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None

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- Justin Kreuter, MD – program planning committee
- Sharon Preuss – program planning committee
- Bobbi Pritt, MD, MSc, DTMH – program presenter, program planning committee
- Cara Schmidt – program planning committee

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None

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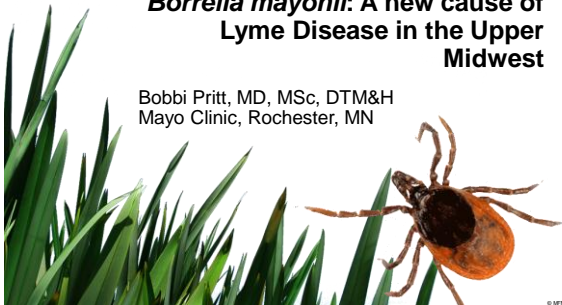
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## ***Borrelia mayonii*: A new cause of Lyme Disease in the Upper Midwest**

Bobbi Pritt, MD, MSc, DTM&H  
Mayo Clinic, Rochester, MN



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## Disclosures

- None

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## Objectives

- Following this presentation, participants should be able to:
  - Name the organisms responsible for Lyme disease in the United States
  - Describe common signs and symptoms of Lyme disease
  - Discuss the unique clinical features of *Borrelia mayonii* infection compared to other causes of Lyme disease

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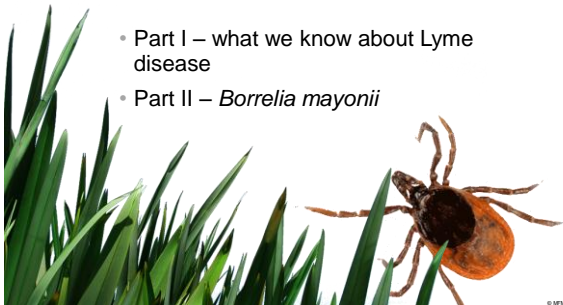
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## Outline of my talk

- Part I – what we know about Lyme disease
- Part II – *Borrelia mayonii*



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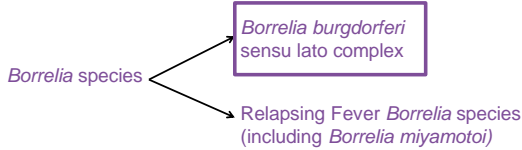
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## Lyme Disease



- Tick-transmitted bacteria (spirochetes) in the *Borrelia burgdorferi sensu lato* complex (Bbsl complex)
  - *Borrelia burgdorferi sensu stricto* - United States
  - *Borrelia afzelii*, *Borrelia garinii* and *B. burgdorferi* - Europe




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## Lyme Epidemiology

- Most common vector-borne illness in the United States and Europe!
- First described in the United States in 1975 (juvenile rheumatoid arthritis)
- Does **not** occur nationwide:
  - Concentrated in the NE and upper midwest.




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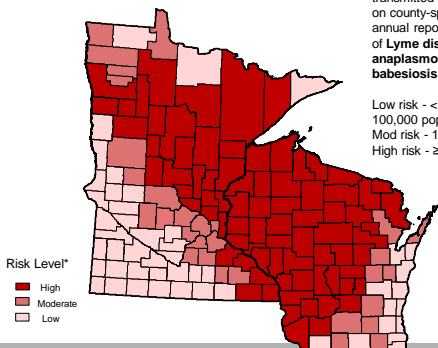
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## Risk of Tickborne disease\*

\* Risk of *I. scapularis* transmitted disease based on county-specific mean annual reported incidence of Lyme disease, anaplasmosis and babesiosis (2007-2013).

Low risk - < 10 cases/100,000 population  
Mod risk - 10 to 24.9  
High risk - ≥ 25




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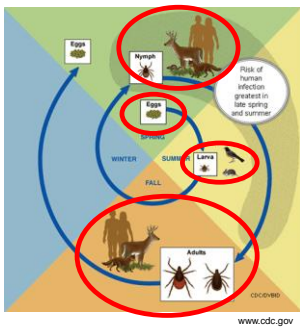
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## Vector

- Transmitted through the bite of an infected **black-legged (deer) tick, *Ixodes scapularis***



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CDC



Graham Hickling, PhD  
University of Tennessee



## Lyme Disease - *B. burgdorferi* Localized Stage

- Erythema migrans (70-80%)
- Flu-like syndrome (malaise, headache, fever)
- Lymphadenopathy



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## Disease Progression

- Early disseminated stage (days to weeks post-tick bite)
- If untreated, may result in:
  - Additional EM lesions
- Bell's palsy
- Meningitis, encephalitis
- Arrhythmia, myocarditis, pericarditis



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## Late Disseminated Stage (months to years post tick bite)

- Intermittent arthritis (60%)
  - Severe joint pain and swelling
  - Large joints, particularly knees.
- Chronic neurological complaints (5%) 
  - Numbness, tingling in the hands or feet
  - Difficulty with short-term memory
- Unique manifestations:
  - *B. garinii* – neurologic manifestations
  - *B. afzelii* – Acrodermatitis chronica atrophicans



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## Treatment

- Early disease with erythema migrans
  - Doxycycline, amoxicillin, or cefuroxime
  - 14 to 21 days
- Lyme meningitis and other manifestations of early neurologic Lyme disease
  - IV Ceftriaxone for 14 days

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## Post-treatment Lyme disease syndrome

- Lingering symptoms after treatment (10-20%)
  - Myalgias, arthralgias
  - Cognitive defects
  - Sleep disturbance
  - Fatigue
- Cause is unknown; may be autoimmune response
- No evidence of ongoing *B. burgdorferi* infection




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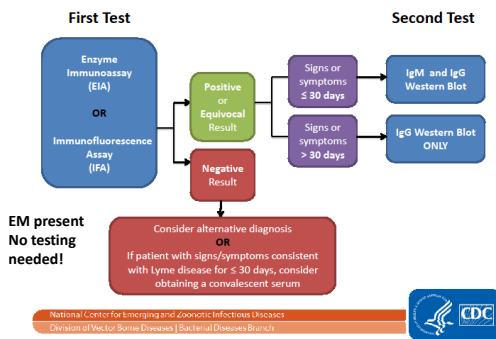


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### Two-Tiered Testing for Lyme Disease




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## Lyme Disease PCR

- Allows for direct detection of the infectious organisms (vs. serology)
- Positive during acute stage of illness – don't rely on development of antibodies which can take several weeks.
- However, has relatively low sensitivity
  - Blood is positive in only 50% of acute cases with EM
  - CSF is positive only 1/3 of patients with early neuroborreliosis
- Adjunctive test; not generally used routinely for Lyme disease diagnosis
- Mayo Clinic PCR assay - performed on blood, CSF, synovial fluid and tissue




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# Lyme PCR

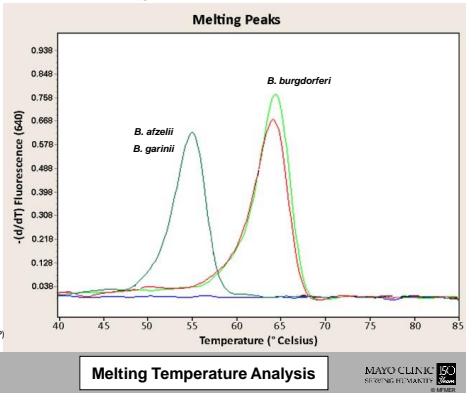
Multiplex real-time PCR Targeting the plasminogen-binding protein (pppA1)



Jon Rosenblatt, MD



Lynne Sloan, MT (ASCP)




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## How the story began...




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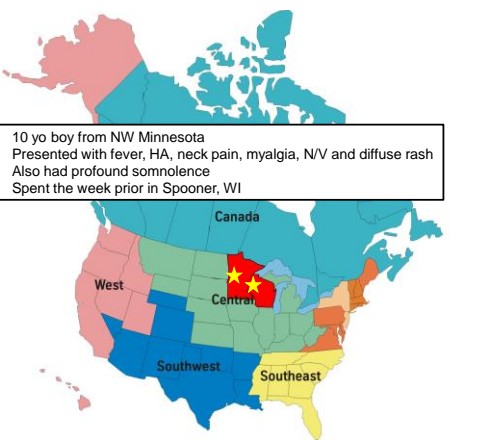
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- 10 yo boy from NW Minnesota
- Presented with fever, HA, neck pain, myalgia, N/V and diffuse rash
- Also had profound somnolence
- Spent the week prior in Spooner, WI




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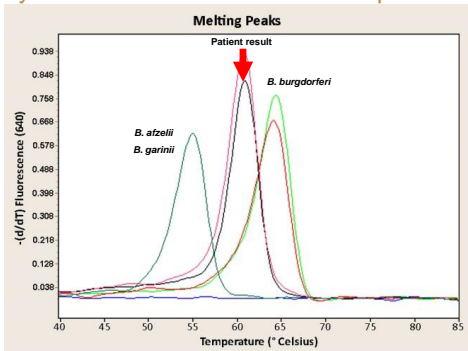
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### Lyme PCR - EDTA whole blood specimen



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### Patient History, continued

- Patient was hospitalized for 4 days
- Treated with ceftriaxone (1d), followed by 21 d of amoxicillin
- Complete recovery

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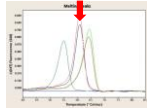
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### More cases identified



- PCR positive in whole blood:
  - July 2013 – 11 yo male from WI
  - Retrospective review: July 2012 – 65 yo male from ND (exposure in MN)
- Synovial fluid specimen from Mayo Clinic Eau Claire
  - June 2013 – 21 yo woman from WI

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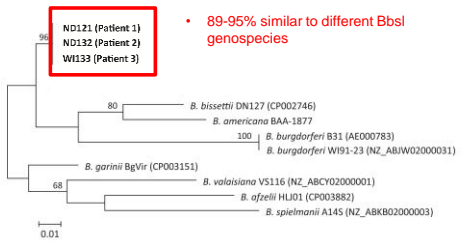
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### Plasminogen binding protein gene (*oppA1*) – 149 bp



• 89-95% similar to different Bbsl genospecies

Bootstrap support values >50% are shown. The scale bar corresponds to 0.01 substitutions per nucleotide position.




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Now what?




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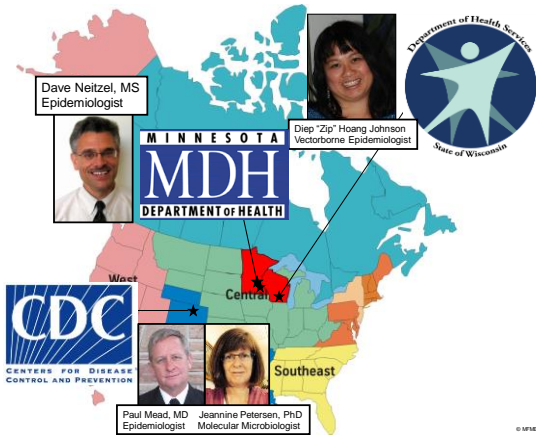
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### Emergence of a New Pathogenic Ehrlichia Species, Wisconsin and Minnesota, 2009

Bobbi S. Pritt, M.D., Lynne M. Sloan, B.S., Diep K. Hoang Johnson, B.S., Ulrike G. Munderloh, Ph.D., Susan M. Paskewitz, Ph.D., Kristina M. McElroy, D.V.M., Jevon D. McFadden, M.D., Matthew J. Binnicker, Ph.D., David F. Neitzel, M.S., Gongping Liu, Ph.D., William L. Nicholson, Ph.D., Curtis M. Nelson, B.S., Joni J. Franson, B.S., Scott A. Martin, M.D., Scott A. Cunningham, B.S., Christopher R. Steward, B.S., Kay Bogumill, R.N., Mary E. Bjorgaard, R.N., Jeffrey P. Davis, M.D., Jennifer H. McQuiston, D.V.M., David M. Warshauer, Ph.D., Mark P. Wilhelm, M.D., Robin Patel, M.D., Vipul A. Trivedi, M.D., and Marina E. Ereemeeva, M.D., Ph.D., Sc.D.

*N Engl J Med* 2011;365:422-429

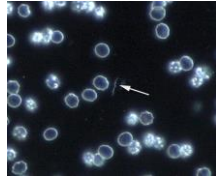


### Further studies

- Blood from 2 patients sent for culture and sequencing
- Also performed at Mayo Clinic

Lynne Sloan, MT (ASCP)





Spirochetes visualized in blood from 1 patient (2/70 hpf of blood, diluted 1:10) = ~85,000 spirochetes/mL

Cultures positive for blood from 2 patients




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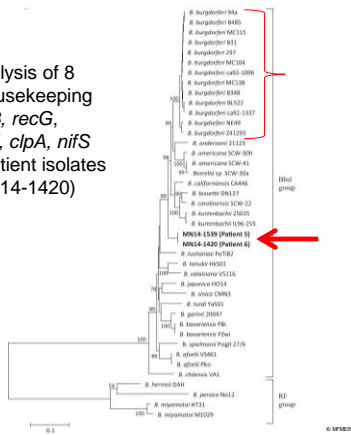
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- Phylogenetic analysis of 8 concatenated housekeeping genes: *uvrA*, *rplB*, *recG*, *pyrG*, *pepX*, *clpX*, *clpA*, *nifS* amplified from patient isolates (MN14-1539, MN14-1420)




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## Multi-Locus Sequence Analysis (MLSA)

- 8 gene MLSA performed
  - Previously used for defining *Bbsl* genospecies
  - Highest pairwise similarity was to *B. burgdorferi* (94.9 to 95.2%)
  - Threshold for separating genospecies = 98.3% similarity
  - Confirmed that this is a novel *Bbsl* genospecies
  - Proposed name: *Borrelia mayonii*




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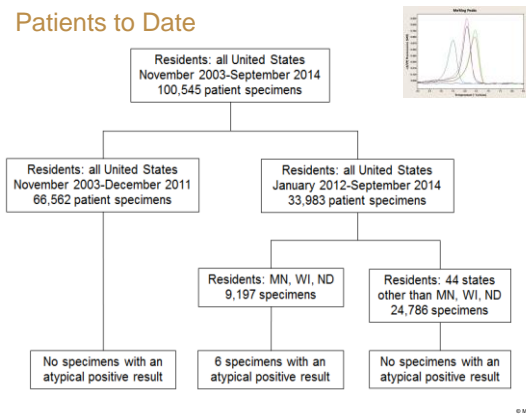
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## Patients to Date




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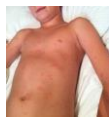
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## Clinical Features of the 6 patients

- Ages 10-67; 4 males, 2 females
- 5 presented with an acute febrile illness
- 3 had potential neurologic involvement (confused speech, profound somnolence, visual difficulties)
- 4 had rash
  - Only 1 suggestion of EM
- 1 had arthralgia
- All reported exposure to ticks/tick habitat
- 5 of 6 recovered with antibiotic therapy
  - 1 reported residual joint pain




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Lynne Sloan, MT (ASCP)



Diiep "Zip" Hoang Johnson  
Vectorborne Epidemiologist



Susan Paskowitz, Professor  
211 Rowell Labs  
1001 Lakeside Drive  
Madison, WI 53706

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## Tick Processing

Archived ticks also tested




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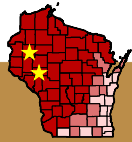
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Overall = 2.9%



Results of PCR testing of *Ixodes scapularis* ticks collected in two Wisconsin counties, 2009-2014

Collection Site County	Collection Date	Adult <i>I. scapularis</i> PCR results		Nymphal <i>I. scapularis</i> PCR results	
		<i>Borrelia mayonii</i> No. Pos/Total (%)	<i>Borrelia burgdorferi</i> No. Pos/Total (%)	<i>Borrelia mayonii</i> No. Pos/Total (%)	<i>Borrelia burgdorferi</i> No. Pos/Total (%)
Barron	October 2013	1/170 (0.6)	68/170 (40.0)	NC	NC
Barron	June-July 2014	14/267 (5.2)	89/267 (33.3)	3/81 (3.7)	22/81 (27.1)
Eau Claire	2009-2010	0/28 (0)	5/28 (17.9)	1/112 (0.9)	11/112 (9.8)
<b>Total</b>		<b>15/465 (3.2)</b>	<b>162/465 (34.8)</b>	<b>4/193 (2.1)</b>	<b>33/193 (17.1)</b>




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## Major findings and conclusions




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Remarkable feature #1

- *Borrelia mayonii* causes Lyme disease in the upper midwest

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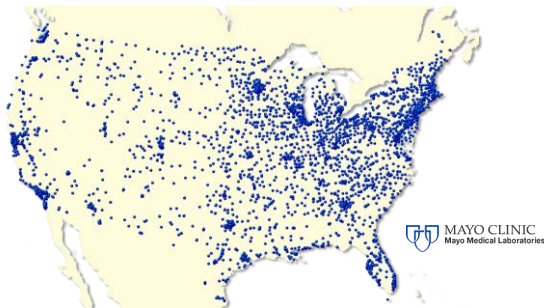
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- *B. mayonii* was not detected in 24,786 specimens from patients outside of MN, WI, and ND (2012-14)



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Remarkable feature #2

- Patients with *Borrelia mayonii* infection detected to date have had more severe disease than Lyme disease caused by *Borrelia burgdorferi*
  - Potential neurologic involvement (3 patients)
  - Illness requiring hospitalization (2 patients)

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### Remarkable feature #3

- Rashes were more diffuse
- Only 1 of 4 patients had classic EM lesion
  - Rashes involved the face, trunk, extremities, palms and soles (1 patient)




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### Remarkable feature #4

- *Borrelia mayonii* has been found predominantly in **whole blood**
- Historically, <0.1% of blood specimens have tested positive for *Borrelia burgdorferi*




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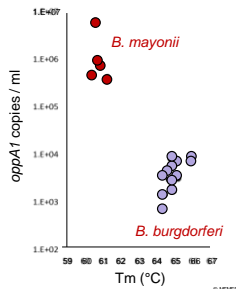
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### Remarkable feature #5

Patients with *B. mayonii* infection in blood had higher *oppA1* copies/mL when compared to patients with *B. burgdorferi* detected in blood during the same time period (**mean 180-fold higher**); ( $p < 0.0016$ )

<i>oppA1</i> (Lyme) PCR	Crossing point	Melting temp
<i>B. burgdorferi</i>	35.23	64.42
<i>B. mayonii</i>	<b>29.58</b>	<b>60.75</b>
<i>B. burgdorferi</i>	33.69	65.05
<i>B. burgdorferi</i>	33.82	65.86
<i>B. burgdorferi</i>	34.09	64.93
<i>B. mayonii</i>	<b>30.88</b>	<b>61.24</b>
<i>B. burgdorferi</i>	35.06	65.16
<i>B. burgdorferi</i>	33.77	65.08
<i>B. burgdorferi</i>	33.61	64.75
<i>B. mayonii</i>	<b>29.82</b>	<b>60.83</b>
<i>B. burgdorferi</i>	33.59	65.92
<i>B. burgdorferi</i>	34.51	64.55
<i>B. mayonii</i>	<b>26.48</b>	<b>60.56</b>
<i>B. burgdorferi</i>	34.77	64.20
<i>B. mayonii</i>	<b>30.63</b>	<b>60.38</b>
<i>B. burgdorferi</i>	35.14	64.65
<i>B. burgdorferi</i>	35.96	64.91
<i>B. burgdorferi</i>	37.29	64.22




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# THE LANCET Infectious Diseases

## Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study

Bobbi S Pritt, Paul S Mead, Diep K Hoang Johnson, David F Neitzel, Laurel B Respicio-Kingry, Jeffrey P Davis, Elizabeth Schiffman, Lynne M Sloan, Martin E Schriefer, Adam J Reptogle, Susan M Paskewitz, Julie A Ray, Jenna Bjork, Christopher R Steward, Alecia Deedon, Xia Lee, Luke C Kingry, Tracy K Miller, Michelle A Feist, Elitza S Theel, Robin Patel, Cole L Irish, Jeannine M Petersen



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## How to avoid tick-borne disease

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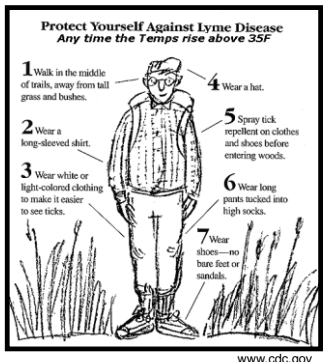
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## Other Helpful Hints

- Showering after being outdoors may help you notice/locate ticks
- Also protect your pets that go outdoors
- Remove embedded ticks right away using tweezers

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## Acknowledgements



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Amy Livermore, MS

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Alecia Deedon, BS

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Xia Lee, MS

**North Dakota Department of Health**  
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Michelle A. Feist, BS



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