Killing Borrelia burgdorferi – Is it possible?

Eva Sapi Ph.D.
University of New Haven
Borrelia burgdorferi the spirochete that causes Lyme disease

- In 1982, the etiologic agent of Lyme disease was discovered by Willy Burgdorfer who isolated spirochetes belonging to the genus *Borrelia* from the mid-guts of *Ixodes* ticks.
- He showed that these spirochetes reacted with immune serum from patients that had been diagnosed with Lyme disease. Subsequently, the etiologic agent was given the name *Borrelia burgdorferi*.

*Borrelia burgdorferi*, FA stain (CDC)
Borrelia burgdorferi (B31) genome

- Has a chromosome of 910,725 base pairs, with at least 17 linear and circular plasmids with a combined size of more than 533,000 base pairs.

- Both the linear chromosome and escort of plasmids of *B. burgdorferi* have been recently sequenced.

- The main chromosome of *B. burgdorferi* is estimated to contain 853 genes that encode a basic set of proteins.
To test for Borrelia susceptibility to antibiotics: MIC and MBC

Minimal inhibitory concentration = MIC is generally considered the drug concentration at which no motile organisms are observed by dark-field microscopy after 48-72 h of incubation with antibiotics in BSK-H medium at 32-33°C

Agger et al 1992; Levin et al 1993

Minimal bactericidal concentration = MBC was defined as the lowest concentration of antibiotics at which no viable spirochetes could be detected after 2-3 weeks of subcultivation in BSK-H medium, free of antibiotics at 32-33°C

Agger et al 1992; Sicklinger et al 2003
Standardized method to test antimicrobial susceptibility against pathogenic spirochetes

- *B. burgdorferi* isolates were added to modified BSK medium containing each antibacterial agent to a concentration of $10^6 - 10^7$ organisms per ml.

- After 24, 48, 72, and 96 h of incubation at $32^\circ$ C, the number of motile spirochetes can determined by using a Petroff-Hausser chamber, and the percentage of killing was calculated as follows:
  
  $\%$ viability = \frac{(\text{number of motile spirochetes/motile organisms in initial inoculum})}{\text{x 100}}$

Agger et al 1992

In vitro studies for different genospecies/species of Borrelia – MBCs (microgram/ml)

- Doxycycline: 0.25-25.0
- Penicillin: 0.15-6.5
- Azitromycin: 0.015-2.0
- Erythromycin: 0.06->0.5
- Clarithromycin: 0.06-0.5
- Telitromycin: 0.002-0.03
- Amoxicillin: 0.4-8.00
- Ceftriaxone: 0.03-2.00*
- Ciproflaxin: 0.5-16.0
- Tigecycline: 0.05-0.19

In vitro and clinical data – do they agree?

- “Survival of *Borrelia burgdorferi* in antibiotically treated patients with Lyme borreliosis” Preac-Mursic et al 1989
- “In vitro results have no proven correlation with antimicrobial clinical effectiveness *in vivo* since the relationship of MICs or MBCs of drugs against slowly dividing organisms such as B. burgdorferi” Moody et al 1994
- “Culture positive and PCR positive blood after antibiotics therapy” Oksi et al 1999
- “Clinically treatment failures occur in 5 to 10% of EM patients (oral doxycycline or amoxicillin for 14 to 30 days)” Smith et al 2002
But how about the *in vivo* studies?

- Treatment with oxytetracycline, erythromycin or doxycycline in mice **failed to eradicate** acute Borrelia infection or ameliorate the disease. *Moody et al 1994*

- Chloramphenicol and azithromycin **failed to eradicate** the organism but ameliorated the disease. *Moody et al 1994*

- In a dog model of infection, showed that antibiotic-treated dogs (doxycycline and amoxicilllin, 30 days) continued to have **persistent Borrelia-specific DNA** in their tissue albeit at lower levels than observed in untreated animals. *Straubinger et al 1997*
But how about Ceftriaxone (Rocephin)?


**SUMMARY:** A low numbers of noncultivable spirochetes, detected by PCR following antibiotic treatment. For at least 3 months after cessation of antibiotic, these noncultivable forms can be acquired by ticks (xenodiagnosis), transmitted by ticks, survive the molts between larvae to nymphs to adults, infect recipient mice by tissue transplant, transcribe RNA, and express antigen in ticks and tissues in the form of morphologically identifiable spirochetes.
The different forms of Borrelia

- *Borrelia burgdorferi* can convert between cyst, non-motile and normal motile spirochete forms.

- The cystic forms are resistant to most antibiotic treatments and difficult to detect in the body.


*B. burgdorferi after exposure to penicillin concentration of 0.125 mg/l. Coiled up spirochete forming a spherical structure (spheroplast).*

Schaller M; Neubert U. 1994
Spiral *Borrelia burgdorferi*

With Permission from Dr. Alan MacDonald
Separate cystic forms of *Borrelia burgdorferi* without extracellular matrix

With Permission from Dr. Alan MacDonald
**Cystic *Borrelia burgdorferi*** without granules inside

**Cystic *Borrelia burgdorferi*** with granules inside

With Permission from Dr. Alan MacDonald
Granular Borrelia evolving from spiral form

With Permission from Dr. Alan MacDonald
L-form of Borrelia

With Permission from Dr. Alan MacDonald
Can all those forms be infective and can they revert back to spirochetes?

Do we need to eliminate all those different *Borrelia* forms for a successful treatment?
O. Brorson *in vitro* studies

- **1997 Report:** *Transformation of Cystic Forms* of *Borrelia burgdorferi* to Normal, Mobile Spirochetes
  
  “the encysted forms developed into regular, mobile spirochetes after 6 weeks”

- **1998 Report:** *In Vitro Conversion* of *Borrelia burgdorferi* to Cystic Forms in Spinal Fluid, and Transformation to Mobile Spirochetes by Incubation in BSK-H Medium
  
  “Normal, mobile spirochetes were inoculated into spinal fluid, and the spirochetes were converted to cysts (spheroplast L-forms) after 1-24 h. When these cystic forms were transferred to a rich BSK-H medium, the cysts were converted back to normal, mobile spirochetes”
Round bodies of *Borrelia burgdorferi*

- Do they have clinical relevance?
- Can they reproduce? Can they revert back to spirochetes *in vivo*?

*MacDonald A 1988*

*Mursic et al 1996*
Several round bodies along borreliae after 24 h of incubation with ceftriaxone as shown by Transmission Electron Microscope (TEM)

Kersten et 1995
B. burgdorferi exposed to penicillin for 24 h. A vesicle is shown by TEM to adhere to the outer surface of a spirochete.
Round bodies *in vivo*

- **Concurrent Neocortical Borreliosis and Alzheimer's Disease Demonstration of a Spirochetal Cyst Form**
  - *MacDonald A 1988*
  - An unexpected observation was the identification of cystic forms of the Borrelia spirochete in dark-field preparations of cultured hippocampus

- **As clinical persistence of Borrelia burgdorferi in patients with active Lyme borreliosis occurs despite obviously adequate antibiotic therapy** *Mursic et al 1996*

- **Conversion of Borrelia garinii Cystic Forms to Motile Spirochetes *In Vivo. Grunter et al 2001***
  - B. garinii cystic forms maintain their capability to reconvert into normal spirochetes not only in vitro but also in vivo and can therefore be considered infective, at least in BALB/c mice.” “Dormant borreliae evacuated in cysts might be resistant to antibiotics (due to low metabolic activity) and stage under non-optimal environmental conditions. Borrelial cystic forms could therefore be responsible for the frequent failures of antibiotic therapy and for the commonly reported relapses of Lyme disease.
Agents for the cystic forms (RB)

- An *In Vitro* Study of the Susceptibility of Mobile and Cystic Forms of Borrelia burgdorferi to Metronidazole
  *Brorson et al 1999*

- “*B. burgdorferi has the ability to make cystic forms both in vivo and* *in vitro, e.g. when exposed to* *antibiotics commonly used for treating Lyme borreliosis. This phenomenon, combined with the ability of the cysts to reconvert to normal mobile spirochetes* may explain a reactivation of the disease after an illusory cure – and not a “post Lyme syndrome” as postulated by other researchers.”
Additional Brorson et al *in vitro* studies for the antibiotic sensitivity of the cystic (round bodies) form


“Destruction of spirochete *Borrelia burgdorferi* round-bodies by Tigecycline”  
*Brorson et al 2009*

Table 1. Inhibitory and bacteriocidal effect of TG  
(TG concentration in μg/ml)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Spirochete TG culture, incubation time (34°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Minimal inhibitory concentration</td>
<td>n/a</td>
</tr>
<tr>
<td>Minimal bacteriocidal concentration</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Borrelia afzelii* ACA-1 to TG at different incubation times (34°C).

Figure 3 showing round bodies treated with tigecycline (Panel A 0.05 μg/ml and Panel D >0.05 μg/ml) treated for 5 weeks  
*Bar: 5 μm*
Ineffectiveness of Tigecycline against Persistent *Borrelia burgdorferi* in vivo

- Genetically altered **non-cultivatable** *B. burgdorferi* could be isolated from mice treated with ceftriaxone and tigecycline.
- Mice remained **infected with non-dividing, but infectious spirochetes**, particularly when antibiotic treatment was commenced during the chronic stage of infection.

*Barthold et al 2010*
### TABLE 3. *ospA* copies in tissue from mice treated with high-dose tigecycline, low-dose tigecycline, ceftriaxone, or saline at 3 weeks of infection and then sampled 3 months after completion of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mouse</th>
<th>Inoculation site</th>
<th>Heart base</th>
<th>Ventricular muscle</th>
<th>Tibiotarsus</th>
<th>Quadriceps muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tigecycline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dose</td>
<td>1</td>
<td>–</td>
<td>4</td>
<td>–</td>
<td>62</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>32</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>21</td>
<td>4</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>–</td>
<td>4</td>
<td>–</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Low dose</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>386</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>2</td>
<td>49</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>–</td>
<td>122</td>
<td>–</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>–</td>
<td>3</td>
<td>–</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>–</td>
<td>43</td>
<td>–</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td><strong>Ceftriaxone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>13</td>
<td>–</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>38</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>25</td>
<td>–</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>13</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>–</td>
<td>6</td>
<td>–</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>–</td>
<td>5</td>
<td>–</td>
<td>73</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td><strong>Saline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>23</td>
<td>37</td>
<td>55,900</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>24</td>
<td>95</td>
<td>1,230</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>45</td>
<td>19</td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>829</td>
<td>505</td>
<td>2,520</td>
<td>718</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>92</td>
<td>1</td>
<td>326</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>–</td>
<td>289</td>
<td>416</td>
<td>1,500</td>
<td>493</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>–</td>
<td>118</td>
<td>36</td>
<td>805</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>–</td>
<td>147</td>
<td>7</td>
<td>1,100</td>
<td>2,100</td>
</tr>
</tbody>
</table>

*–, sample tested negative.*

---

Barthold et al 2010
TABLE 4. *ospA* copies in tissue from mice treated with high-dose tigecycline, low-dose tigecycline, ceftriaxone, or saline at 4 months of infection and then sampled 3 months after completion of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mouse</th>
<th>No. of <em>ospA</em> copies/mg tissue&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inoculation site</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>High dose</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Low dose</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Saline</td>
<td>1</td>
<td>1,640</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20,600</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13,800</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>41,700</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>962</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

<sup>a</sup>, sample tested negative.
Tibiotarsal and heart tissue from these mice transplanted into a immunocompromised SCID mice

TABLE 6. Summary of culture and *ospA* real-time PCR results from tissues from SCID mice at 4 weeks after transplant of tibiotarsal and heart base tissue from donor mice treated with high-dose tigecycline, ceftriaxone, or saline at 4 months of infection and then collected 3 months after completion of treatment

<table>
<thead>
<tr>
<th>Donor treatment</th>
<th>Culture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of mice positive/no. tested by:</th>
<th>ospA PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Inoculation site</td>
<td>Heart base</td>
</tr>
<tr>
<td>High-dose tigecycline</td>
<td>0/9</td>
<td>1/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Saline</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Combined culture results from blood, inoculation site, and/or urinary bladder.

<sup>b</sup> Cumulative total of mice with 1 or more PCR-positive tissues.
So what can we do now ????

- What other escape route Borrelia could have??
Penicillin effect on *Borrelia burgdorferi*

Rao, PD, Kollisetti, V, Sapi E. Unpublished data 2008
Acrodermatitis Chronica Atrophicans
Immunohistochemistry

“Granular forms of *B burgdorferi* in a “colony” with a “Reddish veil”
Borrelial lymphocytoma with medusa-like colony of Borrelia.
Eisendle et al, “Morphea” a manifestation of infection with Borrelia species”, British J Dermatology 2007

Morphea – with biofilm-like “clump” of Borrelia
Human brain culture demonstrating a potential biofilm of *Borrelia burgdorferi*

Year 1987

Tick gut culture showing *Borrelia burgdorferi* in a potential biofilm

Unit

Year 1981
**Borrelia burgdorferi** B31 colony on agarose

**FIG. 2.** SEM of 3-week-old *B. burgdorferi* B31 colony, small, compact type, on agarose. Bar, 30 μm. Inset: Note the sharp, distinct border (bar, 10 μm).

Kurtti TJ et al 1987
B. burgdorferi in nymphal midgut during feeding in the form of epithelial cell–associated networks

Dunham-Ems et al 2009
Borrelia burgdorferi colonies

Miklossy J et al 2008
Borrelia burgdorferi “Photo 51”
MacDonald A, & Sapi E: Biofilms of Borrelia burgdorferi in chronic cutaneous borreliosis

AJCP 2008 June

- We propose the hypothesis that that *Borrelia burgdorferi* can form biofilm structures in lymphocytomas and acrodermatitis chronica atrophicans.

- Our close examination of these pictures revealed striking similarity to previously published biofilm pictures and our preliminary findings on specific biofilm-like colony formation of *Borrelia burgdorferi* when cultured in the presence of human plasma.
  - The idea is also featured in Under our Skin movie 2008
Potential biofilm formation of *Borrelia burgdorferi*

- A **biofilm** is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface.

- Bacterial **biofilms** are very difficult to treat because they show much greater resistance to antibiotics (up to 1000-fold) than their free-living counterparts.

- Responsible for several chronic diseases, such as chronic lung infection in cystic fibrosis patients, chronic urinary infection, chronic middle ear infection, sinusitis, and even fatal endocarditis.

Azano D, Carpenter K, MacDonald and Sapi E, unpublished pictures, 2008
Doxycycline treated biofilm and spirochete

Red stain: Dead
Green stain: Viable

D. Luecke, Kaur N and E Sapi unpublished data 2010
THE PLAN: 2009

1. Reevaluate all antimicrobial agents for all forms of Borrelia, starting with:
   - Spirochetes
   - Round bodies
   - Biofilm-like colonies

2. Reevaluate the current techniques for culture and antibiotic evaluation:
   - Microdilution method – direct counting (Baclight staining
   - Culturing in plates contra tubes
Microplate contra small tubes

Are they provide different growing environment for *Borrelia*?
Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC) determination by different methods

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Micro-dilution method/Literature data (MIC) μg/ml</th>
<th>Our data (MIC) μg/ml</th>
<th>Micro-dilution method/Literature data (MBC) μg/ml</th>
<th>Our data (MBC) μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micro-dilution method</td>
<td>Direct cell counting</td>
<td>BacLight™ staining</td>
<td>Micro-dilution method</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.06-2</td>
<td>0.4</td>
<td>&gt;25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.006</td>
<td>0.015</td>
<td>&gt;5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.03-2</td>
<td>0.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.06-32</td>
<td>0.3</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Tinidazole</td>
<td>-</td>
<td>0.09</td>
<td>&gt;62.5</td>
<td>&gt;62.5</td>
</tr>
</tbody>
</table>

Sapi E et al 2011
Effect of Doxycycline of the Spirochete and Round Body formation of *Borrelia burgdorferi* (72h)

Sapi E et al 2011
Effect of Metranidazole of the Spirochete and Round Body Formation of *Borrelia burgdorferi* (72h)

![Graph showing the effect of metronidazole on the percentage of control of *B. burgdorferi* strain B31.](image)

Sapi E et al 2011
Effect of Tinidazole of the Spirochete and Round Body formation of *Borrelia burgdorferi* (72h)

Sapi E et al 2011
Effect of Tigecycline of the Spirochete and Round Body formation of *Borrelia burgdorferi* (72h)

Sapi E et al 2011
Effect of Different Antibiotics of the Spirochete and Round Body formation of *Borrelia burgdorferi* - 3 weeks

---

Sapi E et al 2011
Effect of Different Antibiotics of the Spirochete and Round Body formation of *Borrelia burgdorferi* - 3 weeks

Sapi E et al 2011
Evaluation of live/dead spirochete and round body forms of *B. burgdorferi* following various antibiotics treatment

Sapi E et al 2011
Evaluation of live/dead spirochete and round body forms of *B. burgdorferi* following various antibiotics treatment

![Graph showing the percentage of dead and live round bodies after various treatments.](attachment:graph.png)

Sapi E et al 2011
Visualization of spirochete and round body forms of Borrelia following antibiotic treatment (72h)  400x magnification  - Sapi E et al 2011
Effect of different antibiotics on the spirochete formation of *Borrelia burgdorferi* – 72h

Kaur N, Datar A and Sapi E unpublished data 2009
Effect of different antibiotics on the round body formation of *Borrelia burgdorferi* – 72h

Kaur N, Datar A and Sapi E unpublished data 2009
*Borrelia burgdorferi* B31 treated with various antibiotics

Kaur N, Datar A and Sapi E unpublished data 2010
B. burgdorferi early development of biofilm-like structure
dark field 40X
But how about Borrelia biofilm???

How do you test the antibiotic effect on biofilm?

- Crystal violet staining and evaluation technique
  - Measure only the amount of biofilm mass but does not determine whether it is viable – can be useful?

- BacLight live/dead method – it is a very good viability assay for overall viability inside of the biofilm
Effect of antibiotics on the biofilm-like colonies of Borrelia measured by crystal violet staining

Sapi E et al 2011
Effect of antibiotics on the biofilm-like colonies of Borrelia measured BacLight staining.
Doxycycline treated *Borrelia burgdorferi* biofilm

Red stain: Dead
Green stain: Viable

D. Luecke, Datar A, Kaur N and E Sapi  2010
Treatment of *Borrelia* biofilm with various antibiotics and evaluation by crystal violet staining method

Kaur N, Datar A and Sapi E unpublished data 2010
So how can we eliminate Borrelia biofilm?

- Other Antibiotics?
- Herbal agents like Samento, Banderol, Garlic?
- Enzymes to open up the biofilm???
- D - amino acid??? *Kolodkin-Gal I et al 2010 Science*
Comparison of the effect of Doxycycline, Samento extract and Banderol extract on the different morphological forms of *Borrelia burgdorferi* - 96 h

Datar A et al 2010
Effect of Samento, Banderol and Doxycyline on the biofilm formation of *Borrelia burgdorferi* (BacLight staining)

- Control
- Samento 1:300
- Banderol 1:300
- Samento+Banderol 1:300
- Doxy 250μg/ml

Red: dead cells
Green: viable cells

Datar A, Kaur N, Luecke D and Sapi E Townsend Letter 2010
Borrelia burgdorferi treated with 25 microgram/ml of doxycycline for 3 weeks

D. Luecke, Kaur N, Datar A and E Sapi unpublished data 2010
Recent Projects of our Borrelia Biofilm Research

- To test the efficiency of anti-microbial agents on the prevention and elimination of *Borrelia* biofilm

- Study the unique features of *Borrelia* biofilm with the goal to find novel therapeutic targets

THE NEW PROJECT - 2011:

“Differential Gene Expression During Biofilm formation of *Borrelia burgdorferi*”
Lyme Disease Studies at UNH supported by the Turn the Corner Foundation/2008 - 2011

- "Detection of other **novel pathogens** in Ixodes scapularis ticks"
  - *The purpose of this study is to identify microfilarial nematodes (worms) as potential tick-borne human pathogens in the US.*

- "**Biofilm formation** of *Borrelia burgdorferi***"
  - *The purpose of this study is to establish and study Borrelia "Biofilm formation of Borrelia burgdorferi***

**THE NEW PROJECT - 2011:**

"**Differential Gene Expression During Biofilm formation of Borrelia burgdorferi***"
Lyme Disease Studies at UNH supported by the Lyme Disease Foundation/2010-11

“Study of the biofilm structure of *Borrelia burgdorferi*”

- Aim 1: To study the structural components of *Borrelia* biofilm using atomic force, scanning electron, differential interference contrast and fluorescent microscopy techniques.
- Aim 2: Characterization of *Borrelia* biofilm components using RAMAN spectrometry and *in situ* fluorescent staining methods.

“Pilot study: Identification of XMRV virus in *Ixodes scapularis* ticks”

- We proposed test 200 *Ixodes scapularis* ticks for the presence of XMRV virus using real-time PCR technology.
“An in vitro evaluation of antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*”

The goal of this research is to test the *in vitro* effects of various antibiotics on different morphological forms of *Borrelia burgdorferi*.

- **AIM 1:** To test *in vitro* susceptibility of spirochete and cyst forms of *Borrelia burgdorferi* to different antibiotics used for Lyme disease treatment.

- **AIM 2:** To examine the optimal *in vitro* combinations of these antibiotics that can eliminate the different forms of *Borrelia burgdorferi*, as demonstrated by viability and microscopic assays.
Lyme Disease Symposiums at the University of New Haven

- **May 12, 2006** “Current Trends in Lyme Disease Research”
  - 80 attendants
- **May 19, 2007** “Current Trends in Lyme Disease Research”
  - 230 attendants
- **May 17, 2008** “Understanding and Treating Lyme Disease: Choices and Challenges”
  - 250 attendants
- **May 8, 2010** “The Challenges of Lyme Disease: Emerging Research - Pediatric Care”
  - 200 attendants
- **May 21, 2011** “Breakthroughs in Lyme Disease Research”
  - 150 attendants
Special Thanks To:

- University of New Haven and College of A&S for funding our studies.
- Turn the Corner Foundation, CALDA, LDA, CT Lyme Riders Inc for supporting our research.
- Turn the Corner and Schwartz Foundation for providing a “state of the art” microscopes for our morphological studies.
- Dr. Roman Zajac, Dr. Michael Rossi, Dr. Saion Sinha and Jennifer Diana M.S. at Department of Biology and Environmental Science (UNH) for additional support and funds.
  1. Michelle Hessberger MS, Jeremy Bober MS, Michelle Montagna MS
  2. Brandon Guida MS,Yogita Verma PhD, MS, Murali Reddy MS, Meera Raman MS, Marianne Tawadros MS
  3. Donna Rhoads MS, Pushpa Rao MS, Sonali Solanski MS, Raghavender Reddy MS, Kristin Bovat MS, Sumyuktha Komarigiri MS
  4. Diane Azano MS, Katie Carpenter, Jamie Mille MSr, Kyrle Luth
  5. Akshita Datar MS, Navroop Kaur MS, David Luecke, Cedric Mpoy, Namrata Pabbati MS, Bien-Aime Lubraine MS, Seema Patel MS, Sam Anyanwu MS, Ashley Taylor MS, Yram Foli MS, Nicola Ricker MS, Scott Bastian, Shilpa Madari MS, Michael Feulner MS, Amy Rattelle MS
UNH Lyme Disease Research Group
2011 May 21st