#15 April 20 & 21, 1997

10th Annual International Scientific Conference on Lyme Disease & other Tick-borne Disorders

Place: National Institutes of Health, Clinical Center Masur Auditorium
Bethesda, MD

Conference Co-Chairs

James N. Miller, PhD
UCLA School of Medicine

Benjamin J. Luft, MD
SUNY at Stony Brook, School of Medicine

Poster Session Co-Chairs

Ron F. Schell, PhD
University of Wisconsin Medical School

Charles S. Pavia, PhD
New York Medical College

CME Credits

The School of Medicine, SUNY at Stony Brook is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to sponsor continuing medical education for physicians. The School of Medicine, SUNY at Stony Brook designates this continuing medical education activity for a maximun of 19 credit hours in the Category 1 of the Physician's Recognition Award of the American Medical Association.

This conference is made possible by a major grant from Glaxo Wellcome, Inc.

Lyme Disease Foundation
1 Financial Plaza, Hartford, CT 06103
860-525-2000 Fax 860-525-8425 Hotline 800-886-LYME
Lymefnd@aol.com www.Lyme.org

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James N. Miller, PhD - UCLA School of Medicine

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David W. Dorward, PhD - National Institutes of Health, RML
Janis J. Weis, PhD - University of Utah

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Ron F. Schell, PhD - University of Wisconsin Medical School
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Public Forum

Joseph J. Burrascano, Jr., MD - Southampton Hospital
Julie A. Rawlings, MPH - Texas Department of Health

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Max J. Appel, DVM, PhD, *College of Veterinary Medicine, Cornell*
Stephen W. Barthold, PhD, DVM, *Yale University School of Medicine*
Jorge L. Benach, PhD, *SUNY Health Science Center*
Edward M. Bosler, PhD, *SUNY at Stony Brook, School of Medicine*
Willy Burgdorfer, PhD, MD (hon), *National Institutes of Health, Rocky Mountain Laboratories*
Joseph J. Burrascano, Jr., MD, *Southampton Hospital*
Jeffrey J. Collins, PhD, *Glaxo Wellcome, Inc.*
Patricia K. Coyle, MD, *SUNY at Stony Brook, School of Medicine*
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Brian A. Fallon, MD, *Presbyterian Hospital, NYS Psychiatric Institute*
Lesley A. Fein, MD, *Mountainside Hospital*
Denise M. Foley, PhD, *UCLA School of Medicine*
Jesse L. Goodman, MD, *University of Minnesota School of Medicine*
Mark S. Hanson, PhD, *MedImmune, Inc.*
Carrie A. Hughes, PhD, *Georgetown University School of Medicine*
Fred S. Kantor, MD, *Yale University School of Medicine*
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Charles S. Pavia, PhD, *New York Medical College*
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Patricia A. Rosa, PhD, *National Institutes of Health, Rocky Mountain Laboratories*
Ronald F. Schell, *University of Wisconsin School of Medicine*
Steven E. Schutzer, MD, *NJ School of Medicine and Dentistry*
Ellen S. Shang, PhD, *UCLA School of Medicine*
Janice Soreth, MD, *U.S. Food and Drug Administration*
Gerold Stanek, MD, *University of Vienna Hygiene Institute*
Franc Strle, MD, *University Clinical Center Japljevaz, Yugoslavia*
Reinhard K. Staubinger, DVM, PhD, *College of Veterinary Medicine, Cornell* → see Persistence of Bb in Experimentally Infected Dogs after Antibiotic Treatment.
Steven Straus, MD, *National Institutes of Health, Clinical Center*
Irwin T. Vanderhoof, PhD, *NYU Stern School of Business*
Janis J. Weis, PhD, *University of Utah*
Bettina Wilske, MD, *Max von Pettenkofer Institute*

Purpose & Objectives

The purpose of this conference is to present the current status of clinical and basic sciences with regards to several aspects of Lyme disease; the structure-function relationships of the host response and coinfection; patient diagnosis, treatment, and management; and prevention.

Structure-Function Relationship of Host Response and Coinfection

Ticks carry a plethora of infectious organisms. The interactions of these organisms in the vector, the reservoir and the human host will impact on diagnosis, treatment and prevention. Upon completion, participants should be able to:

- relate laboratory and clinical data on coinfection, particularly ehrlichiosis
- identify new approaches to the laboratory diagnosis of Lyme disease
- recognize the complex nature of outer surface proteins of *Borrelia burgdorferi* in relation to host response to infection

Patient Management: Diagnosis and Treatment

Because Lyme disease is an emerging and evolving syndrome with many variable clinical manifestations, it is important for healthcare professionals to keep abreast of the current clinical research on localized and disseminated infection, differential diagnosis and refractory infections. Upon completion, participants should be able to:

- examine the only FDA approved antibiotic for early Lyme disease and the process of obtaining FDA approval for Lyme disease treatment regimens
- diagnose neurologic Lyme disease and its many complex manifestations
- distinguish the differential diagnosis of Lyme disease and chronic fatigue, fibromyalgia and somatization disorders.

Prevention

Prevention of the disease is the cornerstone of the public health mission. The conference will present state-of-the-art information on several approaches to prevention of Lyme disease. Upon completion, participants should be able to:

- identify the spectrum of problems associated with the etiology of chronic Lyme disease, e.g. animal models and clinical data
- assess the implications of genetic diversity of the Lyme disease spirochete on the development of new diagnostic tests and vaccines.

10th Annual International Scientific Conference on Lyme Disease & Other Tick-borne Disorders

Program Agenda

Monday April 28, 1997

7:00 am All day registration, posters. 7.5 CME's for the day

8:00 am Welcome and Keynote
The Tick: A Pandora's Box - Willy Burgdorfer, PhD, MD (hon) National Institutes of Health, RML

Coinfections with *Borrelia burgdorferi*

Session Chair: Stephen Dumler, MD 8:30 am - 9:45 am

Naturally Occurring Coinfections in Reservoir Mice
David H. Persing, MD, PhD ..... *Mayo Clinic*

Human Granulocytic Ehrlichiosis: Clinical and Biological Features of an Emerging Infec
Jesse L. Goodman, MD ..... *University of Minnesota, School of Medicine*

Clinical Aspects of Ehrlichiosis
J. Stephen Dumler, MD ..... *Johns Hopkins Medical Institutions*

Refreshment Break 9:45 am - 10:05 am

Borrelia Structure-Function Relationship & Host Response I

Session Chairs: Drs. Dorward and Weis 10:05 am - 11:45 am

Outer Membrane Proteins of Pathogenic *Borrelia* Species
Ellen S. Shang, PhD ..... *UCLA School of Medicine*

Role of Lipoproteins in Localized Inflammation: Activation of Human Endothelial Cells and Neut
Janis J. Weis, PhD ..... *University of Utah*

***Borrelia burgdorferi*-Host Cell Interactions**
John M. Leong, MD, PhD ..... *University of Massachusetts Medical Center*

Interaction of *Borrelia burgdorferi* with Human Lymphocytes
David W. Dorward, PhD ..... *National Institutes of Health, Rocky Mountain Laborato*

Lunch 11:45 am - 12:45 pm

Program Resumes 12:45 pm - 2:25 pm

Tick Immunity as a Strategy for Prevention of Transmission of Spirochetes and Other Tick-B Pathogens
Fred S. Kantor, MD ..... *Yale University School of Medicine*

Infectivity and Serologic Responses of Rabbits Fed upon by *Bb* Infected Deer Ticks
Charles S. Pavia, PhD ..... *New York Medical College*

Canine Model of LD: Migration of *Borrelia burgdorferi* in Tissue and its Consequences
Reinhard K. Staubinger, DVM, PhD ... *College of Veterinary Medicine, Cornell University*

Acute and Long Term Pathologic Lesions in Human Lyme Borreliosis
Paul Duray, MD ..... *National Institutes of Health, National Cancer Institu*

Refreshment Break 2:25 pm- 2:45 pm

Diagnosis and Treatment I

Session Chairs: Drs. Luft and Liegner 2:45 pm - 5:15 pm

Regulatory "Point-to-Consider" in Clinical Trial Design for Lyme Disease
Janice Soreth, MD ..... *U.S. Food and Drug Administration*

Cefuroxime Axetil: First FDA Approved Antimicrobial for Early Lyme Disease
Jeffrey J. Collins, PhD ..... *Glaxo Wellcome, Inc.*

Infectious Risk after Tick Bite in Middle Europe
Gerold Stanek, MD ..... *University of Vienna Hygiene Institute*

Molecular and Immunological Variability of European *Bb* strains and the Diagnosis and Prophylaxis of Lyme Borreliosis
Bettina Wilske, MD ..... *Max von Pettenkofer Institute*

Diagnosis and Treatment of Early and Disseminated Lyme Disease
Joseph J. Burrascano, Jr., MD ..... *Southampton Hospital*

Clinical Presentation and Treatment of Skin and Nervous System Infections in Lyme Disease
Franc Strle, MD ..... *University Clinical Center, Japljevaz, Yugoslavia*

6:30 pm - 8:00 pm Public Forum at the Hyatt Regency Hotel
Joseph J. Burrascano, Jr., MD - Southampton Hospital
Julie Rawlings, MPH - Texas Department of Health

Tuesday April 29, 1997

7:00 am All Day Registration and Poster Sessions

Diagnosis and Treatment II

Session Chairs: Drs. Luft and Liegner 8:00 am - 10:00 am

Neurologic Lyme Disease: Diagnosis and Treatment
Patricia K. Coyle, MD..... *SUNY at Stony Brook, School of Medicine*

Subtle Injury to Transformed Neural Cells by *Bb*
Jorge L. Benach, PhD..... *SUNY at Stony Brook, Health Science Center*

Psychiatric Aspects of LD and the Use of SPECT Imaging
Brian A. Fallon, MD ..... *Presbyterian Hospital, NYS Psychiatric Institute*

Transplacental Transmission of *Bb* in a Murine Model
Sousan Sayahtaheri Altaie, PhD ..... *U.S. Food & Drug Administration*

Clinical Presentations: A Survey of 1,000 Patients
Irwin T. Vanderhoof, PhD ..... *NYU Stern School of Business*

Refreshment Break 10:00 am - 10:20 am

Present and Future Testing for Lyme Disease
Steve E. Schutzer, MD ..... *NJ School of Medicine and Dentistry*

New Serologic Approaches to the Diagnosis of Lyme Disease
Raymond J. Dattwyler, MD ..... *SUNY At Stony Brook, School of Medicine*

Differential Diagnosis

Session Chairs: Drs. Luft and Liegner 11:10 am - 12:10 pm

Chronic Fatigue Syndrome and Chronic Lyme Disease: Living with Diagnostic Uncertainty
Steven Straus, MD ..... *National Institutes of Health, Clinical Center*

Lyme Disease vs. Fibromyalgia
Lesley A. Fein, MD, MPH ..... *Mountainside Hospital*

Lyme Disease vs. Somatization Disorders
Brian A. Fallon, MD, MPH ..... *NYS Psychiatric Institute*

Awards Presentations 12:10 am - 12:20 pm

Lunch 12:20 pm - 1:20 pm

Persisting (Refractory) Infection

Session Chairs: Drs. Donta and Coyle 1:20 pm -3:10 pm

Complement Resistance in *Borrelia burgdorferi*
Carrie A. Hughes, PhD ..... *Georgetown University School of Medicine*

Resistance of *Borrelia burgdorferi* to Antibiotic Treatment in Beagles
Max J. Appel, DVM, PhD ..... *College of Veterinary Medicine, Cornell*

Expression of *Borrelia* DNA in Mammals
Catherine J. Luke, PhD ..... *University of CA at Irvine*

Promises and Pitfalls of Clinical Trials for Lyme Disease
Benjamin J. Luft, MD ..... *SUNY at Stony Brook, School of Medicine*

Intramural Clinical Program on Chronic Lyme Disease
Adriana R. Marques, MD..... *National Institutes of Health, Clinical Center*

Break 3:10 pm - 3:30 pm

Program Resumes 3:30 pm - 4:40 pm

Randomized, Double-Blinded, Placebo-Controlled, Multicenter Trials of the Safety and Efficacy of Ceftriaxone and Doxycycline in the Treatment of Patients with Seropositive and Seronegative Chronic Lyme Disease
Mark S. Klempner, MD ..... *Tufts New England Medical Center*

Seronegative Chronic Meningoencephalomyelitides
Kenneth B. Liegner, MD ..... *New York Medical Center*

Management of Patients with LD; Reactivation of Latent Infection
Sam T. Donta, MD ..... *Boston University Medical Center*

Genetic Variation: Confounding Patient Management I

Session Chairs: Drs. Bosler and Miller 4:40 pm - 5:30 pm

Genetic Variation of *Bb* within a Population of Ticks
Dan E. Dykhuizen, PhD ..... *SUNY at Stony Brook, Ecology & Evolution*
Edward M. Bosler, PhD ..... *SUNY at Stony Brook, School of Medicine*

Targeted Gene Inactivations in *Borrelia burgdorferi*
Patricia A. Rosa, PhD ..... *National Institutes of Health, Rocky Mountain Laboratories*

Reception for Registrants Hyatt Regency 7:00 pm - 10:00 pm

Wednesday April 30, 1997

7:00 am All Day Registration

Genetic Variation: Confounding Patient Management II

Session Chairs: Drs. Bosler and Miller 8:00 am- 9:30 am

Crystal OspA and Genomic Sequencing of *B. burgdorferi*
Catherine L. Lawson, PhD ..... *Brookhaven National Laboratory*

***Bb sensu lato* Isolates from Missouri**
James H. Oliver, Jr., PhD ..... *Georgia Southern University*

Aspects of Lyme and/or Lyme-like Disease in Missouri
Edwin J. Masters, MD.....*Regional Primary Care, Inc.*

Break 9:30 am - 9:50 am

Prevention

Session Chairs: Drs. Barthold and Phillip 9:50 am - 12:05 pm

Humoral Immune Response to *in vivo Borrelia* Antigens
Stephen W. Barthold, DVM, PhD, ... *Yale University School of Medicine*

***Bb* Decorin Binding Protein as Components of a Second Generation Lyme Disease Vaccine**
Mark S. Hanson, PhD ..... *MedImmune, Inc.*

Acquired Resistance to *Bb* in the Rabbit Model: OspA Vaccine-derived vs. Infection-derived Immunity
Denise M. Foley, PhD ..... *UCLA School of Medicine*

Macrophages and Enriched Populations of T Lymphocytes Interact Synergistically for the Induction of Severe Destructive Lyme Arthritis: Relationship to Vaccination
Ronald F. Schell, PhD ..... *University of Wisconsin School of Medicine*

Development of Spirochetal Vaccines
James N. Miller, PhD ..... *UCLA School of Medicine*

Conclusion 12:05 pm - 12:10 pm

Edwin Masters, M.D.
Regional Primary Care, Inc.
69 Doctors Park
Cape Girardeu, MO 63703

Clinical Aspects of Lyme and/or Lyme-Like Disease in Missouri

Edwin Masters. Regional Primary Care, Inc., Cape Girardeu, MO

Examples of clinical erythema migrans in Missouri are presented along with other clinical presentations and examples of sequelae. Etiological and epidemiological theories are presented and discussed. The possible role of the lone star tick *(Amblyomma americanum)* in the transmission of this illness is explored.

Notes:

10TH ANNUAL INTERNATIONAL SCIENTIFIC CONFERENCE ON LYME BORRELIOSIS & OTHER TICK-BORNE DISORDERS

PREVENTION & PATIENT MANAGEMENT

Abstract For Oral & Poster Presentations

Deadline: December 15, 1996

TICK-BORNE DISEASE:

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__ Other:

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__ Clinical manifestations
__ Laboratory diagnosis
__ Early disease management
__ Late disease management
__ Chronic disease management
__ Other:

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LA-2 EQUIVALENT ANTIBODIES & BACTERIOCIDAL ACTIVITY OF A RECOMBINANT LIPOPROTEIN-OspA VACCINE AGAINST *BORRELIA BURGDORFERI* IN HEALTHY ADULT VOLUNTEERS.

C. Van Hoecke[1]*, D. Fu[1], D. De Grave[1], P. Voet[1] and E. Lebacq[2]
[1]SmithKline Beecham Biologicals, Rixensart, Belgium
[2]Clinique Notre-Dame de Grâce, Gosselies, Belgium

SmithKline Beecham Biologicals have formulated a candidate Lyme vaccine containing 10 µg of purified recombinant lipoprotein-OspA (adsorbed onto aluminium hydroxide), which has been shown to protect mice against challenge by *Borrelia burgdorferi*-infected ticks. Clinical trials in healthy adults revealed that the lipoprotein formulation induces early and large anti-OspA IgG and "LA-2 equivalent" responses. However, a recent study with a similar vaccine showed only a low, short-term borreliocidal activity of these anti-OspA antibodies. We therefore examined the safety, immunogenicity (including the long-term persistence) and borreliocidal activity of the Lyme vaccine in adult volunteers.

The candidate Lyme vaccine was evaluated in 20 subjects, who received a three-dose primary vaccination course given at monthly intervals, and a booster at month 12. Blood samples at 3, 12, 13,18 & 24 months were used to assess the immune response using an inhibition ELISA to determine "LA-2 equivalent" antibody titers. A bacteriocidal assay was also performed and a correlation factor determined between the two tests.

All volunteers remained in good health up to 24 months, with no withdrawals or serious adverse events. All local symptoms were reported as "mild or moderate" and resolved spontaneously within the four-day follow-up. One general symptom was reported, a case of mild headache after the booster dose. There were no unsolicited symptoms, rash or arthralgia reported. An immune response was induced in all vaccinees which persisted until the booster at month 12. The booster increased the GMT by 25-fold and high titers persisted through month 24. The correlation factor between bacteriocidal activity and "LA-2 equivalent" antibodies was 0.86, based on a total number of 78 paired blood samples. In-house data from at least 43 bacteriocidal assays indicated that the test was reproducible within 95% confidence limits.

Thus, the candidate Lyme vaccine appears to be safe and immunogenic, eliciting a strong "LA-2 equivalent" antibody response which has a good correlation with borreliocidal activity. The imminent results of a large, two-year efficacy trial will show whether the apparent induction of borreliocidal antibodies has direct benefits in the clinical condition.

Name C. VAN HOECKE & PIERRE VOET
Title CLINICAL PROJECT DIRECTOR
Affiliation SMITHKLINE BEECHAM BIOLOGICALS
Street RUE DE L'INSTITUT, 89
City RIXENSART State — Zip B-1330 Country BELGIUM
phone (02) 656 8770
fax (02) 656 9132
e-mail vanhoecke@scbio...

Mail to: Lyme Disease Foundation, One Financial Plaza, Hartford, CT 06103-2610
(860) 525-2000 Fax (860) 525-8425 E-mail: lymefnd@aol.com

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A NEW SPIROCHETE ISOLATED IN TIBET, CHINA

P. H. Zhang, X. T. Zhang,* Q. E. Zhang, W. C. Cao
Beijing Institute of Microbiology and Epidemiology, Beijing 100071, P.R. China

An epidemiological investigation concerning a natural focus of disease, was carried out in Tibet, China in 1993-1994. During this investigation, a previously unknown microbe with spirochete morphology was isolated from wild rodents and ticks, and from symptomatic patients in the region which presented with marked lymphadenopathy and a common cold-like illness. This new isolate demonstrated the following characteristics: (1) The organism was fragile and easily broken and demonstrated slow growth in BSK culture medium at an optimal temperature of 28°C. (2) The morphology was characterized by 2-12 regular spirals with both ends terminating in a point. The organism is non-motile and 0.02-0.34 μm in diameter and 0.5 -10.8 μm in length. Transmission electron microscope revealed an incomplete outer membrane and an irregular interior with nodal material. There was no evidence of either flagella or axoneme. (3) Upon direct experimental infection of both rabbits and mice, the animals developed an illness typified by hepatosplenomegaly with areas of acute inflammation present in several anatomical sites. (4) The organism showed a weak antigenic cross-reaction with polyclonal antibodies against international standard B31 but no reaction to the monoclonal antibody VL-G2F9 specific for *Borrelia burgdorferi* when analyzed by the indirect fluorescent antibody test. (5). Specific antibodies against the organism were detected by enzyme-linked crack blot using soluble antigen extracted from the organism. The positive rates of sera collected from local residents and wild rodents were 21.1% and 50.0-66.7% respectively. All sera from patients with leptospirosis and syphilis were negative for the specific antibodies against the new isolate. We conclude that the newly found organism may be an unidentified pathogen, which differs from other spirochetes such as *Borrelia burgdorferi, T. pallidum* and *Leptospira*. Further pathogenic and epidemiological studies are under way.

Name Xi-Tan Zhang
Title Professor
Affiliation Beijing Institute of Microbiology and Epidemiology
Street 20 Dong Da Street, Fengtai District
City Beijing State Zip 100071 Country P.R.of China
phone 861063812060
fax 861068213044
e-mail

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C. Van Hoecke[1]*, D. Fu[1], D. De Grave[1], P. Voet[1] and E. Lebacq[2]
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Name C. VAN HOECKE & PIERRE VOET
Title CLINICAL PROJECT DIRECTOR
Affiliation SMITHKLINE BEECHAM BIOLOGICALS
Street RUE DE L'INSTITUT, 89
City RIXENSART State — Zip B-1330 Country BELGIUM
phone (02) 656 8770
fax (02) 656 9132
e-mail Vanhoecke @scbb...

Mail to: Lyme Disease Foundation, One Financial Plaza, Hartford, CT 06103-2610
(860) 525-2000 Fax (860) 525-8425 E-mail: lyme fnd@aol.com

1

A Murine Model for Studying Transplacental Transmission of Borrelia burgdorferi

Sousan Sayahtaheri Altaie, Ph.D.
Assistant Professor of Pediatrics,
SUNY at Buffalo and
Microbiology Review Officer,
U. S. Food and Drug Administration

Background (1)

Gardner,T. 1995. Lyme Disease. In J. S. Remington and J. O. Klein (eds.) Infectious diseases of the fetus & newborn infant, , 4th ed.

- A review of the congenital and gestational Lyme borreliosis literature yielded 161 cases for which the outcome of the episode of gestational Lyme borreliosis was noted.
- The 46 cases of adverse outcomes of these 161 cases were found to include miscarriage, stillbirth, perinatal death, congenital anomalies, systemic illness, early-onset fulminating or mild sepsis, and late-onset chronic progressive infection.

Background (2)

Gardner,T. 1995. Lyme Disease. In J. S. Remington and J. O, Klein (eds.) Infectious diseases of the fetus & newborn infant, , 4th ed.

Fetal/ Neonatal abnormality	No. with finding/Total (%)
Cardiac	11/46 (24)
Neurologic	9/46 (20)
Orthopedic	6/46 (13)
Ophthalmic	3/46 (7)
Misce. Anomalies	8/46 (17)
Fetal/Neonatal demise	22/46 (48)
Rash	5/46 (11)

Background (3)

Gardner,T. 1995. Lyme Disease. In J. S. Remington and J. O, Klein (eds.) Infectious diseases of the fetus & newborn infant, , 4th ed.

Trimester	Antibio.	No./Total (%) Adv. Out.	No./Total (%) Norm. Out.
First	Yes	8/17 (47)	9/17 (53)
	No	8/10 (80)	2/10 (20)
Second	Yes	5/22 (23)	17/22 (77)
	No	4/5 (80)	1/5 (20)
Third	Yes	0/8 (0)	8/8 (100)
	No	1/2 (50)	1/2 (50)

3

Borrelia burgdorferi Strains

- Strain 297, a Northeastern isolate
  - isolated from human CSF
- Strain W18, a Midwestern isolate
  - isolated from *I. scapularis* nymph off a Veery bird along St. Croix River in Minnesota
- Strain Son-1 from the Pacific region
  - isolated from *I. pasificus* nymph in Sonoma County, California

Mice

- Five- to six-week-old inbred C3H/HeJ mice were obtained from Jackson Laboratories.
  - Animals had access to food and water ad libitum and were exposed to a 12-h light/12-h dark cycle.
  - Three to five days prior to infection and mating, the animals were anesthetized by inhalation of Methoxyflurane and were surgically splenectomized.

4

B. burgdorferi propagation

- The isolates were maintained in our laboratory by animal inoculation and recovery at 32°-34°C in modified Barbour-Stoener-Kelly medium (BSK II) with the following antibiotics: Phosphomycin, 400 µg/mL; 5-Fluorouracil, 100 µg/mL; Trimethoprim, 10 µg/mL; Sulfamethoxazole, 50 µg/mL.

Animal Inoculation

- Splenectomized test animals were inoculated subcutaneously on the right lower quadrant of the ventral surface, with 10^6 - 10^7 *B. burgdorferi* in 0.25 mL BSK II medium.
- Splenectomized control animals were inoculated with 0.25 mL sterile BSK II medium.

5

Culture Method

- Using aseptic techniques various tissues were harvested and rinsed in sterile saline. After rinsing, a small section of the tissue was placed in 4 mL of BSK II and incubated at 32°-34°C for nine weeks. The cultures were examined by darkfield microscopy every three weeks for presence of motile spirochetes.

Experimental Design: In-utero

- Early-gestation
  - Infected 6-7 days after observation of coital plugging and sacrificed 6 days after infection
- Middle-gestation
  - Infected 9-10 days after observation of coital plugging and sacrificed 6 days after infection
- Late-gestation
  - Infected 12-13 days after observation of coital plugging and sacrificed 6 days after infection

(6)

Experimental Design: In-utero

- Sacrificed adults by exsanguination and soaked them in ethanol until necropsy
  - Cultured adult brain, ear, heart, bladder, liver, kidney, and spleen after rinsing in sterile saline
- Harvested fetuses and placentas aseptically
  - After rinsing in sterile saline, cultured full minced fetuses in early gestation and cultured 1/2 fetuses in mid- and late- gestation.
  - After rinsing in sterile saline, cultured 1/2 placentas

Experimental Design: Postnatal

- Infected and mated adults the same day
- If coital plugging did not occur within a week of mating, animals were discarded.
- Allowed pregnancies to go to term and sacrificed adults and pups at 1, 7, 14, and 21 days post delivery

Experimental Design: Postnatal

- Sacrificed adults by exsanguination and soaked in ethanol until necropsy (10-20 min.)
  - Cultured adult B, E, H, BL, L, K, and S after rinsing in sterile saline
- Sacrificed pups by exsanguination and soaked in ethanol until necropsy (20-30 min.)
  - Cultured, Ear and skin from scruff in one tube, B, H, BL, K, and S in another tube, and milk content of the stomach in a third tube
  - Milk was cultured on all 1-day-old and some of the 7- and 14- day-old pups.

B = Brain
E = Ear
H = Heart
BL = Bladder
L = Liver
K = kidney
S = Spleen

Results: In-utero (1)

- 100% of experimentally infected animals were culture positive.
- None of the uninfected mating pairs were culture positive; i.e., no sexual transmission.
- All the fetuses and placentas were culture negative.

(8)

Results: In-utero (2)

- However, by PCR, from 34 experimentally infected females in groups A and C, 7 (21%) transmitted *B. burgdorferi* to their fetuses and/or placentas.
  - 4/11 (36%) during early-gestation
  - 3/12 (25%) during middle-gestation
  - No transmission during late-gestation
- No PCR-positive samples in group B

Results: In-utero (3)

- Of the 7 female mice that transmitted *B. burgdorferi*,
  - one was infected with strain 297
  - three were infected with strain W18
  - three were infected with strain Son-1
- Low numbers but no difference among strains, thus data from strains were pooled

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Results: In-utero (4)

PCR positive samples/Total No.

Gestation	F & P	P only	F only	Total
Early	1/30 (3%)	3/30 (10%)	1/30 (3%)	5/30 (17%)
Middle	2/57 (3%)	1/57 (2%)	2/57 (3%)	5/57 (9%)
Late	0/47 (0%)	0/47 (0%)	0/47 (0%)	0/47 (0%)
Total	3/134 (2%)	4/134 (3%)	3/134 (2%)	10/134 (7%)

Results: Postnatal (1)

- 100% of experimentally infected animals were culture positive.
- None of the uninfected mating pairs were culture positive; i.e., no sexual transmission.

H.H.S. proved milk transmission in 1997 - Work done by FDA

(10)

Results: Postnatal (2)

- From the 25 experimentally infected females in which the milk was cultured in groups A and C, 2 (8%) transmitted *B. burgdorferi* to their pups on day one via their milk.
  - 2 out of 4 pups, group C, strain 297
  - 1 out of 8 pups, group A, strain W18
- None of the milk samples collected after day 1 were culture positive.
- Transmission rate via milk from infected dams to offspring was 2.3% (3/131).

Results: Postnatal (3)

- Milk samples collected on days 1 and 7 were positive for anti-*B. burgdorferi* antibodies by ELISA.
  - No milk samples collected for ELISA from pups older than 7 days
  - Milk collected from control animals up to day 14 were negative by ELISA for anti-*B. burgdorferi* antibodies.

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Results: Postnatal (4)

- Among 49 experimentally infected dams from groups A and C, 5 (10.2%) transmitted *B. burgdorferi* to their pups in-utero
  - 3/5 dams were infected with strain 297
    - first litter : 2/6 pups were culture positive
    - second litter: 1/2 pups was culture positive
    - third litter: 1/7 pups was culture positive

Results: Postnatal (5)

- 2/5 dams were infected with strain Son-1
  - first litter: 1/7 pups was culture positive
  - second litter: 2/6 pups were culture positive
- None of the dams infected with strain W18 had culture positive pups.
- All control pups were culture negative.
- Thus, the in-utero transmission rate from infected dams to offspring was 3% (7/224) in this study.

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Results: Postnatal (6)

- Close contact transmission
  - Four of 24 litters from mating pairs in group B had culture positive pups (rate, 17%)
  - (9/132 pups; rate, 7%)
    - one male infected with strain 297
    - one male infected with strain W18
    - two males infected with strain Son-1
  - All four females were culture-, ELISA-, and PCR- negative.

Results: Postnatal (7)

- Close contact transmission
  - Litter 1, 7-days-old : 3/5 culture positive, strain 297
    - 2 only E/S positive; 1 E/S and other tissues positive
    - no milk or serum was collected
  - Litter 2, 7-days-old: 1/8 culture positive, strain W18
    - E/S positive; Milk and serum ELISA-negative
  - Litter 3, 7-days-old: 3/6 culture positive, strain Son-1
    - E/S positive; Milk and serum ELISA- negative
  - Litter 4, 21-days-old: 2/4 culture positive, strain Son-1
    - E/S positive; *no milk collected, serum* ELISA-negative

E/S = Ear and skin from the scruff

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Summary (1)

- Experimental infection rate is 100% in this model.
- The three tested strains seem to behave similarly in this model.
- If groups A and C are combined from both phases
  - from 83 experimentally infected females 12 (14%) transmitted *B. burgdorferi* to their offspring inutero
  - the in-utero transmission rate from infected females to offspring is 5% (17/358) in this model.

Summary (2)

- In-utero transmission seems to occur during the early- and middle-gestation periods.
- From the 25 experimentally infected females in groups A and C, 2 (8%) transmitted *B. burgdorferi* to their pups via colostrum.
- Transmission rate via colostrum from infected dams to offspring is 2% (3/131).

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Summary (3)

- Close contact transmission does not occur among mice >3-week-old in this model.
- From 24 experimentally infected males in group B, 4 (17%) transmitted *B. burgdorferi* to their pups.
- The close contact transmission rate from infected males to offspring is 9/132 (7%).

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