the primary infection—is marked by a disseminated infection.

With respect to ocular manifestations, conjunctivitis, keratitis, iritis, uveitis, vitritis, endophthalmitis, ischemic optic neuropathy, optic neuritis, oculomotor palsy, and retinal vasculitis have been reported (3–10). The diagnosis in all reported cases was made by clinical signs and serological tests for antibody to *B. burgdorferi*. This case report presents a patient in whom *B. burgdorferi* was first isolated from an iris biopsy. Additionally we report about the isolation of *B. burgdorferi* after corticosteroid and antibiotic therapy in patients with latent disseminated Lyme disease and interesting ophthalmological findings.

PATIENTS AND METHODS

Patients

See Table 1.

Serological Tests

Antibodies to *B. burgdorferi* in blood and cerebrospinal fluid (CSF) were determined by indirect immunofluorescence test (IFT) as described previously (11). To avoid unspecific false positive reactions, the test samples were absorbed with *Treponema phagedenis*. Antibody titers ⩾1:64 were regarded as significantly elevated, titers of 1:32 as borderline. Intrathecal production of antibodies against *B. burgdorferi* was assessed by comparing the CSF/serum ratio of enzyme-linked immunosorbent assay (ELISA) IgG values (units per milliliter) with the CSF/serum ratio of total IgG (CSF/serum index). A CSF/serum index of <2 was considered normal and >2 was considered elevated.

Bacteriological Examination

The iris biopsy and the samples of CSF and skin biopsy were examined for *B. burgdorferi* by darkfield microscopy and by culture in MKP medium as previously described (12,13). The cultures were incubated at 33°C for ⩾5 weeks and examined weekly by darkfield microscopy and subcultures.

Isolates were identified with monoclonal antibodies L321F11 and L221F8 by Western Blot (14). The susceptibility of the strains to different antibiotics were tested by MIC (minimal inhibitory concentration) using in vitro test in tube (13). Tests for monoculture of the isolates were done on solid media PMR agar (15).

Other Laboratory Examinations

The CSF was examined for white blood cells and total protein. Isoelectric focusing was used to determine oligoclonal IgG bands in the serum and CSF. Concentration of albumin and IgG in serum and CSF were determined by kinetic nephelometry. Serological examinations for syphilis and rheumatoid factor were performed.

RESULTS

The clinical and microbiological data are presented in Table 1.

TABLE 1. *Clinical and microbiological findings*

Patient no/age	Disease		Treatment	Antibodies to *B. burgdorferi*				Isolation of *B. burgdorferi* from
				Serum		CSF		
	Systemic	Ocular		IgM	IgG	IgM	IgG	
1/24	None	Panuveitis-Iridocyclitis	Corticosteroid Doxycycline	NE	593 U*	ND		Iris biopsy
2/55	*E. migrans*	Iritis-uveitis	Doxycycline Corticosteroid Ceftriaxone	NE	NE	ND		Skin biopsy
3/17	None	None	Cefotaxime	NE	1:64°	NE		CSF
4/60	None	Painful eyes	Ceftriaxone	NE	1:64°	NE		CSF
5/62	Arthralgias lymphadenopathy	Conjunctivitis	Penicillin	NE	NE	NE		CSF
6/25	Radicular pain	Iritis	Ceftriaxone Ceftriaxone	NE	1:64°	NE		CSF

ND, not done; NE, negative; CSF, cerebrospinal fluid.
* ELISA (positive > 200 U).
° IFT-ABS (positive is ⩾ 1:64, borderline 1:32, negative ⩽1:16)

Spirochetes isolation was successful between 2 and 16 subcultures in MKP medium. The isolates showed typical protein pattern of *B. burgdorferi* in SDS-page and were identified with monoclonal antibodies L321F11 and L221F8 as *B. burgdorferi*. The in vitro susceptibility of the isolates to antibiotics was the same as in other strains tested (13,16,17). The growth of *B. burgdorferi* on PMR agar is shown in Fig. 1.

Borreliae were isolated after antibiotic and corticosteroid therapy, from iris biopsy of one patient with chronic recurrence uveitis and acute panuveitis, as well as from skin biopsy of one patient with *E. migrans* and ocular manifestations, and also from the CSF of patients with "latent neuroborreliosis." However, the cell count in the CSF was normal and in all six reported patients the specific IgM antibody titers in serum were negative.

Serological examinations for rheumatoid factor and syphilis were negative.

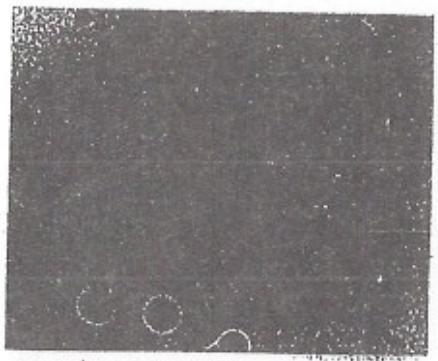

FIG. 1. *Borrelia burgdorferi* growth on PMR-agar.

From the Max v. Pettenkofer Institut für Hygiene u. Medizinische Mikrobiologie (V.P.-M., B.W., S.R., R.B.), Neurologische Klinik (H.W.P.), Klinikum Großhadern, and Augenklinik (H.S.), der LM-Universität München; Augenklinik (R.B.), der RK-Universität Heidelberg, Germany.

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Case 1

A 24-year-old woman developed blurred vision in the right eye in 1991. She had a history of several years of chronic recurrent anterior and posterior uveitis. Since 1985 she had suffered from recurrent bilateral iridocyclitis and had been on immunosuppressive therapy. Lyme antibody titers had not been determined. Three years later in 1988, the IgG antibody titers against *B. burgdorferi* in serum were positive, while the IgM titer was negative. The patient was treated with systemic doxycycline at a dose of 200 mg/day for 4 weeks; the IgG antibody titer decreased. In 1989 the patient received doxycycline, again at a dose of 200 mg daily for 4 weeks, after the Lyme IFT-IgG had been repeatedly positive. In August 1991 the patient was admitted to hospital because of acute panuveitis with iridocyclitis, anterior chamber and vitreous cells, subtotal posterior synechiae, and a lens covered by a dense membrane. Fundoscopy revealed macular pucker, a cystoid macular edema, and an exudative inferior retinal detachment. A sector iridectomy and prepupillary membranectomy were performed to improve fundus visualization to rule out a rhegmatogenous retinal detachment. Laboratory investigations included aqueous humor for antibody titer determination against *B. burgdorferi* as well as excised iris tissue for culture isolation of borreliae. The Lyme ELISA (IgG) in serum was 593 U (norm <200 U) and in aqueous humor, 42 U.

B. burgdorferi was cultured from the iris excision and prepupillary membrane after prolonged incubation in 16 subcultures of MKP medium (13) (Fig. 2).

Borreliae could be visualized in Levaditis stained biopsy (Fig. 3). Gram stains of biopsy specimens showed no organisms and bacterial cultures (aerobic-anaerobic) showed no growth of other bacteria. *E. migrans* was not noted, although a tick bite was recalled.

Case 2

A 55-year-old woman developed *E. migrans*, 4 weeks after a tick bite. Three days later a skin biopsy and Lyme IFT and ELISA was done, and at the same time oral doxycycline 200 mg/day for 10 days was initiated. The Lyme IgM and IgG were negative; however, *B. burgdorferi* was isolated from the skin biopsy. *E. migrans* disappeared after 3 weeks.

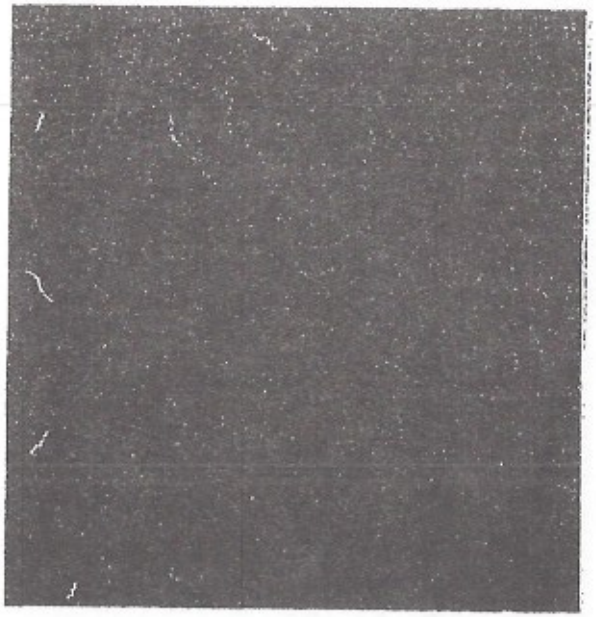

FIG. 2. *Borrelia burgdorferi* from iris biopsy (darkfield ×600).

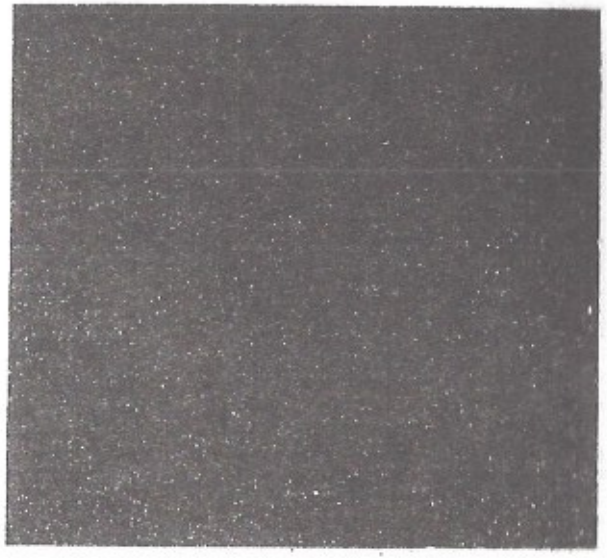

FIG. 3. *Borrelia burgdorferi* (Levaditi stained iris biopsy).

At the follow-up examination, 4 weeks after the antibiotic therapy, a subsequent biopsy (taken in the immediate vicinity of the prior biopsy) for culture of *B. burgdorferi* was negative, as was the Lyme serology.

The patient responded well for 1 year but then developed iritis and uveitis of the left eye. She also noted episodes of vertigo and tinnitus. Cultures and stains for bacteria and fungi were negative, the Lyme IgM and IgG were normal. For the following 6 weeks the inflammation was treated with high doses of topical and systemic corticosteroids with moderate effect. After 1 month of therapy she developed iritis and uveitis of the right eye. Intravenous ceftriaxone 2 g/day were administered for 3 weeks due to her Lyme history and symptomatology in both left and right eyes. The patient has remained well since, without further recurrence.

Case 3

A 17-year-old man had noted several tick bites during the months of August to December after having jogged in the woods. In December (within 2 weeks) he developed a bilateral tinnitus. A complete EENT examination as well as neurologic examination was normal. Upon physical examination, the patient was afebrile and meningeal signs were not present. Bilateral tinnitus was the only clinical symptom. Lumbar puncture revealed normal cell counts (3/3), and total protein (21 mg/dl). Oligoclonal IgG bands were not detected and no intrathecal specific antibodies against *B. burgdorferi* could be demonstrated.

Serum Lyme IFT IgG was positive (1:64). IgM was negative (<1:32). *B. burgdorferi* was isolated from CSF after 2 weeks incubation in MKP medium. The patient was treated with cefotaxime 3 × 2 g per day i.v. over 5 days followed with cephalexin for 8 days. Control cultures for *B. burgdorferi* 3 months later were negative. The same Lyme serological test results were obtained as those prior to therapy.

Case 4

A 60-year-old otherwise healthy man developed recurrent episodes of red, painful eyes in 1990 and was treated with topical corticosteroids. In early 1991 he developed short-lived vertigo with headaches.

A tick bite or *Erythema migrans* had never been seen. The neurological examination was completely normal.

Serum Lyme IgG was 1:64 and IgM was negative. Because of positive serum Lyme IFT IgG, CSF

was investigated (see Table 2). Lumbar puncture revealed normal values for cell counts and total protein. Antibody to *B. burgdorferi* in CSF were not detected, however, when CSF was cultivated in MKP medium, *B. burgdorferi* could be isolated.

There was no serological evidence for a bacterial or viral infection, except Lyme borreliosis. The ceftriaxone was administered at 2 g/day for 14 days. At the follow-up examination 4 months after the antibiotic therapy, cultures for *B. burgdorferi* were negative and the complaints disappeared.

Case 5

A 62-year-old woman had a tick bite in February 1990. Within 10 days she developed fever and cervical lymphadenopathy followed by conjunctivitis, arthralgias, numbness on toes and fingers, and extreme fatigue. The patient received oral penicillin for 12 days in February and again in May. In June 1991 the patient was referred to a neurological clinic due to persistent arthralgias and numbness. The neurological examination showed hypaesthesia and hypalgesia on toes and fingers of both sides as well as pallhypesthesia on malleoli left 4/8 and right 5/8. The rest of the neurological findings were normal. Lumbar puncture released 64 mg/dl protein and 6/3 cells. Antibody titers to *B. burgdorferi* in serum and CSF were negative, but *B. burgdorferi* was isolated from CSF after 4 weeks incubation in MKP medium. Now ceftriaxone was given 2 g/day i.v. for 14 days. Antibiotic treatment resulted in marked reduction of arthralgias and numbness. Cultures from CSF were negative.

Case 6

A 25-year-old man was admitted to hospital because of intensive radicular pain and blurred vision. He also had minor headaches, but he denied having fever or chills. The neurologic finding was a discrete hypaesthesia on the left forearm and bilateral iritis was present. One year earlier he had a 2-week episode of blurred vision, which cleared with oral prednisone therapy.

The patient had no history of tick bite and none of *E. migrans*. The IFT IgG antibody titers against *B. burgdorferi* in serum were positive (1:64); antibody titers in CSF were negative. Lumbar puncture revealed normal values for cell counts and total protein; nevertheless, *B. burgdorferi* was isolated from CSF. The isolation was successful on the second subculture in MKP medium. Ceftriaxone was administered 2 g daily intravenously for 14 days.

Five months after the therapy he remained well and a culture for *B. burgdorferi* from CSF was negative.

DISCUSSION

The diagnosis of Lyme disease is based on clinical symptoms, epidemiology, specific IgG and IgM antibody to *B. burgdorferi* in serum and CSF and isolation of borreliae. The diagnosis may be difficult in the late phase of the disease, particularly for ophthalmogists and rheumatologists. Characteristic of Lyme borreliosis is that its clinical picture is rarely complete and symptoms are overlapping, which makes diagnosis more difficult. A borrelial infection is usually confirmed by determining *B. burgdorferi* antibodies. However, interpretation of serological tests and results may not be straightforward. False-positive and false-negative results occur. Negative serologic results do not necessarily exclude *Borrelia* infection (18–20). As shown here and previously reported, antibiotic therapy may abrogate the antibody response to the infection, but *B. burgdorferi* may persist. In clinically unclear cases, much greater significance is therefore attached to the isolation of *B. burgdorferi*.

We were able to isolate *B. burgdorferi* from CSF and skin biopsies months to years after the antibiotic therapy and disappearance of *Erythema migrans*. The lack of repeated insect bite and *Erythema migrans*, negative AB-titers against *B. burgdorferi* and negative CSF examination suggest persistence of *B. burgdorferi* rather than reinfection.

How often *B. burgdorferi* may persist in the CSF, skin, or other tissues after therapy or its effect in producing atypical manifestations of disease is not known. The reason for the persistence of *B. burgdorferi* in patients after the treatment with antibiotics is not completely understood. A number of factors may play a role, e.g., virulence of *B. burgdorferi*, tissue penetration of antibiotics, insufficient antibiotic therapy (either duration or dose), intracellular localization of borreliae (21), possibility of *B. burgdorferi* survival in tissue and certain types of cells, and not at least the immunity of patients. The capacity of *B. burgdorferi* to hide in various human tissue (heart muscle, spleen, brain) (22–24) and an insufficient antibiotic tissue level are critical for the therapy.

Antibiotic treatment with amoxicillin or doxycycline has been recommended for *E. migrans*, penicillin G, and cephalosporins, ceftriaxone, and cefotaxime for central nervous system infection and late stages. It is known that the therapy of late stages of the Lyme borreliosis can be complicated. Not seldom, there are known recurrences of the disease and persistence of *B. burgdorferi*, also after adequate antibiotic therapy (18,25).

The persistence of *B. burgdorferi* and clinical recurrence in *E. migrans* stage are rarely noted (18,20), as the therapy seems to be mostly effective and sufficient. In certain circumstances the clinical and laboratory investigations (3–4 months after the completion of antibiotics therapy), are probably too short for the enddiagnosis. Furthermore, in most *E. migrans* patients a control of the therapy effect is never done. However, we must take into consideration that changes in clinical symptomatology (after months) can lead the patient to change doctors.

The current antibiotic therapy (antibiotic, dose, duration) is very different, so we have very different clinical and laboratory findings. However, the randomized trials comparing various antibiotics in their clinical response, minimal inhibitory concentration (MIC) and the serum and CSF concentration support the selection for stage-specific treatment. According to the data of recent clinical studies, the cephalosporins are more efficient than penicillin G in late (26,27) but not in early Lyme borreliosis (28). Dattwyler et al. (27), Diringer et al. (29) and Pal et al. (30) reported that ceftriaxone and cefotaxime were effective in treating patients with meningoencephalitis and late borreliosis who did not respond to penicilin G therapy.

The CSF concentrations of penicillin G, cefotaxime, and ceftriaxone in our studies demonstrate that both cephalosporins penetrate to a greater extent than penicillin. The CSF levels are evidently above the MIC 90 values for *B. burgdorferi*. The concentration of penicillin G did not reach the MIC 90 in any of our patients (31,32). Data from controlled clinical studies are still scanty, and the observation period after the therapy is often too short. Furthermore, proof of a successful therapy is based not only on the disappearance of clinical symptoms but also on the elimination of *B. burgdorferi*; this is difficult to achieve and seldom performed. However, the recurrence of the disease, longtime persistence of *B. burgdorferi* in untreated as well as in treated patients, unpredictable progression of the disease and the isolation of *B. burgdorferi* from CSF (without inflammatory signs) of patients with *E. migrans* (n.p.), it seems appropriate to treat patients in Stage I as effectively as possible. The isolation of *B. burgdorferi* from CSF in *E. migrans* without inflammatory signs support an early dissemination of the borreliae.

The results of randomized prospective therapy studies and case reports show that also with adequate antibiotic therapy cure is often impossible with one treatment course. An interval therapy with substantially larger doses of antibiotics—2 × 200 mg doxycycline, 2 × 800 mg amoxicillin for 7 days/2 times with an antibiotic-free interval of 7 days—is advisable. The patients with an active central nervous infection, carditis, or eye manifestations should be treated intravenously with ceftriaxone or cefotaxime (1 × 4 g or 2 × 3 g/day, 7 days/2 times with an antibiotic-free interval of 7 days).

This treatment regimen, which takes into consideration the long generation time of *B. burgdorferi* and the antibiotic mechanism of action can probably be more effective than the regimen used. The higher doses of antibiotics reach correspondingly effective higher serum, CSF, and tissue antibiotic concentrations and the repeated doses of antimicrobials killed the survivor borreliae. Likewise a combination of two antibiotics must be taken in consideration. The interval therapy and/or a combination therapy are often used, are obligatory in the treatment of complicated or chronical bacterial infection. However, currently recommended treatment regimens are inadequate for some patients; the therapy ought to be realized and controlled more individually.

In conclusion, our first isolation of *B. burgdorferi* from eye tissue confirms invasion of the ocular space by *B. burgdorferi*. Furthermore, this isolate demonstrates that in eye conditions we have to think of Lyme infection—on *B. burgdorferi* persistence and on an adequate therapy with antibiotics. Further cases, with varied clinical symptoms and courses, show that negative serological tests do not exclude *B. burgdorferi* infection.

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was investigated (see Table 2). Lumbar puncture revealed normal values for cell counts and total protein. Antibody to *B. burgdorferi* in CSF were not detected, however, when CSF was cultivated in MKP medium, *B. burgdorferi* could be isolated.

There was no serological evidence for a bacterial or viral infection, except Lyme borreliosis. The ceftriaxone was administered at 2 g/day for 14 days. At the follow-up examination 4 months after the antibiotic therapy, cultures for *B. burgdorferi* were negative and the complaints disappeared.

Case 5

A 62-year-old woman had a tick bite in February 1990. Within 10 days she developed fever and cervical lymphadenopathy followed by conjunctivitis, arthralgias, numbness on toes and fingers, and extreme fatigue. The patient received oral penicillin for 12 days in February and again in May. In June 1991 the patient was referred to a neurological clinic due to persistent arthralgias and numbness. The neurological examination showed hypaesthesia and hypalgesia on toes and fingers of both sides as well as pallhypesthesia on malleoli left 4/8 and right 5/8. The rest of the neurological findings were normal. Lumbar puncture releaved 64 mg/dl protein and 6/3 cells. Antibody titers to *B. burgdorferi* in serum and CSF were negative, but *B. burgdorferi* was isolated from CSF after 4 weeks incubation in MKP medium. Now ceftriaxone was given 2 g/day i.v. for 14 days. Antibiotic treatment resulted in marked reduction of arthralgias and numbness. Cultures from CSF were negative.

Case 6

A 25-year-old man was admitted to hospital because of intensive radicular pain and blurred vision. He also had minor headaches, but he denied having fever or chills. The neurologic finding was a discrete hypaesthesia on the left forearm and bilateral iritis was present. One year earlier he had a 2-week episode of blurred vision, which cleared with oral prednisone therapy.

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