HHS Working Group on Lyme and Other Tickborne Diseases

Special Webinar on Lyme Disease and *Borrelia* Persistence
Disclaimer

• The views expressed in this webinar are those of the participants and do not reflect the official policy or position of the Department of Health and Human Services, or the U.S. Government.
Lyme Disease in the U.S.

- Caused by the spirochete bacteria *Borrelia burgdorferi*
- Harbored in small mammals, and transmitted by ticks that are on deer
- Over 30,000 cases reported to CDC in 2012
- 7th most common reportable disease in U.S.
- Cases have been increasing steadily both in numbers and distribution
Lyme Disease Clinical Challenges

• Symptoms range from an erythema migrans rash, early in the course of infection, to neuritis, carditis, and arthritis in later, disseminated stages of illness.
• Prompt treatment with 2-4 weeks of oral doxycycline results in symptomatic cure of the great majority of patients.
• A subset of patients, especially those diagnosed and treated in later stages of illness, may have persistent fatigue, muscle aches, short-term memory problems, and other nonspecific symptoms.
• One of the highest priority research needs in the field of Lyme disease is to elucidate the specific cause or causes of symptoms in these patients and to determine the safest and most effective treatment options.
Purpose of this webinar: To discuss the state of the science of persistence of infection by Borrelia burgdorferi

Objective: Better understanding should lead to improved diagnostics, safer and more durable therapeutics, and an improved vaccine
Speakers and Topics

Convener:
**Dr. Ben Beard**, CDC, NCEZID, Division of Vector-Borne Diseases

Moderator:
**Dr. Joseph Breen**, NIH, NIAID

Speakers:
**Dr. Stephen Barthold**, University of California, Davis
*The Comparative Biology of Borrelia burgdorferi Persistence*

**Dr. Linda Bockenstedt**, Yale School of Medicine
*Design of Animal Studies to Assess Borrelia burgdorferi Persistence*

**Dr. Monica Embers**, Tulane University
*Studies of B. burgdorferi Persistence in the Nonhuman Primate*

**Dr. Adriana Marques**, NIH, NIAID
*Searching for Persistence of Infection in Lyme disease*

**Dr. Linden Hu**, Tufts University
*Borrelia burgdorferi Persistence: Consensus and Controversy – where do we go from here?*
Questions can be submitted online through the webinar interface. Please provide your identity and indicate the speaker to whom you are addressing your question.
For More Information on Lyme Disease:

CDC Lyme Disease site

NIH/NIAID Lyme disease page
The Comparative Biology of
*Borrelia burgdorferi* Persistence

Stephen W. Barthold, DVM, PhD
Distinguished Professor, Emeritus
Persistence:

Essential for *Borrelia burgdorferi* Life Cycle

In Ticks
In Many Different Hosts
Minimal or No Inflammation During Persistent Infection
COMPARATIVE BIOLOGY

*Borrelia burgdorferi* Persistence
Persistence in Humans:

Without Rx: Yes

Following Antibiotic Rx: ?
“…it is probably unrealistic to expect that antimicrobial therapy per se will eliminate every single microorganism from the Infected host…

“…the role of antimicrobial therapy in vivo can be thought of in terms of tipping the balance in favor of the host’s own defenses against a particular pathogen.”


Normal biology of *Borrelia burgdorferi*:
- Immune evasion
- Persistence

Can antimicrobial therapy be expected to be completely effective?
Preponderance of Evidence...

Multiple Labs, Multiple Species, Multiple Drugs

Similar Results

Borrelia burgdorferi DNA (PCR)-positive  
Culture-negative

VAILABLE ?
VIABLE?

✓ Spirochetes in Tissues of Treated Mice

✓ Xenodiagnosis:
  DNA Spirochetes in Ticks (IFA)

✓ DNA Transmission
  Tissue Transplants from Treated Mice to Recipient Mice

✓ *Borrelia burgdorferi* RNA Transcription in Tissues of Treated Mice

✓ Resurgence of *B. burgdorferi* DNA in Tissues at 12 Months After Treatment of Mice

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**Immunohistochemically-Labelled**

*B. burgdorferi* Spirochete in Tissue of an Antibiotic-Treated Mouse

“The studies show no evidence of recrudescence or persistence of clinical or histological findings of an active inflammatory process consistent with *B. burgdorferi* infection…”

“Therefore, even if a few residual *B. burgdorferi* spirochetes or their DNA debris persist after antibiotic treatment in animal systems, they no longer appear to be capable of causing disease.”
Relative Host Cytokine RNA Transcription
In *B. burgdorferi* DNA-Positive Tissues of Mice
12 months after Antibiotic Rx *

* relative to tissues from age-matched, uninfected controls

Hodzic, et al. (2014) Resurgence of persisting non-cultivable *Borrelia burgdorferi* following antibiotic treatment in mice.
PLOS ONE 9:e86907.
Every study design may have flaws, but animal model findings are compelling and justify further investigation into mechanisms & significance of *Borrelia burgdorferi* persistence

“Many anecdotes make a statistic”

Nikolaas Tinbergen, Nobel Laureate


Symptoms after Lyme Disease Treatment

Persistence of live spirochetes that can multiply and cause recurrent disease?

Persistence of remnants of spirochetes that cause inflammation?

The host immune response?
Animal Models to Study Borrelia Persistence: Experimental Factors Influencing Outcome

- **Borrelia burgdorferi**
  - Origin and Strain
  - Inoculum dose

- **Method of Transmission**
  - Inoculation of cultured Borrelia or tick-transmission

- **Mammal**
  - Species
  - Immune competency
    - Naturally-arising variation
    - Genetic modification
    - Drug-induced
  - Reservoir competency
    - Can the species transmit infection back to feeding ticks?

- **Antibiotics**
  - Bacteriostatic vs bactericidal
  - Oral, parenteral
  - Dosing/drug pharmacokinetics
  - Stage of infection

- **Methods for Evaluation of Persistence**
  - Evaluation of the animal
    - Culture, DNA/RNA, microscopy, immune response, xenodiagnosis with ticks
  - Demonstration of persistent viable infectious Borrelia
    - Immunosuppression of animal
    - Tissue transplant
    - Feeding of xenodiagnostic ticks on an uninfected mammalian host
Antibiotic Dosing & Infection Duration

2002 J Infect Dis
Detection of Attenuated, Noninfectious Spirochetes in *Borrelia burgdorferi*–Infected Mice after Antibiotic Treatment

Linda K. Bockenstedt,¹ Jialing Mao,¹ Emir Hodzie,² Stephen W. Barthold,³ and Durland Fish²

2012 J Clin Invest

• **Tick Transmitted Infection**
  
  C57BL/6 MyD88−/− (immunodeficient mice), 1 antibiotic-treated mouse was culture and xeno positive; only Borrelia DNA detected in other antibiotic-treated mice, xenodiagnostic ticks negative for DNA and by culture

  Normal C57BL/6 mice: trace DNA found in only 1 antibiotic-treated mouse; control infected mice were culture and xeno positive

• **Infection Introduced With Cultured Borrelia**
  
  C3H MyD88−/− mice, treatment (ceftriaxone) started at ~4 months of infection;

  DNA in tissue of all antibiotic-treated mouse; Borrelia remnants adjacent to cartilage; negative xenodiagnosis and tissue transplant. Control infected mice were culture and xeno positive.

• C3H mice, tick-transmitted infection, DNA + in tissue; xenodiagnosis + by DFA for limited period (3 months);

• no recrudescence of infection with immunosuppression

• Antibiotic pharmacokinetics not optimized
Alan Barbour: “Remains of Infection”

Stages of dying

- Loss of cell wall
  - Replication, reversion
- No cell wall
  - No replication or reversion
- Cell lysis or immune breakup
  - Blebs or vesicles containing DNA, protein
- Degradation
  - DNA degrades, protein remains
Does Duration of Infection Prior to Treatment Matter?

Experiments conducted in normal and immuno-deficient C3H mice

**C3H mouse results:**
- Detected DNA in only one antibiotic-treated mouse evaluated 7 weeks after antibiotics. Tissue transplants into uninfected mice all negative.
- All control infected mice were culture +; tissue transplant recipients culture +

**C3H MyD88-/- results:**
- Live borrelia detected after 2 doses (1 day) of antibiotics when treatment started at 3 weeks of infection; on day 3 of antibiotics when treatment started at 4 months of infection. All control mice culture +
- No viable spirochetes detected thereafter, but remnants containing antigen and DNA present. Recipients of tissue transplants from antibiotic-treated mice all negative.
- Remnants were less abundant and in some mice undetectable by 9 months after antibiotic therapy

*Bockenstedt, unpublished data*
Cultured Borrelia ≠ Tick-Transmitted Borrelia

Cultured Borrelia

• No selection pressure
  – “clonal” populations not always genetically identical
  – Growth variants
• Can enrich for “persisters”

Tick-Transmitted Borrelia

• Tick selection pressure
  – Bottlenecks at midgut epithelium and hemocael prior to salivary gland entry
  – Changes in gene expression
• Tick saliva

Adapted from Lewis, Nat Rev Microbiol 2007;5:48-56
Designing Animal Experiments for Insight into Human Lyme Disease

One infected *Ixodes nymph*

- Human Infection

- Investigating antibiotic efficacy

1. High dose culture inoculation, late log phase

2. Ticks infected directly using cultured Borrelia

3. Large numbers of ticks to transmit infection

4. Immunodeficient hosts
Host Genetic Variation Influences Disease

Lysosomal β-glucuronidase regulates Lyme and rheumatoid arthritis severity

Kenneth K.C. Bramwell,¹ Ying Ma,¹ John H. Weis,¹ Xinjian Chen,¹ James F. Zachary,² Cory Teuscher,³ and Janis J. Weis¹

¹University of Utah, Salt Lake City, Utah, USA. ²University of Illinois, Urbana, Illinois, USA. ³University of Vermont, Burlington, Vermont, USA.

Normal enzyme activity and recycling of glycoproteins

Low enzyme activity leads to accumulation of inflammatory glycoproteins in the cell

C57BL/6 Mouse
Lyme arthritis-resistant

C3H/HeN Mouse
Lyme arthritis-susceptible
The Human “Ecosystem” in Health and Disease: *Raising Questions We Haven’t Yet Considered*

**About HIPC**

The Human Immunology Project Consortium (HIPC) program was established in 2010 by the NIAID Division of Allergy, Immunology, and Transplantation as part of the overall NIAID focus on human immunology. The purpose of the HIPC is to capitalize on recent advances in immune profiling methods in order to create a novel public resource that characterizes diverse states of the human immune system following infection; prior to and following vaccination against an infectious disease; or prior to and following treatment with an immune adjuvant that targets a known innate immune receptor(s).
Studies of *B. burgdorferi* Persistence in the Nonhuman Primate

Monica E. Embers, Ph.D.
Division of Bacteriology & Parasitology
Tulane National Primate Research Center
Post-treatment Lyme disease Syndrome (PTLSD)

- Potential causes include:
  - Induction of inflammatory responses by lingering dead spirochetes or spirochetal antigen
  - Continuation of active spirochetal infection
  - Irreversible sequelae from previous active infection (autoimmune)
Antibiotic efficacy

- Doxycycline is microbiostatic; efficacy relies on immune clearance of static bacteria

- *B. burgdorferi* evades the immune response in many ways—persistence is the norm in immunocompetent hosts

- Dormant bacteria more tolerant of microbiostatic antibiotics

- *B. burgdorferi* survives for many months inside ticks without nutrient replenishment or replication

- *B. burgdorferi* can be found deep in connective tissues and joints (tissue penetration of antibiotic?)
The rhesus macaque model of Lyme disease

- Rhesus macaques most closely mimic the multi-organ character of human disease.
- Unlike other animal models, disease hallmarks such as erythema migrans, carditis, arthritis, and neuropathy of the peripheral and central nervous systems are all observed in macaques.
- The spirochete burden in tissues following dissemination is very small, as in humans.
- The advantages of this model are:
  - (1) Compared to mice, the disease course, including duration and quantity of Bb in the blood more similar to that of humans;
  - (2) Compared to human samples, the infection history (e.g. exact point of exposure, duration) is known.
Persistence of *Borrelia burgdorferi* in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection

Monica E. Embers¹, Stephen W. Barthold⁴, Juan T. Borda², Lisa Bowers¹, Lara Doyle⁵, Emir Hodzic⁶, Mary B. Jacobs¹, Nicole R. Hasenkampf¹, Dale S. Martin¹, Sukanya Narasimhan⁵, Katherine M. Philipp- Falkenstein³, Jeanette E. Purcell³, Marion S. Ratterree³, Mario T. Philipp¹

1 Division of Bacteriology & Parasitology, Tulane National Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana, United States of America; 2 Comparative Pathology, Tulane National Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana, United States of America; 3 Veterinary Medicine, Tulane National Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana, United States of America; 4 Center for Comparative Medicine, Schools of Medicine and Veterinary Medicine, University of California Davis, Davis, California, United States of America; 5 Section of Rheumatology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, United States of America

Abstract

The persistence of symptoms in Lyme disease patients following antibiotic therapy, and their causes, continue to be a matter of intense controversy. The studies presented here explore antibiotic efficacy using nonhuman primates. Rhesus macaques were infected with *B. burgdorferi* and a portion received aggressive antibiotic therapy 4-6 months later. Multiple methods were utilized for detection of residual organisms, including the feeding of lab-reared ticks on monkeys ( xenodiagnosis), culture, immunofluorescence and PCR. Antibody responses to the *B. burgdorferi*-specific C6 diagnostic peptide were measured longitudinally and declined in all treated animals. *B. burgdorferi* antigen, DNA and RNA were detected in the tissues of treated animals. Finally, small numbers of intact spirochetes were recovered by xenodiagnosis from treated monkeys. These results demonstrate that *B. burgdorferi* can withstand antibiotic treatment, administered post-dissemination, in a primate host. Though *B. burgdorferi* is not known to possess resistance mechanisms and is susceptible to the standard antibiotics (doxycycline, ceftriaxone) in vitro, it appears to become tolerant post-dissemination in the primate host. This finding raises important questions about the pathogenicity of antibiotic-tolerant persisters and whether or not they can contribute to symptoms post-treatment.


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*¹Current address: Biologic Resources Laboratory, University of Illinois, Chicago, Illinois, United States of America

- over 21,000 views

- Received “Faculty of 1000” recommended status
Tick-mediated infection, treatment, and evaluation for persistent infection

Status: serology, pathology complete; assessing necropsy tissues for presence of *B. burgdorferi*
Tick-mediated infection, treatment, and evaluation for persistent infection

Antibody responses to four different Bb antigens over 60 weeks following infectious tick bite. Animals IH11 (dark blue) and IK66 (red) were treated with doxycycline between weeks 16-20. The other animals (IP55, IP67 and IN16) were not treated. All values shown are triplicate averages with the mean pre-immune value per individual animal subtracted.
Only one of the ten animals developed a bona fide EM lesion, while others exhibited some diffuse erythema.

Culture of skin biopsy tissue resulted in positive detection for 5 of 10 monkeys.

Detection of Bb by DNA PCR was positive for 8 of 10 monkeys.
Histopathology from infected monkeys

Untreated

IP55: Perineural inflammation of the Right Ulnar nerve, 10X

IN16: Axillary lymph node hyperplasia, 2x (upper) and lung hyperplasia (lower), 10X

Treated

IH11: Cervical Spinal nerve inflammation, 5X (upper) and lung focal inflammation (lower), 5X

IK66: Focal inflammation in the skeletal muscle of the thigh (above) and arm (below), 10X
Results

Very few ticks positive for xenodiagnosis—why?

Anti-tick immunity

May affect xenodiagnosis and not transmission
Infectivity of "persisters" in NHP (Koch’s postulates)

Status: Experimental protocol complete; assessing ticks and recipient animal tissues for *B. burgdorferi*
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Searching for persistence of infection in Lyme disease

Ticks as a medical device for xenodiagnosis of Lyme disease

Adriana Marques, MD
LCID, NIAID
Post Lyme disease symptoms (PLDS)

The pathogenesis of these symptoms is an area of great controversy.

• Studies of patients with erythema migrans have shown that 0-40% of the patients have persistent or intermittent non-specific symptoms of mild to moderate intensity 6-24 months after therapy.

• Culture and PCR (direct detection of the bacteria) have very low sensitivity outside skin and blood samples in early disease, or PCR in synovial fluid of patients with Lyme arthritis. Current antibody-based assays cannot be used to determine successful eradication of the organism.

• Four antibiotic treatment trials of PTLDS patients showed that retreatment provides little if any benefit and carries significant risk. The four trials included 195 patients (48 + 115 + 32).

• Evaluating patients with post treatment Lyme disease symptoms can be quite complex. One of the main questions is whether in some of the patients, persistent infection could be the cause of the symptoms.
Lyme Disease - Xenodiagnosis

- Studies have shown that with PCR methods, spirochetes/DNA may persist after therapy in dogs, mice and monkeys. These spirochetes (?) are not cultivable, but could be acquired by xenodiagnosis and transmitted to SCID mice.
- What would be the results of xenodiagnosis in humans? Xenodiagnosis could provide researchers with a new tool with which to study the mechanisms of disease.
- Ticks are not simply “crawling needles and syringes”. Tick saliva is chemoattractant for the organism and feeding ticks, despite taking only small quantities of blood, sample much more in extracellular fluid and have the potential to aggregate and concentrate bacteria from a wide area, improving sensitivity.
- Nothing was known about the parameters of xenodiagnosis of Lyme disease in humans.
- We set up a phase I study to develop the technique and to assess the safety of the procedure.
Xenodiagnosis to detect *Borrelia burgdorferi* infection: a first-in-human study

Xenodiagnosis to Detect *Borrelia burgdorferi* Infection: A First-in-Human Study

Adriana Marques,¹ Sam R. Telford III,² Siu-Ping Turk,¹ Erin Chung,³ Carla Williams,⁴ Kenneth Dardick,⁵ Peter J. Krause,⁶ Christina Brandeburg,³ Christopher D. Crowder,⁷ Heather E. Carolan,⁷ Mark W. Eshoo,⁷ Pamela A. Shaw,⁸ and Linden T. Hu³

¹Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; ²Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, and ³Department of Medicine, Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, Massachusetts; ⁴SAIC-Frederick, Inc, NCI-Frederick, Frederick, Maryland; ⁵Mansfield Family Practice, Storrs, ⁶Department of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale School of Medicine, New Haven, Connecticut; ⁷Ibis Biosciences, Inc., a subsidiary of Abbott Company, Carlsbad, California; and ⁸Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

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Study Protocol

• Participants were enrolled at 3 sites in Massachusetts, Connecticut, and Maryland. Written informed consent was obtained from all participants.
• The study was approved by the IRB at each center and was conducted under an IDE approved by the FDA. An independent medical monitor reviewed interim data for safety.

Study Populations:
• EM, 1 to 4 months post antibiotic therapy
• Post-treatment Lyme disease syndrome
• High C6 antibody (index above 3 for at least 6 months post antibiotic therapy)
• Erythema migrans, very early therapy (potential positive control)
• Lyme arthritis, no therapy (potential positive control)
• Healthy volunteers

Timeline to initiation of the study:
• Sept 2008 to April 2009: Collaboration discussion, protocol development, initial internal reviews.
• April 2009 to August 2010: Regulatory reviews
• September 2010- Site initiation visits.
• November 2010- first enrollment.
Xenodiagnostic Ticks

- Pathogen-free *Ixodes scapularis* larval ticks were obtained from a laboratory-maintained colony at the Cummings School of Veterinary Medicine.

- One-third of larval ticks from each egg mass were tested for *Borrelia burgdorferi*, *Babesia* spp., *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, *Bartonella* spp., *Rickettsia* spp., deer tick virus and orbiviruses by PCR.

- SCID mice were infested with subsets of larvae from each batch and monitored for 1 month for illness.

- A subset of the ticks was also tested by PCR and electrospray ionization mass spectroscopy (PCR/ESI-MS) for *Francisella tularensis*, *Babesia* spp, *Borrelia* spp, *Spirochetes*, *Rickettsia* spp., and alphaproteobacteria at IBIS.
Tick Placement Procedure

- At Day 1, 25–30 larval ticks were placed under the dressing, close to an area where disease was observed (EM site, close to an affected joint), if possible.
- Participants returned to the clinic for recovery of the ticks at 3-6 days. At the last day, skin punch biopsy was performed at a feeding site.
- Participants followed at 7-10 days, 4-6 weeks and 3 months after tick removal.

First participant enrolled: Only 2 ticks feed, the others get stuck on the adhesive. We will spend the next 6 months testing different versions of the dressings and placement procedures.

After the addition of a foam ring to create a barrier between ticks and adhesive, we were able to get between 30 and 50% of ticks to feed successfully.
Testing of xenodiagnostic ticks

Ticks tested using 2 different protocols:

Protocol 1: Live fed ticks molted to nymphs. Nymphs were placed on SCID mice. The nymphs were then tested by culture and PCR. The SCID mice were tested at 2 weeks (ear punch biopsies) and at 4 weeks (skin, ankle joint, heart, and bladder tissues) by culture and PCR. PCR target ospA, and positive results were confirmed by using a 2nd primer set for ospA, and testing for flaB, recA, and ospC.

- Review showed significant problems with molting and recovering of nymphs after feeding on SCID mice, the protocol was amended to remove these steps, and perform direct analysis of the fed larva.

Protocol 2: Ticks were tested by:
- Culture, PCR, injection of lysates into SCID mice with subsequent culture and PCR
- AND/OR
  - Isothermal amplification PCR electrospray ionization mass spectrometry (IA/PCR/ESI-MS performed by IBIS) - this assay uses 8 PCR primer pairs that target 7 Borrelia genes, and can be used to distinguish genotypic variation
Participants Characteristics

36 individuals underwent xenodiagnosis: 21 men and 15 women, median age 55 years. 7 patients underwent more than one procedure.

- 10 patients with high C6
- 10 patients with PTLDS
- 5 patients with EM after antibiotic therapy
- 1 patient with EM in early treatment ("positive control")
- 10 healthy volunteers

- We were not able to enroll untreated Lyme arthritis patients ("positive control").

High C6 group: enrolled a median of 4.5 years after their original diagnosis and received a median of 2.5 courses of antibiotics. The most common presenting manifestation was Lyme arthritis.

PTLDS group: enrolled a median of 3.8 years after their original diagnosis and received a median of 2 courses of antibiotics. The most common initial presenting manifestation of Lyme disease was EM. The most common symptoms at enrollment were fatigue, difficulty concentrating, memory complaints, and arthralgias.
Xenodiagnosis

• Xenodiagnosis was well tolerated.
• The most common adverse event was mild itching at the site, seen in 58% of subjects, with a median duration of three days.
• All subjects successfully completed the tick placement.
• There were no withdrawals during the study.
• There were no serious adverse events associated with the procedure.
• Larval ticks required 4-5 days to feed to repletion.

23 participants with Lyme disease had at least one tick tested.

• 19 negative
• 2 indeterminate (we could not rule out laboratory contamination).
• Ticks from healthy volunteers tested by protocol 1 and 2 were negative.
• All tissues from SCID mice tested negative by PCR and culture.
• All skin biopsies were negative by culture and culture PCR. The 6 biopsies tested by direct IA/PCR/ESI-MS were negative.
Results

EM on therapy:
Individual was completing 4th day of antibiotic therapy when the ticks were collected.
Positive by IA/PCR/ESI-MS from a single tick and from a pool of three ticks.
The single tick contained a mixture of two *B. burgdorferi* genotypes. Six other ticks were tested by culture and PCR.
Repeat xenodiagnosis 7 months after antibiotic: IA/PCR/ESI-MS testing of 10 ticks was negative

PTLDS:
Positive in 2 separate xenodiagnostic procedures (8 months apart).

Xeno 1- One nymph positive by PCR of the nymph lysate culture.
IA/PCR/ESI-MS: identified the DNA as a novel genotype of *B. burgdorferi*.

Xeno 2- 1 tick positive by IA/PCR/ESI-MS, consistent with the previously found genotype. Another tick was tested by PCR and culture and was negative.
Summary

• We developed a protocol for xenodiagnosis with *I. scapularis* larva in humans that is well tolerated.
• Adverse events were minimal and limited predominantly to itching at the tick bite sites.
• Up to 30 larval ticks can be applied and 30 and 50% of ticks feed successfully.
• Larval ticks required 4-5 days to feed to repletion in humans.
• Initial results show that the majority of the patients with Lyme disease treated with antibiotic therapy are negative by xenodiagnosis.

Caveats:
• In general, a small number of ticks per participant were tested, particularly in the early participants. In animal studies, the numbers of fed ticks tested were important for sensitivity of xenodiagnosis.
• There is no gold standard for comparison of results.
• DNA only: Insufficient evidence regarding the presence of viable spirochetes.

Future:
Studies to identify whether persistence of bacteria or products can be used to predict persistence of symptoms.
Xenodiagnosis may be used as a tool to:
- Develop better tools
- Test hypotheses and new strategies for therapy
We thank the study participants for their enthusiastic involvement!
"To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science."

Albert Einstein

‘It is difficult to say what is impossible, for the dream of yesterday is the hope of today and the reality of tomorrow.’

Robert H. Goddard
B. burgdorferi Persistence: Consensus and Controversy

Where do we go from here?
Borrelial Persistence: Consensus

Some findings are very consistent across laboratories, antibiotic regimens, delivery systems and hosts.

- Antibiotics greatly decrease the number of bacteria/amount of DNA in treated animals

- C6 antibody titers decrease after antibiotic therapy
Borrelial Persistence: Consensus

- DNA persists and can be detected by xenodiagnosis, PCR and/or IBIS
- Protein antigens persist and can be detected by immunofluorescence
- Bacteria are not cultivatable
Antibiotic regimens have differed and there are questions about appropriateness of the doses

Transmission (of DNA) from a xenodiagnostic tick to an uninfected animal has been seen in only one publication. All other studies have not been able to transmit bacteria to new hosts
Implications for human Lyme disease

No animal model for post-treatment Lyme disease syndrome (PTLDS).

Therefore, no linkage of persistence of bacteria/DNA/proteins to symptoms of PTLDS
Implications for human Lyme disease

• Antibiotic dosing issues
  - how many humans take antibiotics as prescribed?
  - lack of adjustment of human antibiotic dosing for weight, vol of distribution.

• Inability to culture after antibiotic therapy
Inability to culture bacteria

Possible explanations (all are unlikely or break established rules but something must be true):

• Laboratory contamination

• DNA/RNA/proteins from bacteria persist for many months after the organisms are dead

• Bacteria persist but are altered by antibiotic exposure to no longer be cultivatable
Persistence of Bacterial products without bacteria

- DNA from killed *B. burgdorferi* injected into mice quickly becomes undetectable

- DNA/RNA from other sources (e.g. CPG DNA, fetal/maternal studies) show rapid clearance from hosts

- Studies of multiple foreign proteins have shown rapid clearance from animal and human hosts to the level of non-detectability

- No detection of *B. burgdorferi* DNA from skin biopsies at the tick bite site. Also xenodiagnostic positives are difficult to explain as blood PCRs all negative after treatment.
Survival of non-cultivatable bacteria

- “Persisters” seen in many different bacteria after antibiotic treatment
- Phenotypic variants that form during normal bacterial growth and are not genetically altered
- Likely multiple pathways to forming persister cells, but many revolve around slowed cell division
- Persisters may be a reservoir for reactivation and in other bacteria, are cultivatable after removal of antibiotics
Survival of non-cultivatable bacteria

• Some studies have suggested plasmid loss as a reason for non-cultivatability of *B. burgdorferi*, but reliability of detection of plasmids in the setting of low #’s of bacteria is unclear and results are inconsistent.

• Some evidence for antibiotic selection of non-replicating bacterial persisters in other bacteria (e.g. *Salmonella*, Helanie *et al.*, Science, 2014). However, these eventually resumed growth.
Survival of non-cultivatable bacteria

• Only one study showing evidence of resumed “growth” after antibiotics. If “persisters” are an evolutionary mechanism to allow a reservoir of bacteria to survive contact with naturally occurring sources of antibiotics, a regrowth phase would be key.
Studies of Borreliial Persistence: What’s next?

• Better understanding of clearance of Borreliial proteins from killed organisms

• New strategies for trying to cultivate bacteria after antibiotic therapy—do they need a signal to jump-start re-growth or are we trying to raise the dead?

• Better tests for detecting the presence of B. *burdorferi* products in humans
Studies of Borrelial Persistence: What’s next?

- Patient studies to identify whether persistence of bacteria or products can be used to predict persistence of symptoms.