An Update of Lyme Disease in BC and Ongoing Challenges in Laboratory Diagnosis

Muhammad Morshed, PhD, SCCM

Clinical Professor
Department of Pathology & Laboratory Medicine
University of British Columbia
Program Head, Zoonotic Diseases & Emerging Pathogens
Laboratory Services, BC Centre for Disease Control

Nov 25, 2010
Acknowledgements

• Bonnie Henry
• Sunny Mak
• Quantine Wong
• Yvonne Simpson
• Min-Kuang Lee
• Marsha Taylor
• ZEP Technologists and Co-op students
Overview

• Background of Lyme Disease in BC
• BC existing data on Lyme disease
• Risk of Lyme in BC
• Lyme disease testing policy
• Discussion on laboratory testing issues
• Conclusions
What is Lyme Disease?

Lyme disease is a complex, multistage multisystem inflammatory infectious disease caused by a spirochete, *Borrelia burgdorferi* of both humans and animals. This disease is transmitted by the bite of infected *Ixodes* ticks.
Lyme Disease

- Lyme disease or lyme borreliosis is caused by three species of bacteria in *Borrelia* genus.
- The cause of Lyme disease in North America is *Borrelia burgdorferi sensu stricto*, which is spread by ticks.
- In British Columbia, *Ixodes pacificus* (black legged ticks) are the carrier known to transmit *Borrelia burgdorferi ss*.
- Rodents are the natural reservoir for Lyme disease.
- An infected tick must be attached to the host for at least 24-36 hours for *Borrelia* transmission to occur.

*Borrelia burgdorferi* – magnification 400x. Corkscrew/helical shaped gram-negative bacteria 10-25µm long.
The different *Borrelia burgdorferi* sensu lato species in the United States, Asia, and Europe

<table>
<thead>
<tr>
<th>Borrelia species</th>
<th>Pathogenic (manifestation)</th>
<th>Geographic region</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. burgdorferi</em></td>
<td>Yes (arthritis)</td>
<td>United States, Europe, possibly Asia</td>
</tr>
<tr>
<td><em>B. garinii</em></td>
<td>Yes (neuroborreliosis)</td>
<td>Europe, Asia</td>
</tr>
<tr>
<td><em>B. afzelii</em></td>
<td>Yes (ACM)</td>
<td>Europe, Asia</td>
</tr>
<tr>
<td><em>B. japonica</em></td>
<td>No</td>
<td>Asia (Japan)</td>
</tr>
<tr>
<td><em>B. valaisiana</em></td>
<td>Uncertain</td>
<td>Europe, Asia</td>
</tr>
<tr>
<td><em>B. lusitaniae</em></td>
<td>Minimally</td>
<td>Europe (south)</td>
</tr>
<tr>
<td><em>B. spielmanii</em></td>
<td>Minimally (erythema migrans)</td>
<td>Europe (central)</td>
</tr>
<tr>
<td><em>B. andersonii</em></td>
<td>No</td>
<td>United States</td>
</tr>
<tr>
<td><em>B. bissetii</em></td>
<td>Minimally</td>
<td>United States</td>
</tr>
<tr>
<td><em>B. tanukii</em></td>
<td>No</td>
<td>Asia (Japan)</td>
</tr>
<tr>
<td><em>B. turdi</em></td>
<td>No</td>
<td>Asia (Japan)</td>
</tr>
<tr>
<td><em>B. sinica</em></td>
<td>No</td>
<td>Asia (China)</td>
</tr>
<tr>
<td><em>B. californiensis</em></td>
<td>No</td>
<td>United States (west)</td>
</tr>
</tbody>
</table>
Lyme Disease Progression

Stage 1: Early localized infection

- Not all patients with Lyme disease will develop all symptoms; about 7% of infected individuals will be asymptomatic.
- Incubation period for Lyme disease is one to two weeks.
- Flu-like symptoms: fever, fatigue, headache, muscle soreness and malaise.
- Lyme disease can progress to later stages even if the patient does not display above symptoms.

Circular skin rash called erythema migrans occurs at the site of the tick bite. About 80% of infected patients develop the rash 3 to 30 days after the tick bite.
Lyme Disease Progression

Stage 2: Early disseminated infection

- Borrelia bacteria may spread to the different organs through bloodstream and localizes sites other than the bite site

Stage 3: Late persistent infection

- Severe and chronic symptoms develop after several months in 5% of untreated or inadequately treated patients.
- Lyme encephalopathy: problems with concentration and short-term memory, fatigue.
- Polyneuropathy: shooting pains, numbness, tingling in the extremities.
- Chronic encephalomyelitis: cognitive impairment, weakness in the legs, change in gait, facial palsy and bladder problems.
- Lyme arthritis
Lyme Disease Transmission

- Spirochete can successfully transmitted through tick bites and so far only through tick bites

- Person-to-Person
  - cannot get infected from touching, kissing or having sex with a person who has Lyme disease No evidence

- During Pregnancy & While Breastfeeding - No evidence

- From Blood –
  - In principle yes, but no evidence so far

- From Pets – No evidence

- Other Transmission
  - There is no credible evidence that Lyme disease spirochete can be transmitted through air, food, water, or from the bites of mosquitoes, flies, fleas, or lice
Lyme Disease Treatment and Vaccination

- Infection can be eliminated with antibiotic treatment. For adults - doxycycline and for children - amoxicillin. Other alternatives being cefuroxime and cefotaxime.

- *Borrelia burgdorferi* has high levels of outer surface protein A expression. A vaccine was therefore developed based on OspA protein by GlaxoSmithKilne. It was pulled from the market by the developer in 2002 reportedly due to poor sales.

- Currently there is interest in developing a broadly protective Lyme disease vaccine against tick saliva that coats the bacteria, in particular Salp15 protein. This type of vaccine would also offer protection against some other pathogens transmitted by ticks.

Crystal structure of outer surface protein A (OspA)
History

- 1975: Juvenile cases of arthritis found in Lyme Town, Connecticut
- Epidemiology linked with tick bite and EM
- 1981: A spirochete was isolated from the tick gut
- Spirochete proved to be the causative agent of Lyme disease
Lyme Disease History in BC

- BCCDC sent personnel to Dr. Burgdorfer’s Lab in 1984 for training on Bb diagnosis
- Started providing IFA test in 1985
- Introduced EIA and WB in 1992
- First Bb isolated from Tick in 1993
- Lyme disease become reportable in 1995
- First Bb isolated from migratory bird in 1999
Lyme disease found in B.C.

By Holly Horwood
Staff Reporter

Scientists say Lyme disease has arrived in B.C. and "thousands" may be unknowingly infected with it—or a mysterious twin ailment.

Twenty-three British Columbia residents have been diagnosed with the disease, but there are many more who may not know they have it.

And some researchers say Canadian residents could find themselves threatened at an increasing rate by ticks on the mainland and the coast.

"We believe that Lyme disease is over-diagnosed and under-recognized," said Dr. Muhammad Manzoor, head of the British Columbia Centre for Disease Control.

While Canadian residents can contract Lyme disease by travelling to endemic areas, some say it is only a matter of time before it becomes prevalent in B.C.

"The disease is here to stay," said Dr. Manzoor.

Lyme disease makes its way to Island

The tick that carries Lyme disease discovered on Vancouver Island.

With more ticks, Manzoor says, the chance of contacting Lyme disease increases—and the chance of symptoms persisting after the disease has been treated.

Lyme disease remains a rarity in Ontario but for those suffering from the bug-borne illness, life is a living hell.

"It's a very serious disease," he said. "It's a very difficult disease to treat."

He says that if anything, Lyme is under-diagnosed, even in Canada where the numbers are scant.

A recent study in the U.S. on the disease has revealed that 60 percent of those who are diagnosed have Lyme disease.

"A lot of people with a few achy joints and other aspects of chronic fatigue are walking around believing they have Lyme when they really don't," Manzoor said.

If Lyme-carrying ticks are concentrated in eastern New York and states such as Minnesota and Wisconsin have emerged as hot spots, why isn't the disease more prevalent in nearby Ontario?

"One of the reasons is that we're not screening for it," Manzoor said. "And if we were, we'd find a lot more cases."
The relative size of the animals approximates their significance as hosts for the different tick life cycle stages in a typical woodland habitat.
Established* and reported** distribution of the Lyme disease vectors Ixodes scapularis (I. dammini) and Ixodes pacificus, by county, United States, 1907-1996

*at least 6 ticks or 2 life stages (larvae, nymphs, adults) identified.
**at least 1 tick identified.
National Lyme disease risk map with four categories of risk

Note: This map demonstrates an approximate distribution of predicted Lyme disease risk in the United States. The true relative risk in any given county compared with other counties might differ from that shown here and might change from year to year. Risk categories are defined in the accompanying text. Information on risk distribution within states and counties is best obtained from state and local public health authorities.
Prime Vectors in BC

• *Ixodes pacificus*

• More rarely found:
  - *Ixodes angustus*
  - *Ixodes auritulus*

• Infectivity level low

• *Dermacentor* not a competent vector
Main Host for Lume spirochete in BC

- *Peromyscus maniculatus* (Deer mice)

- Found all over BC
- Infectivity level low

Distribution of *Peromyscus* spp in NA
Dermacentor andersoni

Distribution in BC

Data source: Dr. Muhammad Monshid, PHSA Public Health Microbiology and Reference Laboratory – 1985-2006 field sampling and clinical specimens. Map created July 2010.
Positive
*Borrelia burgdorferi*
Ticks and Rodents in British Columbia

- Feeding or questing tick
- Submitted tick
- Trapped rodent

Isolation and/or Detection of *Borrelia burgdorferi* from Tick and Mice Populations in B.C. (1993 – 1996)

<table>
<thead>
<tr>
<th>Year</th>
<th>Tick</th>
<th></th>
<th>Mice</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture Positive</td>
<td>Total</td>
<td>Culture Positive</td>
<td>Total</td>
</tr>
<tr>
<td>1993</td>
<td>21</td>
<td>3218</td>
<td>2</td>
<td>321</td>
</tr>
<tr>
<td>1994</td>
<td>12</td>
<td>2543</td>
<td>16</td>
<td>1360</td>
</tr>
<tr>
<td>1995</td>
<td>5</td>
<td>3178</td>
<td>7</td>
<td>888</td>
</tr>
<tr>
<td>1996</td>
<td>2</td>
<td>1117</td>
<td>0</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>10056</td>
<td>25</td>
<td>2766</td>
</tr>
</tbody>
</table>

0.40 % \[\text{Tick}\] 0.90 % \[\text{Mice}\]
### Isolation and/or Detection of *Borrelia burgdorferi* from Tick and Mice Populations in B.C. (1997 – 2007)

<table>
<thead>
<tr>
<th>Year</th>
<th>Tick</th>
<th></th>
<th></th>
<th>Mice</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture Positive</td>
<td>Total</td>
<td>Culture Positive</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>3</td>
<td>1491</td>
<td>3</td>
<td>309</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>3</td>
<td>1509</td>
<td>2</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>2</td>
<td>808</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>3</td>
<td>611</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>1</td>
<td>511</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>3</td>
<td>430</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>1</td>
<td>436</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>7</td>
<td>1219</td>
<td>0</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>5</td>
<td>733</td>
<td>0</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>336</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>518</td>
<td>0</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8602</td>
<td>5</td>
<td>833</td>
<td></td>
</tr>
</tbody>
</table>

**Overall Percentages:**
- **Tick:** 0.35%
- **Mice:** 0.60%
Ticks Received for ID and *B. burgdorferi* Culture from BC Physicians, Veterinarians and Residents (2000-2007).
Forecasted Ecological Niche for *Ixodes pacificus* in British Columbia based on the Distribution of Tick Samples.
Forecasted Ecological Niche for *Ixodes angustus* in British Columbia based on the Distribution of Tick Samples

89% model accuracy
Validation (39): 35 optimal, 2 potential, 2 not suitable
Forecasted Ecological Niche for *Borrelia burgdorferi* in British Columbia based on the Distribution of Positive Tick Samples

94% model accuracy
Validation (39): 33 optimal, 3 potential, 3 not suitable

Anti-borrelia Serology Tests on Deer Mice in BC

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Mice Sera Tested</th>
<th>IFA Positive</th>
<th>WB Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>33</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2005</td>
<td>34</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2005</td>
<td>34</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>2005</td>
<td>17</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2007</td>
<td>46</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>3.66%</td>
<td>164</td>
<td>34</td>
<td>6</td>
</tr>
</tbody>
</table>
Number of Tests per patient
1997 to 2010

<table>
<thead>
<tr>
<th>Count</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4802</td>
<td>822</td>
<td>202</td>
<td>78</td>
<td>28</td>
<td>17</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
C6 Peptide tests (2006-2010) Run by a Reference Laboratory

- Total tests done : 56
- Total Positive : 02
## Lyme Disease in BC 2007-todate

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>15</td>
</tr>
<tr>
<td>2008</td>
<td>06</td>
</tr>
<tr>
<td>2009</td>
<td>10</td>
</tr>
<tr>
<td>~2010</td>
<td>5~</td>
</tr>
</tbody>
</table>
Rates in Washington and BC per 100,000 population

1. Washington State data from the State Department of Health

2. BC incidence total (endemic and travel) incidence rates for LD by year
Examples of the Impact of Pretest Probabilities of Late Lyme Disease on the Frequency of False Positive ELISA Test Result

<table>
<thead>
<tr>
<th>Pretest Probability (Prevalence)*</th>
<th>Total</th>
<th>Number of True Positives**</th>
<th>Number of False Positives**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>80</td>
<td>76</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>0.50</td>
<td>57</td>
<td>48</td>
<td>9 (16%)</td>
</tr>
<tr>
<td>0.20</td>
<td>34</td>
<td>19</td>
<td>15 (44%)</td>
</tr>
</tbody>
</table>

* A hypothetical scenario assumed 80 and 50, or 20 patients with actual Lyme disease in three groups of 100 patients Selected by using objective or subjective clinical criteria of Lyme disease.

** Random-effects combined estimate of test’s sensitivity of 95% and specificity of 81% was taken from Tugwell et al., 1997.
International Diagnosis Approaches (CDC/ASTPHLD/CPHLN etc....)

• Two tier test approach
  - Step 1: EIA or C6 Peptide, or IFA....
    • If + or equivocal
  - Step 2: Western Blot
  - Accredited laboratory
  - QC/QA
Performance of 2-tiered testing for detecting antibodies to *Borrelia burgdorferi* in serum: Specificity

<table>
<thead>
<tr>
<th>Disease classification</th>
<th>No. of samples</th>
<th>Two-tiered test</th>
<th>% sensitivity</th>
<th>% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Other than Lyme</td>
<td>559</td>
<td>5</td>
<td>554</td>
<td>-</td>
</tr>
<tr>
<td>Anti-cardiolipin antibody</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Anti-nuclear antibody</td>
<td>116</td>
<td>2</td>
<td>114</td>
<td>-</td>
</tr>
<tr>
<td>Healthy endemic</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Healthy nonendemic</td>
<td>243</td>
<td>0</td>
<td>243</td>
<td>-</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Rapid plasma reagin reaction</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>94</td>
<td>1</td>
<td>93</td>
<td>-</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Syphilis</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Tickborne relapsing fever</td>
<td>14</td>
<td>2</td>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>

**Improved Serologic Testing for Lyme Disease · JID 2003:187**
Performance of 2-tiered testing for detecting antibodies to *Borrelia burgdorferi* in serum: Specificity

<table>
<thead>
<tr>
<th>Disease Classification</th>
<th>No of sample</th>
<th>Two tiered test</th>
<th>% sensitivity</th>
<th>% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lyme disease</td>
<td>280</td>
<td>189</td>
<td>91</td>
<td>68</td>
</tr>
<tr>
<td>Acute (EM)</td>
<td>80</td>
<td>30</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>Early Conval.</td>
<td>106</td>
<td>71</td>
<td>35</td>
<td>67</td>
</tr>
<tr>
<td>Early neurologic</td>
<td>15</td>
<td>13</td>
<td>2</td>
<td>87</td>
</tr>
<tr>
<td>Early Neur. Conv.</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>Arthritis</td>
<td>33</td>
<td>31</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>Arthritis Cov.</td>
<td>24</td>
<td>23</td>
<td>1</td>
<td>96</td>
</tr>
<tr>
<td>Late Neurologic</td>
<td>11</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
Commonly Used Lab Tests

Techniques

- (Clinical)
- EIA or ELISA, CLIA or IFA or C6 Peptide ELISA
- Western Blot
- PCR
- Culture
Techniques - EIA

• EIA -- serum
  - IgG and IgM
  - Sensitivity depends on stages
  - Specificity poor
  - Lyme vaccine
  - Treatment

• EIA -- CSF

• Others
Techniques -- Western blot

• Several important bands
  - IgM 2 of 3 bands (23, 39, or 41 kDa)
  - IgG 5 of 10 bands (18, 23, 28, 30, 39, 41, 45, 58, 66, or 93 kDa)

• Time frame

• Antigenic differences

• Recombinant
IgM:
< 4wks

IgG:
>4 wks

*B. burgdoferi* >5
Techniques - Culture

- Skin, CSF, blood
  - BSK II medium
  - requires 12 weeks
  - detected by dark-field microscopy
  - confirmed by molecular tests
- Very specific
- Undefined sensitivity
Techniques - PCR

- Skin, CSF and joint fluid
- Limited by numbers
- Extraction important
- Primers
CSF

PCR Amplification

Sequencing

Blast

Human Gene
Other Tests for Lyme Disease

- Borreliacidal Antibody Assay (Gunderson test)
- T-cell Activation Test
- Lyme Urine Antigen Test (LUAT)
- Immune complex / antigen-antibody test
- Tissue Biopsy and Staining
- Bowen Test
- Many more........
PUBLIC HEALTH LABORATORY

LYME DISEASE DX

PRIVATE LABORATORY

I'M RIGHT

I'M RIGHT
Global Challenges

• Appropriate Sample
• Unavailability of highly sensitive and highly specific test- early infections
• Genetic variability (European strain)
• Social network around Lyme
• Laboratories with questionable QC/QA
Lab Test Conclusion

• Technically difficult
• Pre-test probability
• Global strain Variability
• Interpretation key
• Should Interpret data with caution!
Prevention

• To prevent tick bites the following measures are strongly recommended:
  - Walk on cleared trails;
  - Wear a hat, long sleeves and pants and light coloured clothing;
  - Tuck pant legs into socks or boots;
  - Use an insect repellant containing DEET on clothing and exposed skin
Use Common sense to avoid tick bite

Remove tick carefully if found

Use centre of the Clear trail
Wear Light color clothing

Tucking pant legs into socks
Use Insect repellents
12th International Conference on

LYME BORRELIOSIS
AND OTHER
TICK-BORNE DISEASES

September 26-29, 2010 Ljubljana, Slovenia
ICLB 2010

• > 300 participants
• 41 oral presentations
  - 32 on Lyme (Eco-epidemiology, Pathogenesis, Diagnosis, Treatment and Prevention)
  - 9 others (TBE; RF; Anaplasma; Babesia etc)
• 145 posters
  - Over 100 LB
  - 11 TBE
  - Few others
ICLB 2010

• 3 panel discussion with Keynote speakers
  - Treatment
  - Laboratory Diagnosis
  - CFS and Chronic Lyme (?)
ICLB 2010
Outcome on Panel Discussion

• Treatment
  − There is no benefit for Long Term Treatment

• Laboratory Diagnosis
  − Two Tier testing is still valid
  − Data present on EIA screen followed by C6 peptide test

• CFS and Chronic Lyme (?)
  − IgM test may not be necessary in low endemic areas
  − IgG is an excellent test
Overview

- Background of Lyme Disease in BC
- BC existing data on Lyme disease
- Risk of Lyme in BC
- Lyme disease testing policy
- Discussion on laboratory testing issues
- Conclusions
Summary

• Lyme disease is present in BC in low levels
• Clinical Diagnosis is important for diagnosing an acute case
• Two tier Laboratory testing is universally accepted tests for the Laboratory diagnosis of Lyme diseases
• Prevention is the key to protect anyone from Lyme disease
Thank you