Declaration

- D Webster
  - AbbVie honorarium for NB Provincial HCV Advisory Board
- T Hatchette
  - Research funding from GSK for influenza related vaccine effectiveness
  - AbbVie honorarium for lecture on risk of TB in the use of biologic agents

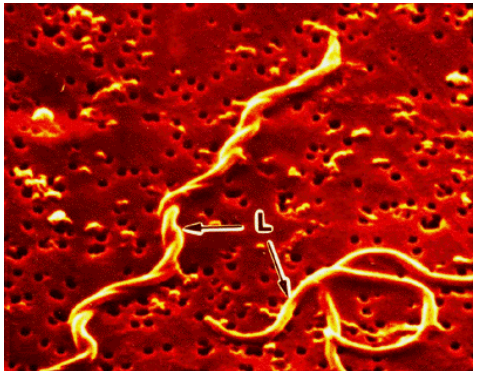
Objectives

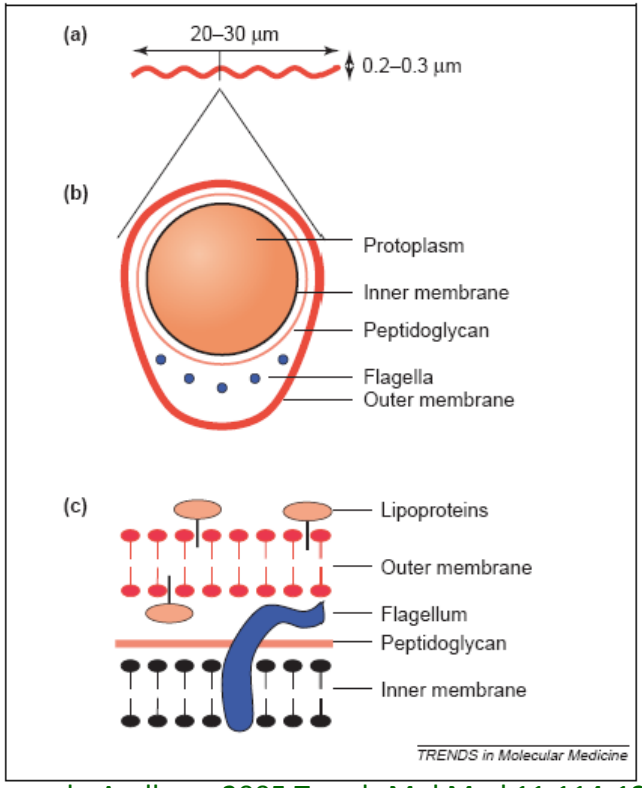
- Objectives
  - To gain a deeper understanding of tick-borne illness
  - To recognize the local implications
  - To understand the diagnostics and local related research

Lyme Disease

- 1977
  - Steere described clustering of Lyme arthritis.
- Fall 1981
  - Burgdorfer noted spirochete in body fluid of black-legged tick identified as Lyme Dz causative agent
- May 28, 1999
  - Isolation of *Borrelia burgdorferi* from *Ixodes scapularis* removed from a bird in Nova Scotia
- Now the most common arthropod-borne illness in NA

Borrelia burgdorferi sensu lato complex

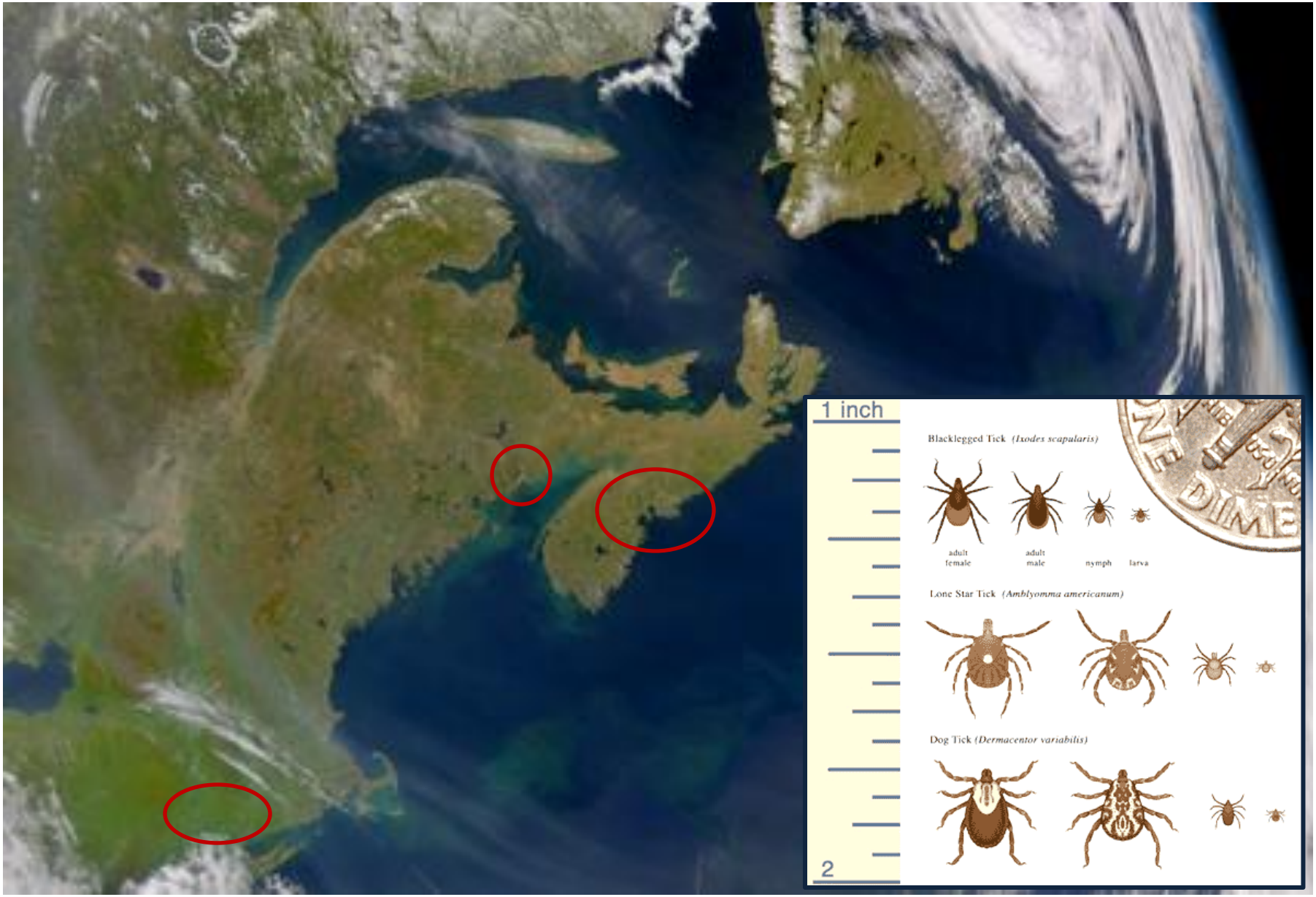




Guerau-de-Arellano, 2005 Trends Mol Med 11:114-120

The genospecies of *Borrelia burgdorferi* and their tick vectors and locations

	Principal tick vector	Location
Three pathogenic species		
Borrelia burgdorferi sensu stricto	*Ixodes scapularis*	Northeastern and north central US
	Ixodes pacificus	Western US
	Ixodes ricinus	Europe
Borrelia garinii	*Ixodes ricinus*	Europe
	Ixodes persulcatus	Asia
Borrelia afzelii	*Ixodes ricinus*	Europe
	Ixodes persulcatus	Asia
Eight minimally pathogenic or nonpathogenic species		
Borrelia andersonii	*Ixodes dentatus*	Eastern US
Borrelia bissettii	*Ixodes spinipalpis*	Western US
	Ixodes pacificus	
Borrelia valaisiana	*Ixodes ricinus*	Europe and Asia
Borrelia lusitaniae	*Ixodes ricinus*	Europe
Borrelia japonica	*Ixodes ovatus*	Japan
Borrelia tanukii	*Ixodes tanukii*	Japan
Borrelia turdae	*Ixodes turdus*	Japan
Borrelia sinica	*Ixodes persulcatus*	China

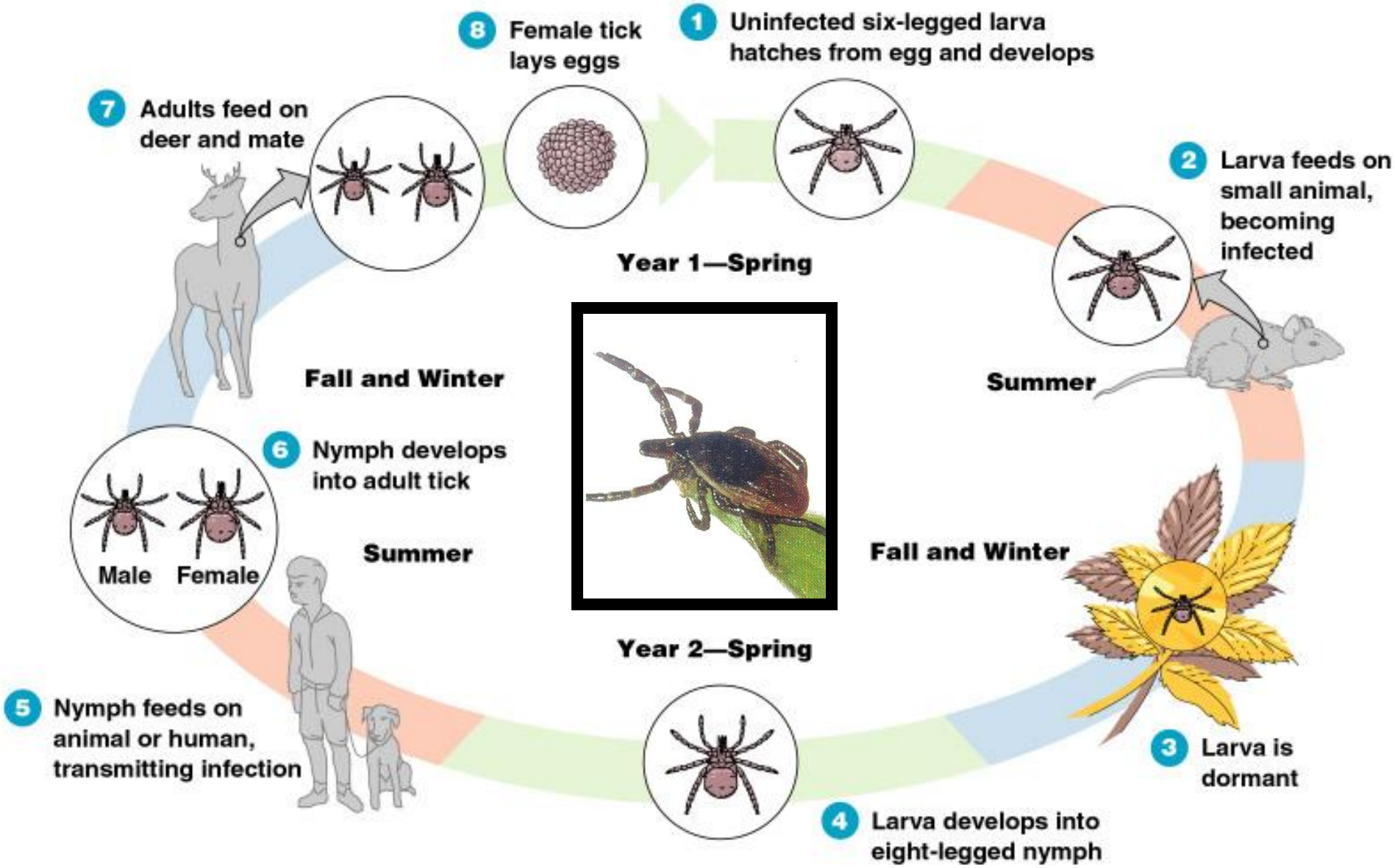

1 inch
2
Blacklegged Tick (Ixodes scapularis)
adult female
adult male
nymph
larva
Lone Star Tick (Amblyomma americanum)
Dog Tick (Dermacentor variabilis)

Ixodes scapularis Survey

- Millidgeville July 16, 2008
- Drag sampling
  - 29 larvae
  - 95 nymphs
- 20% of nymphs were positive for *Borrelia burgdorferi* by PCR
  - Comparable to endemic areas in Nova Scotia & Ontario

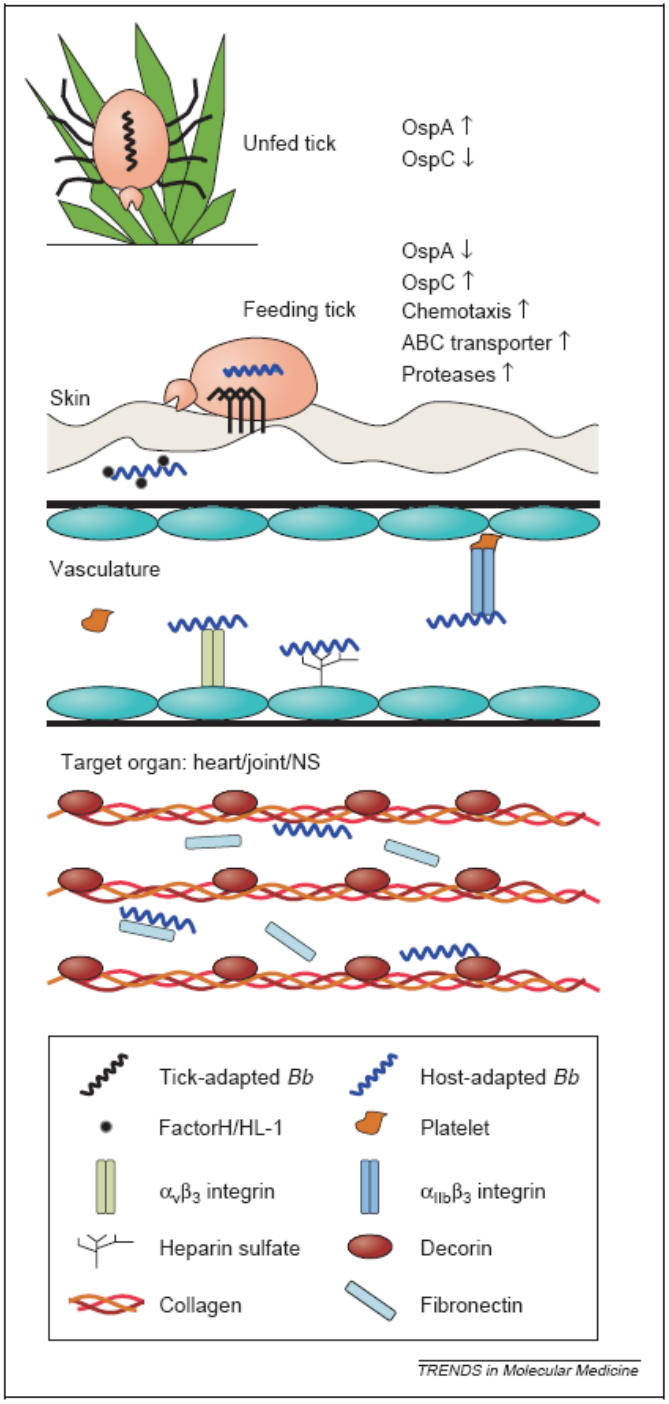
Ixodes scapularis
Deer Ticks

- Larvae:
  - Usually feed on mice
  - Need to have their first blood meal to get infected…then infected for life.
- Nymphs
  - Transmits most disease perhaps in part because they are so difficult to detect
- Adults
  - Feed and mate on large animals
  - Likely often already positive from nymphal phase on the mice



(a) The tick, *Ixodes scapularis*, has a two-year life cycle in which it requires three blood meals. The tick is infected by its first blood meal, and can pass on the infection to a human in its second.

Figure 23.13a

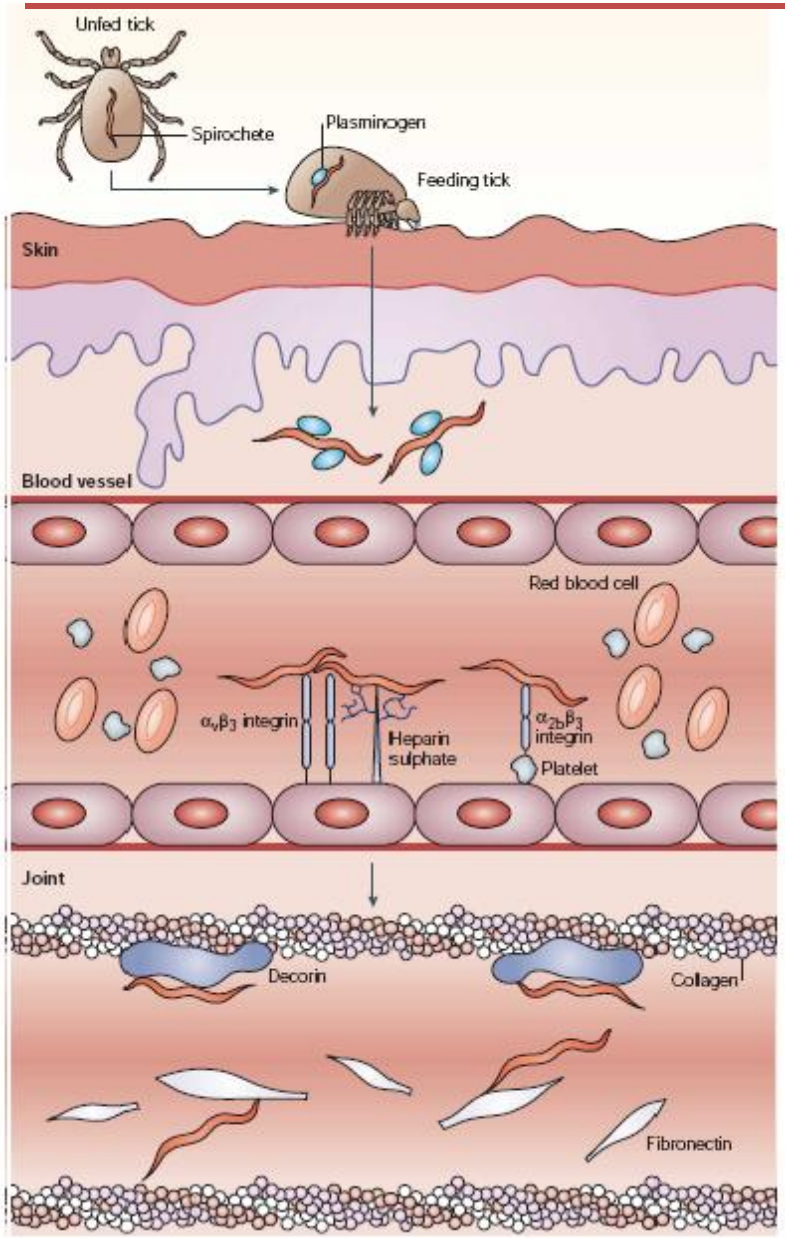


Mechanisms of Disease

- Bacteria undergo changes
  - OspC required for attachment in mammalian host
- Bacteria must travel from midgut to salivary glands
  - 48-72 hrs
  - As the tick fills, it seeks to rid itself of excess water by salivation back into the patient
- Ticks attached for < 24 hrs DO NOT transmit infection

(Guerau-de-Arellano, 2005 Trends Mol Med 11:114-120)

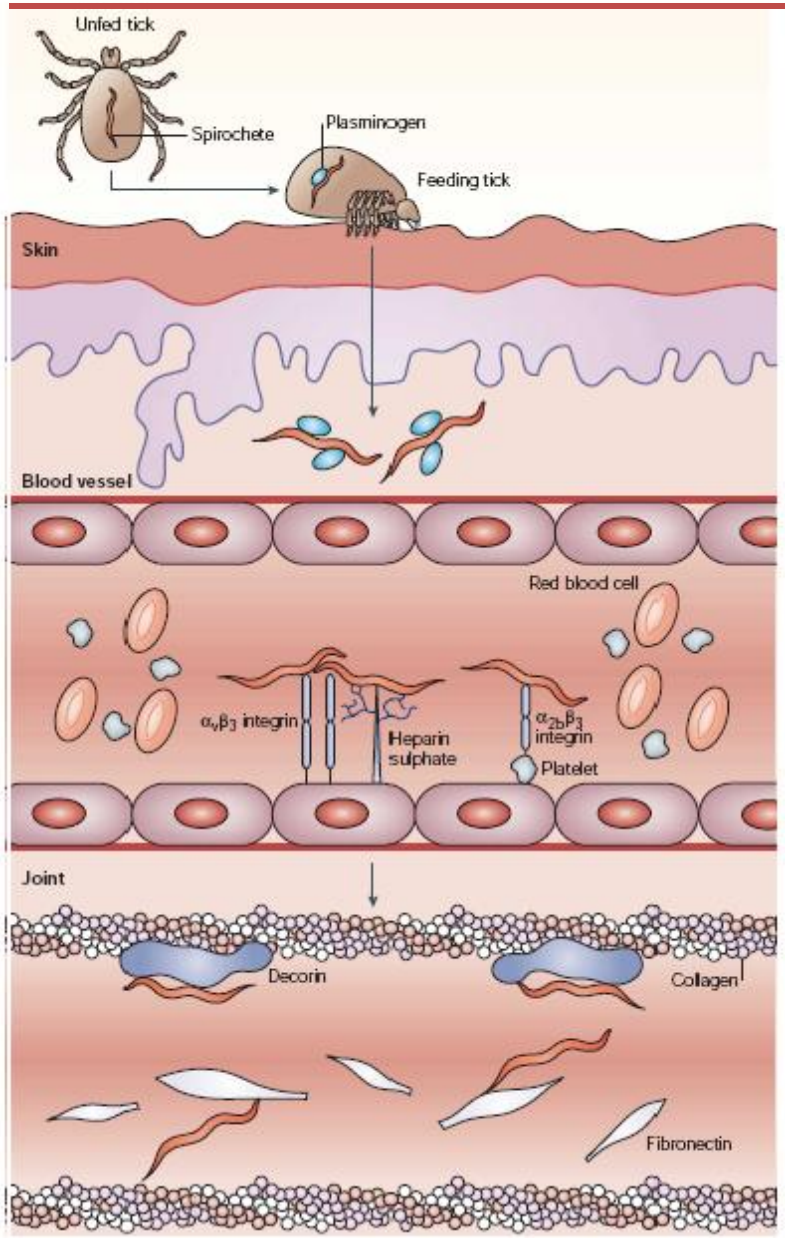
Mechanism of Disease



- Invasion helped by Tick saliva
- Uses human plasminogen to facilitate entry and spread
  - host plasmin to traverse host extracellular matrix
- After injection by the tick, spirochetes migrate outward, producing EM.

Steere and Glickstien, 2004 Nat Rev Immuno. 4:143-152

Mechanism of Disease



- Infection is then spread hematogenously to other organs.
- Organisms bind to host receptors on endothelial cells
  - P66 - integrin
  - Bgp - heparin sulphate
  - Fibronectin protein
  - Decorin binding proteins
- Spirochetes have particular tropism for skin, nervous tissue, and the AV node and joints.

Clinical Manifestations

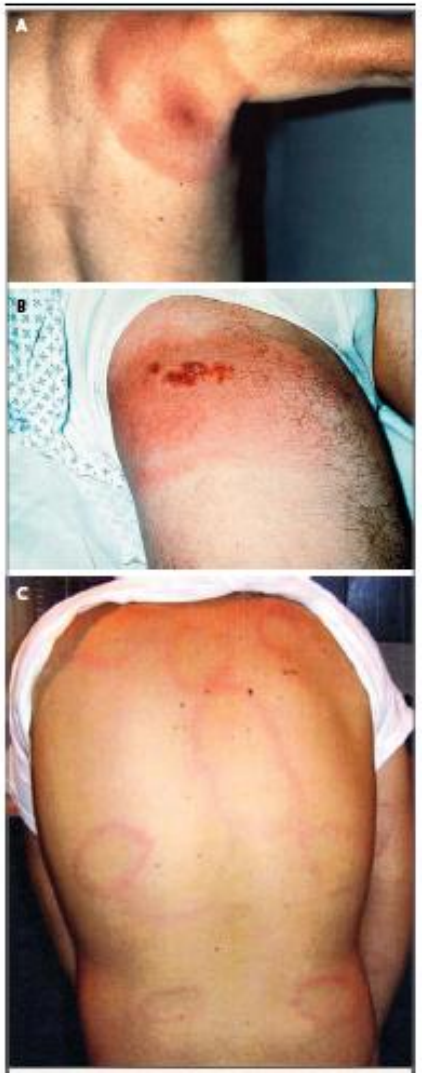
Lyme Clinical Manifestations

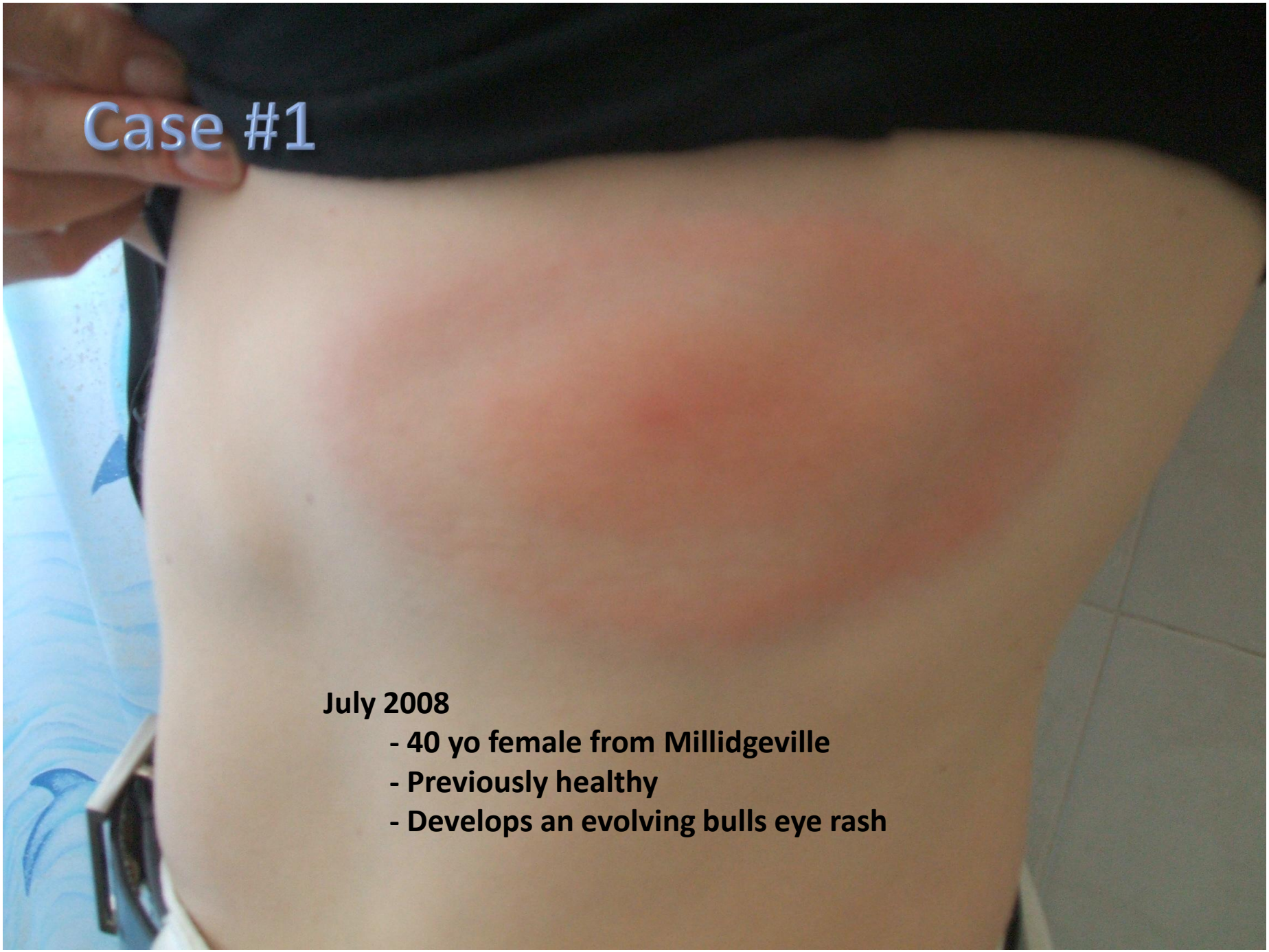
System	Early Localized 3–30 days post bite	Early Disseminated Days-months post bite
Cardiac		AV block, myopericarditis, panacrditis
Constitutional **Lymphatic**	**Flu-like Sx** Regional Lymphadenopathy	**Malaise,fatigue** Regional/general Lymphadenopathy
MSK	Myalgia	Migratory polyarthralgias/polyarthritis
Neurologic	Headache	Meningitis, Bell's Palsy, cranial neuritis, radiculoneuritis
Dermatologic	**Erythema Migrans**	**Secondary annular legions**

Asymptomatic infection can also occur.

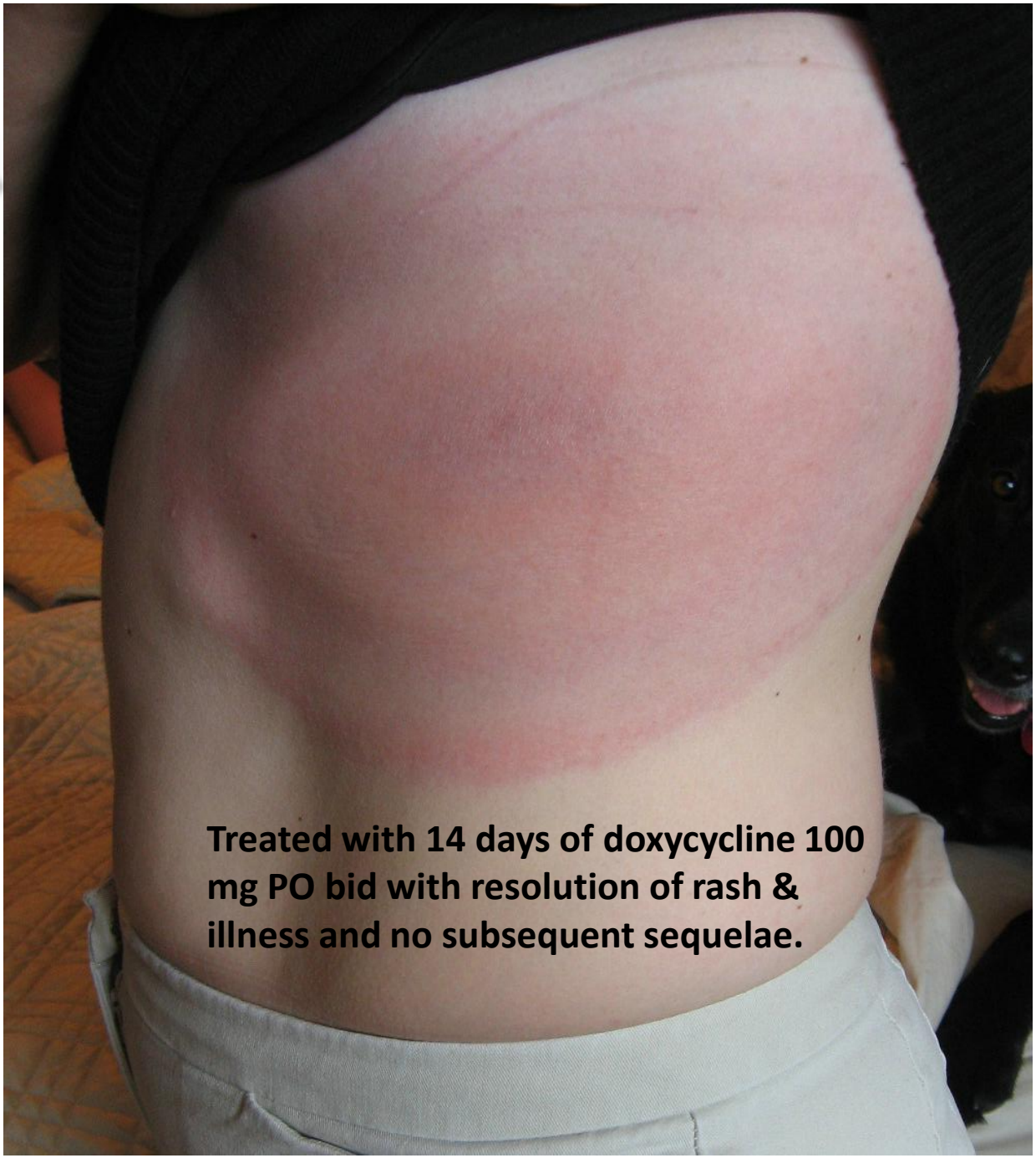
Erythema Migrans

- Typically within 7-14d (3-30) of bite
- Should be ay least 5 cm for secure diagnosis
- Usually expands in size over 24-48 hours
- Secondary lesions may be < 5 cm
- Can vary in appearance
  - oval, round
  - ± cental clearing
  - ± partially purpuric
  - ± central vesicles/pustules



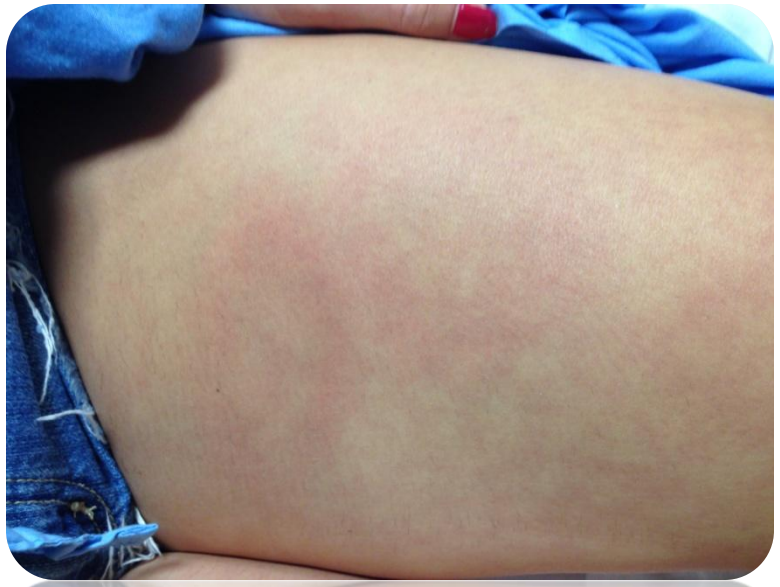

Case #1
July 2008
- 40 yo female from Millidgeville
- Previously healthy
- Develops an evolving bulls eye rash

Case #1


Treated with 14 days of doxycycline 100 mg PO bid with resolution of rash & illness and no subsequent sequelae.

Case #2

- August 23, 2014 – 23 yo female from Millidgeville
  - 2 day hx of facial droop
  - No fever
  - No headache
  - No meningismus
  - Doxycycline x 21 days
  - Lyme serology reactive
  - Facial palsy resolved



Early Neurologic Lyme Disease

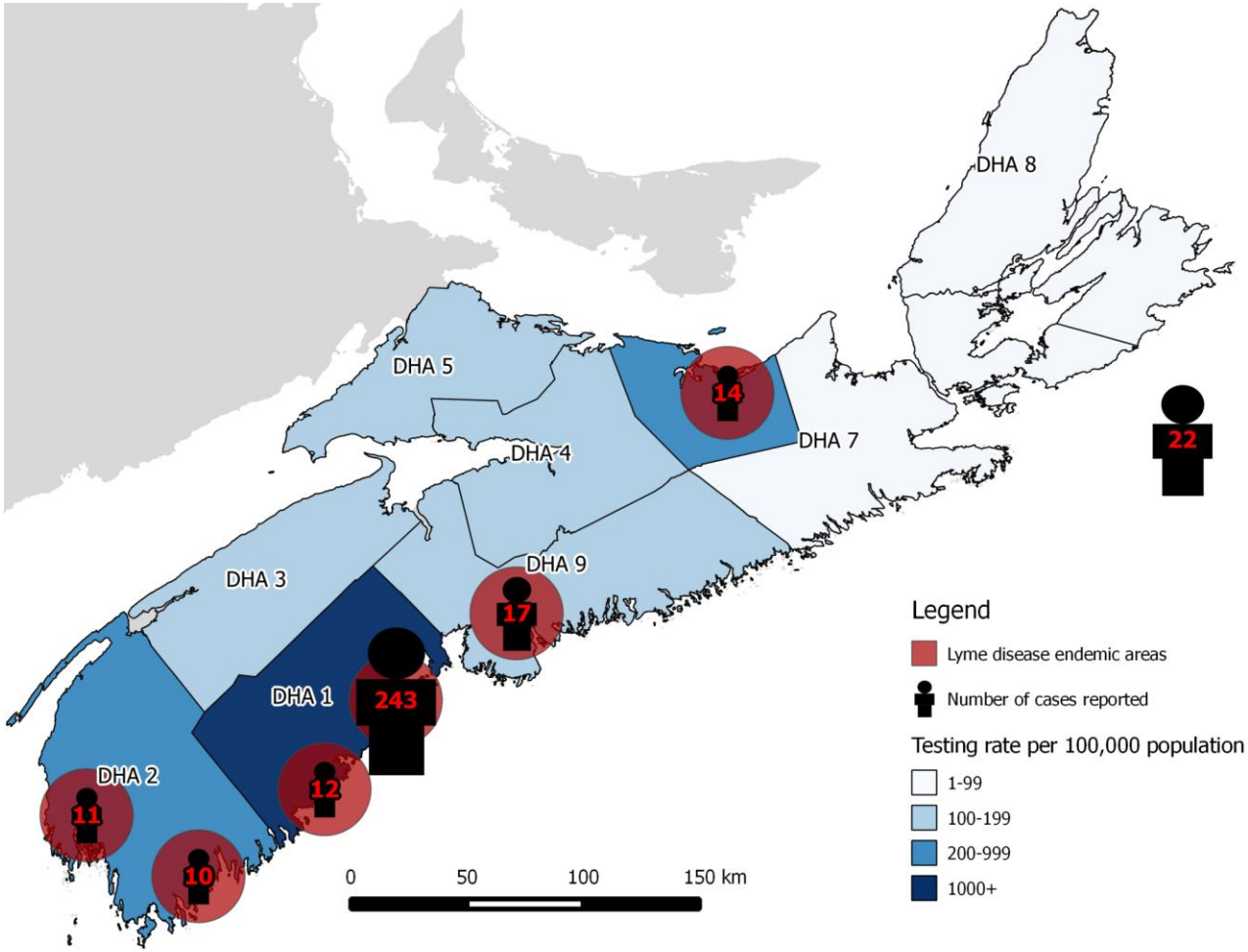
- Peripheral Nervous System
  - Radiculopathy
  - Cranial neuropathy
  - Mononeuritis monoplex
- Central Nervous System
  - Lymphocytic meningitis
  - Encephalomyelitis

Late Lyme Disease

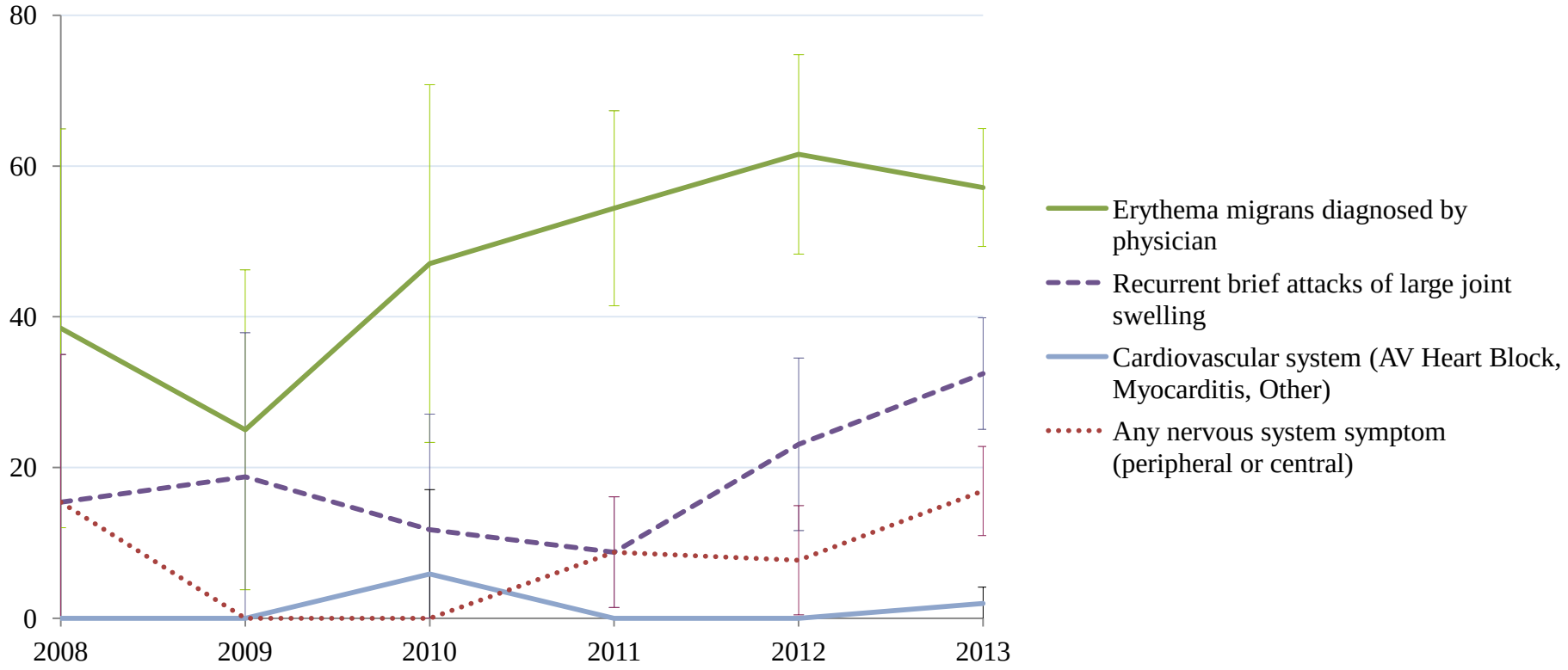
- Neurologic (uncommon in NA)
  - Encephalomyelitis
  - Peripheral neuropathy
  - Encephalopathy
- Rheumotologic
  - Monoarticular or oligoarticular arthritis
  - Typically the knee
  - Often intermittent if untreated
  - Inflammatory synovial fluid

Local Epidemiology

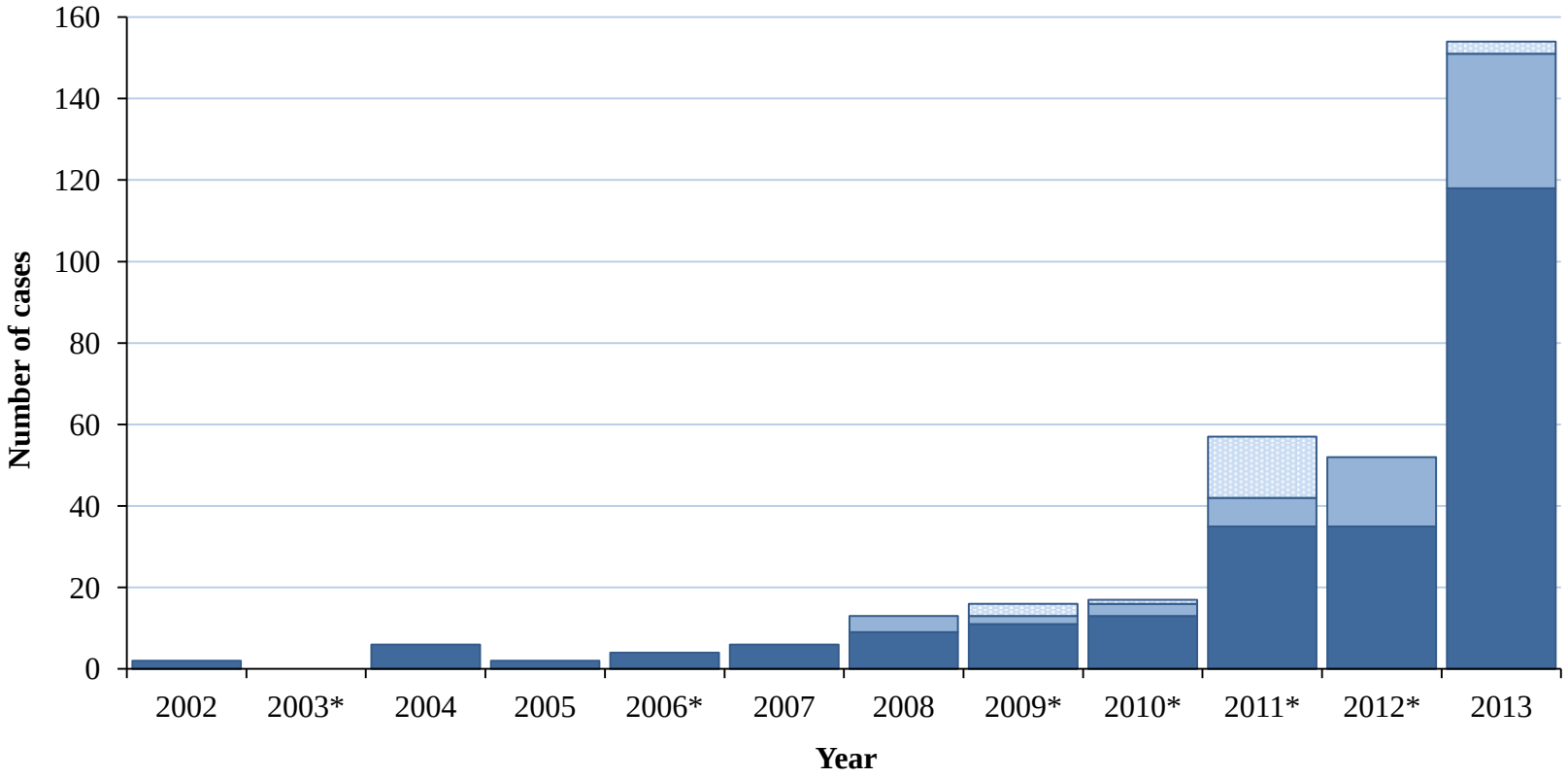
Reported cases of Lyme disease (2002-2013), Nova Scotia



Percentage of LD cases in NS with symptom complex by year, 2008-2013



Lyme Disease in Increasing Recognized in NS



*Years when new LD endemic areas were declared.

NS Serosurvey

	Laboratory test type				Seroprevalence estimates	
	Total screening (Zeus EIA) n	Zeus EIA positive or indeterm n (%)	C6 positive or equivocal n (%)	IgG WB borderline n (%)	IgG WB borderline	C6 ELISA
DHA1	191	21 (11.0)	1 (0.5)	0	0 (0-1.9)	0.52 (0-2.9)
DHA 2	199	19 (9.5)	2 (1.0)	0	0 (0-1.8)	1.01 (0.1-3.6)
DHA 3	261	42 (16.1)	2 (0.8)	0	0 (0-1.4)	0.77 (0.1-2.7)
DHA 4	120	12 (10.0)	1 (0.8)	1 (0.8)	0.83 (0-4.6)	0.83 (0-4.6)
DHA 5	44	1 (2.3)	1 (2.3)	0	0 (0-8.0)	2.27 (0.1-12.0)
DHA 6	74	3 (4.1)	0	0	0 (0-4.9)	0 (0-4.9)
DHA 7	72	3 (4.2)	0	0	0 (0-5.0)	0 (0-5.0)
DHA 8	201	22 (10.9)	1 (0.5)	0	0 (0-1.8)	0.5 (0-2.7)
DHA 9 /IWK	693	92 (13.3)	9 (1.3)	1 (0.1)	0.14 (0-0.8)	1.3 (0.6-2.5)
Nova Scotia*	**1855**	**215 (11.6)**	**17 (0.9)**	**2 (0.1)**	**0.14 (0.02-0.51)**	**0.98 (0.56-1.60)**

*Provincial seroprevalence estimates weighted by age, sex, and DHA, accounting for oversampling in DHAs 1, 2, and 3.

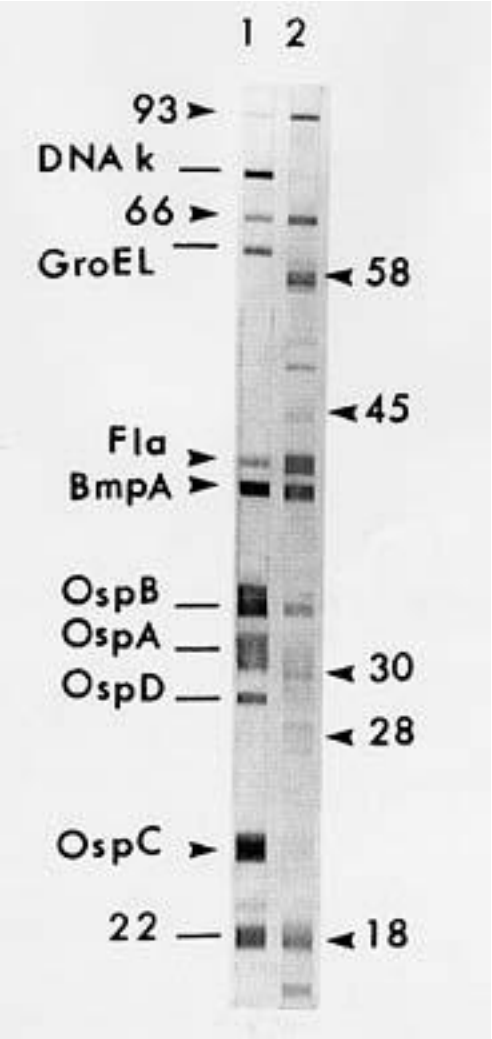
Diagnostics

Lyme Diagnosis

- Serology
  - Two tier testing
    - Sensitive EIA – whole cell or C6 peptide
    - Western blot
  - Culture
    - Poor sensitivity, some methods non reproducible
  - PCR
    - Only real use is in persistent arthritis

Specific Confirmatory Test

- Tier II Western blot
- CDC Interpretative Criteria
  - IgG WB
    - 5 of 10 bands present
  - IgM WB
    - 2/3 bands present
  - They are subjective /semi quantitative
  - Indeterminate bands are not reported



IgG Western blot

Performance of Serology Depends On Disease Stage

Poor sensitivity in Early Infection

Disease State	Whole Cell EIA	+IgM WB	+ IgG WB	Two Teir Testing
Acute (EM)	33-49%	43-44%	0-13%	29-40%
(EM) Convalescent after antibiotics	76-86%	75-84%	15-21%	29-78%
Neurological involvement	79%	80%	64-72%	87%
Arthritis	100%	16%	96-100%	97%

(Aguero-Rosenfeld et al., 2005 CMR 18:484-509)

Prospective Study of Serologic Tests for Lyme Disease

Allen C. Steere, Gail McHugh, Nitin Damle, and Vijay K. Sikand

Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston

- 1999-2001 East Lyme Connecticut
- 76 patients who had culture confirmed EM
- 147 with late Lyme or other illnesses
  - objective clinical findings and positive serology
- PLD (Post LD or Chronic LD)
  - pain or neurocognifive or fatigue < 6 months after appropriate ABX Rx
- Controls

CID 2008 47:188-195

Testing is Accurate for Late Lyme

Disease State	C6 EIA	EIA + IgM WB	EIA + IgG WB	EIA &IgM or IgG WB
Acute (stage 1)	19-38%	11-38%	6-15%	17-43%
Convalescent after antibiotics	47-63%	39-70%	17-20%	53-75%
Disseminated infection (acute neurologic or arthritis) (stage 2)	100%	85%	85%	100%
Persistent infection (stage 3)	100%	23%	100%	100%

CID 2008 47:188-195

Testing is Accurate for Late Lyme

Disease State	C6	+	EIA + IgG WB	EIA &IgM or IgG WB
Acute (stage 1)			6-15%	17-43%
Convalescent after antibiotics	4	%	17-20%	53-75%
Disseminated infection (acute neurologic or arthritis) (stage 2)	100%	85%	85%	100%
Persistent infection (stage 3)	100%	23%	100%	100%

2/13 had facial nerve palsy only!

CID 2008 47:188-195

Testing is Accurate for Late Lyme

Disease State	C6 EIA	EIA + IgM WB	EIA + IgG WB	EIA &IgM or IgG WB
Chronic LD (n=14)	43%	50%	36%	71%
Not Lyme (n=75) (CFS, FM,MS,RA)	1%	0%	0%	0%
Healthy (endemic area) (n=86)	5%	1%	1%	2%
Healthy (non-endemic area) (n=50)	2%	0%	0%	0%

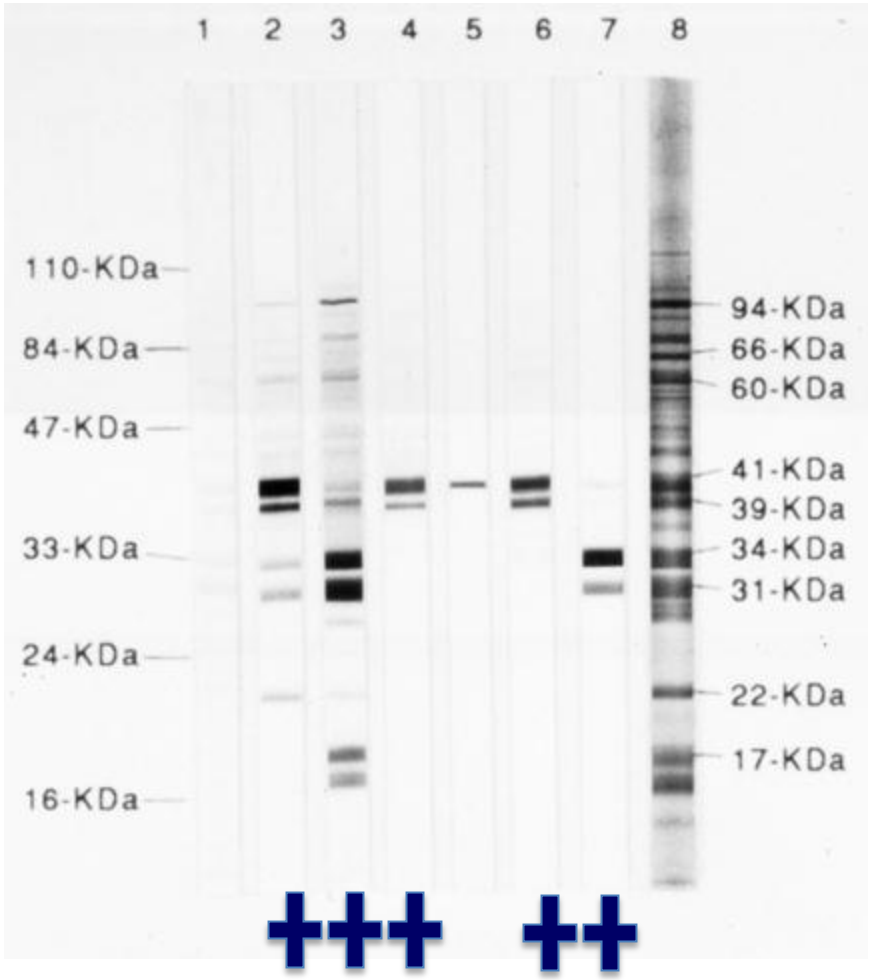
CID 2008 47:188-195

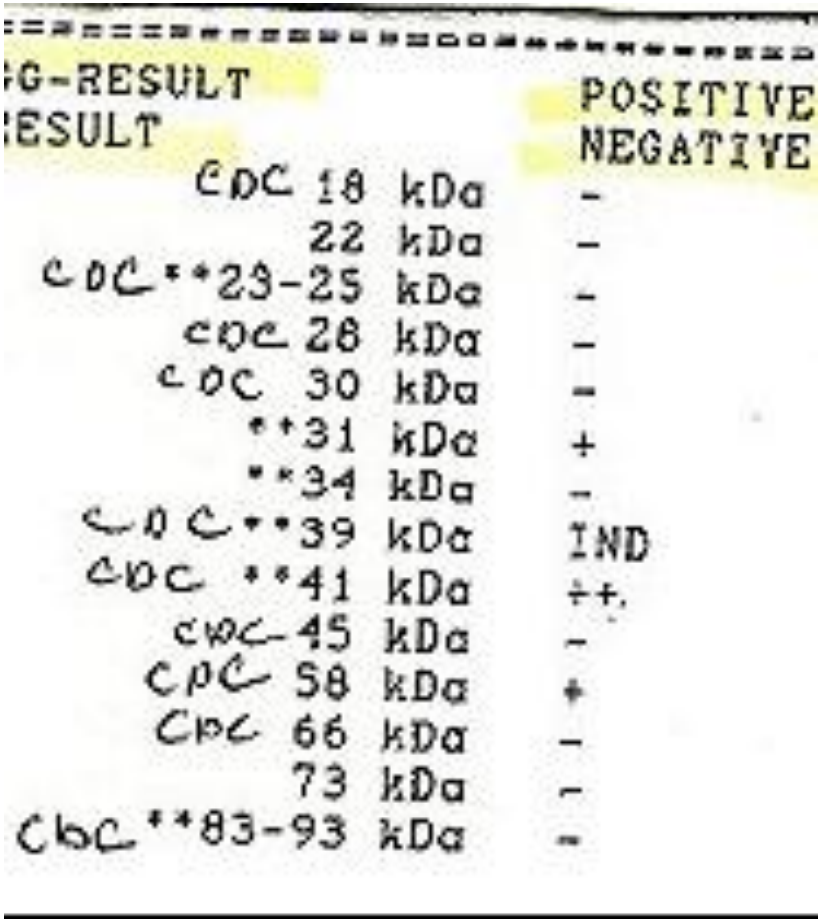
Diagnosis of Re-infection Is A Challenge

- Re-infection identified in 5 prospective studies of Lyme disease in the US
  - Rate of re-infection/year - 1.2 – 3.1%
  - Usually EM at a different site
- No pattern has been identified to differentiate re-infection from previous infections
- Serology can persist for decades
- Seroconversion or a 4x rise in IFA titre could indicate re-infection

Nadelman and Wormser, 2007 CID 45:1032-1038
Krause et al., 2006 AJTMH 75:1090-1094

IGeneX Interpretive Criteria





Caveats to Lyme

- Patients with symptoms of greater than 4 weeks duration should have IgG
  - Except if treated early in the course of their illness
- IgM can persist for years
- IgM can be false positive
- Early treatment may not seroconvert

Limitations of Serology

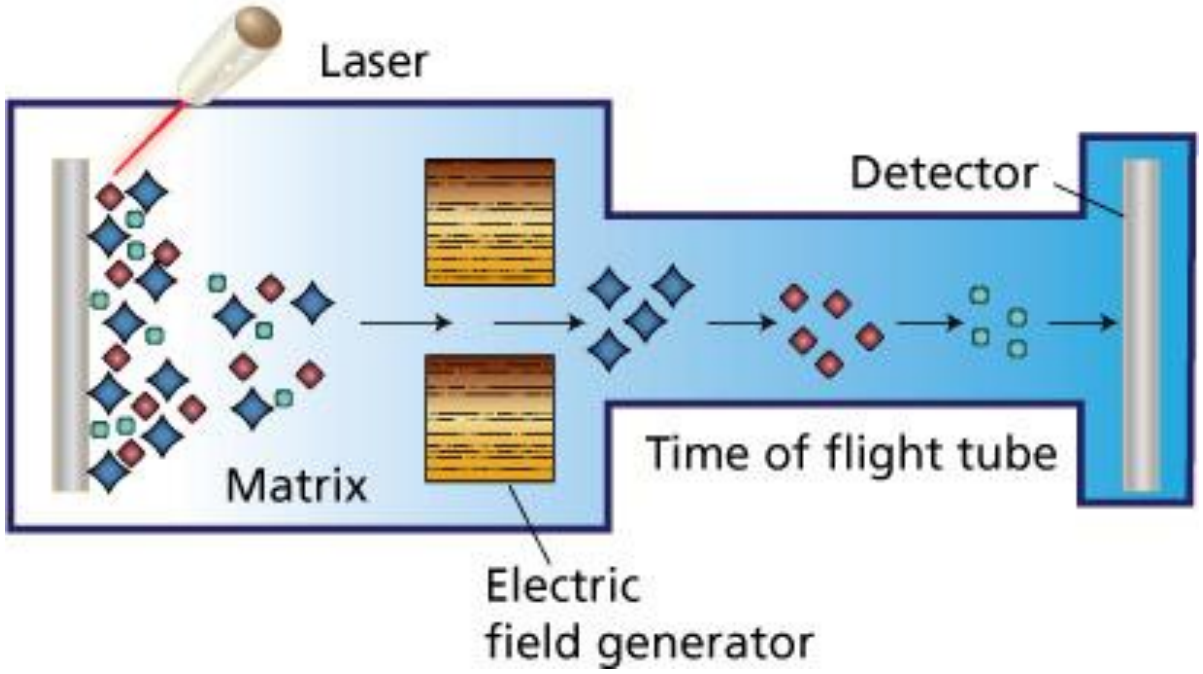
- False Positives (Specificity):
  - Cross-reacting antibodies
    - Other spirochetal disease, viral illnesses and autoimmune diseases (SLE, RA)
    - 5% general population may test positive for LD by ELISA!
- False Negatives (Sensitivity):
  - Slow antibody response
  - Early antibiotic therapy can abort seroconversion!
  - Unclear about sensitivity for other "strains" of *B. burgdorferi*

Novel Approach to Tick Identification

MALDI-TOF & Tick Identification

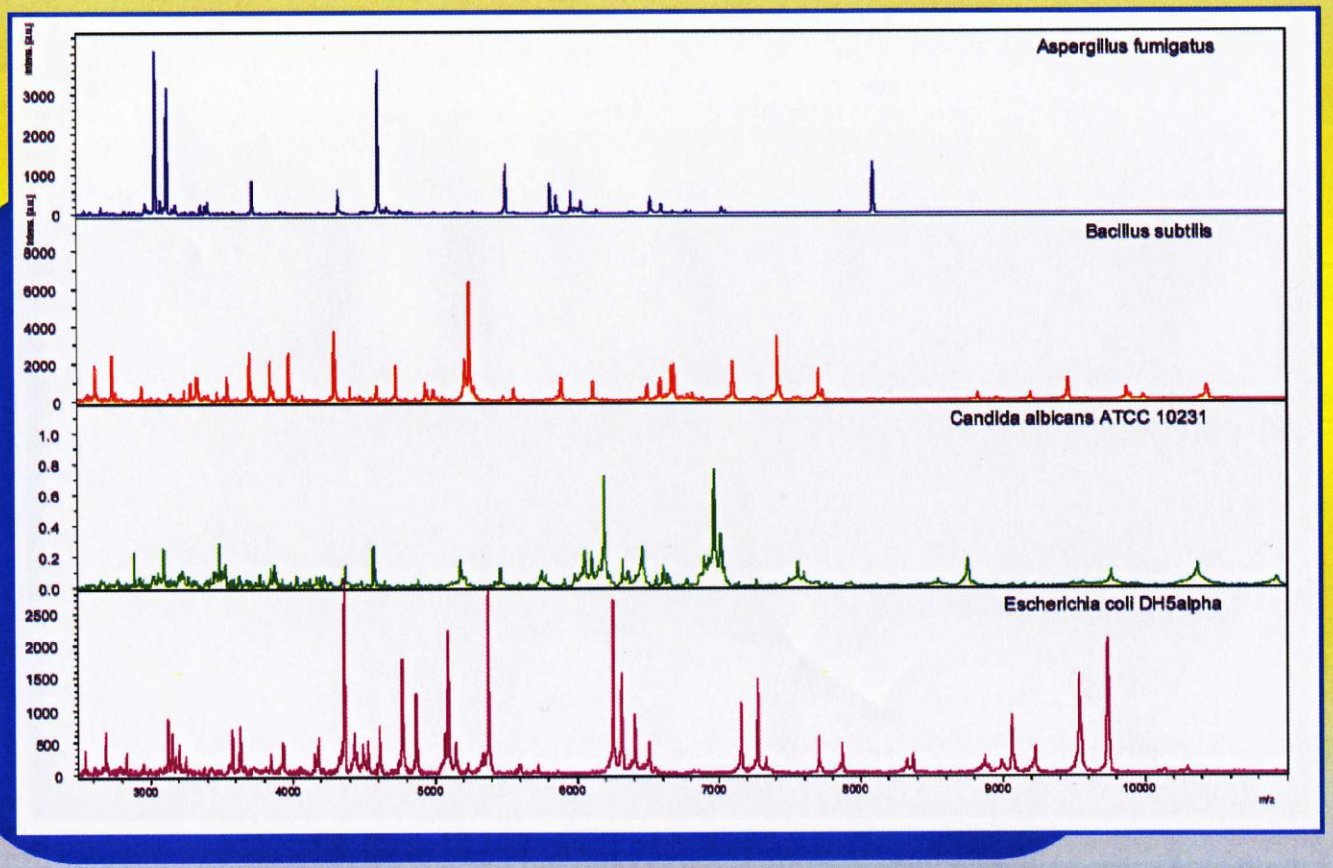
- Matrix-assisted laser desorption ionization – time of flight (MALDI-TOF) mass spectroscopy uses mainly ribosomal proteins
- Compares the mass peaks achieved by test strains to those of known strains
- Organism identification within 20 minutes of starting the process
- The resultant identification is meant to be robust, as it relies on high abundance proteins
- Potential use for tick identification

MALDI-TOF Principle



Broad Applicability of MALDI-TOF MS Profiling

Filamentous fungi, yeast, gram+ and gram- bacteria



Local Study

MALDI-TOF MS appears to be an effective tool for the rapid identification of tick vectors that requires no previous expertise in tick identification

A. americanum

I. scapularis

D. variabilis

Unengorged

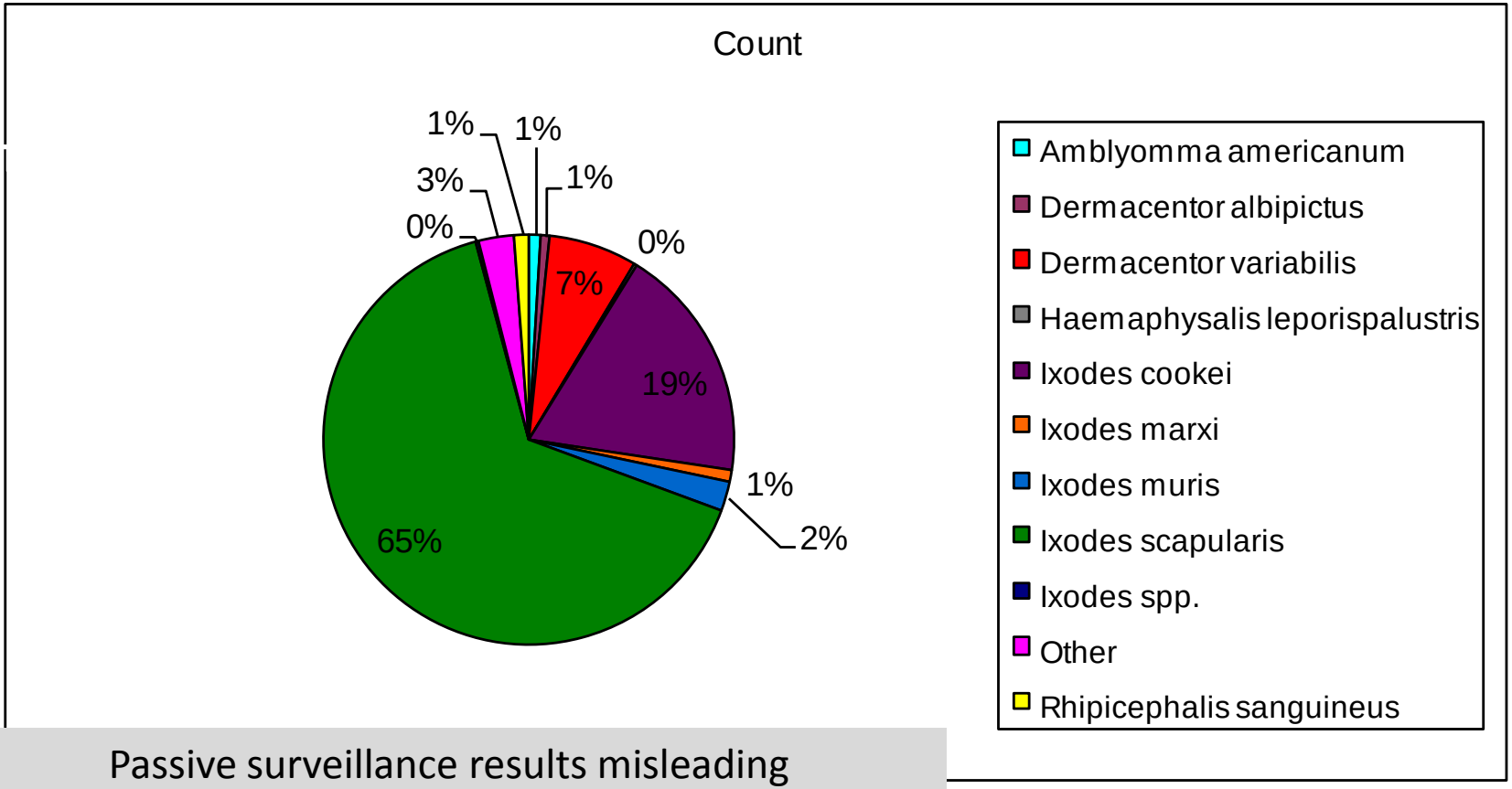
Engorged

1 mm

I. scapularis nymph

RELATIVE ABUNDANCE OF TICK SPECIES IN NEW BRUNSWICK

1 January 2008 to 1 September 2009



Passive surveillance results misleading
Rabbit and Moose Ticks are likely most abundant

Tick Study

MALDI-TOF Mass Spectrometry for the Rapid Identification of Tick Vectors

Amina Yssouf et al Journal *Clinical Microbiology 2013,51(2):522- 528*

Six species were tested, using the legs, identification verified by a molecular platform

Our Study

- Certain areas in New Brunswick are endemic for Lyme disease
  - Millidgeville area of Saint John
  - Grand Manan Island
- Objective:
  - to speciate ticks
  - to asses the ability of MALDI to distinguish between ticks infected with *B. burgdorferi* from those that are not

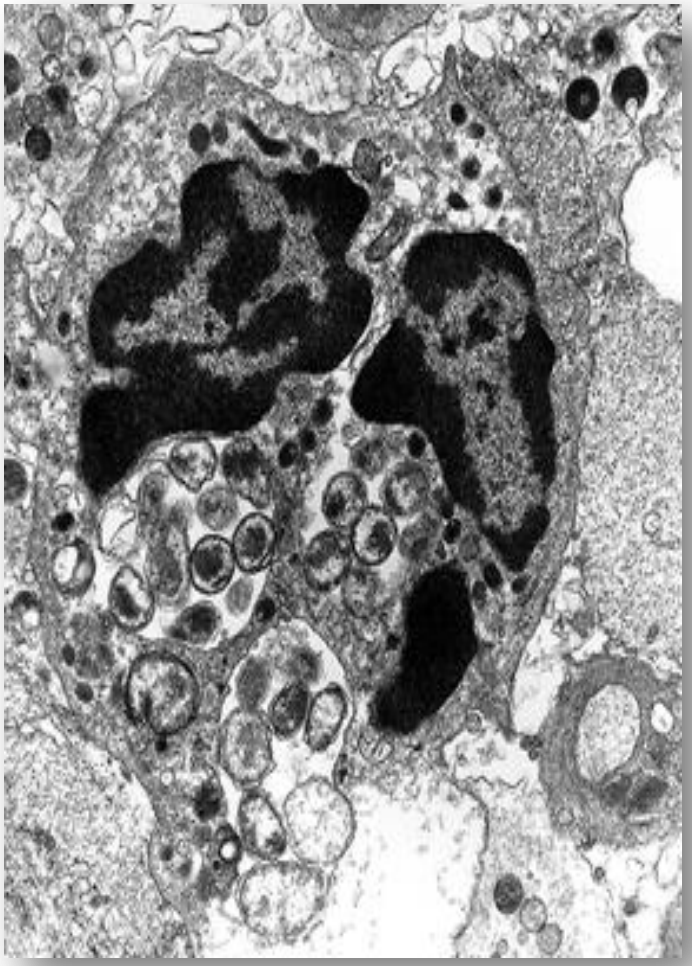
Other Emerging Tick Borne Illness In Atlantic Canada

Case #3

- August 2013
  - Aseptic meningitis
- September 2013
  - Re-admitted with weakness
  - Neurology & ID consults
    - Multiple tests ordered
      - Lyme serology → non-reactive
      - Anaplasma titre → reactive **titre 1:64**

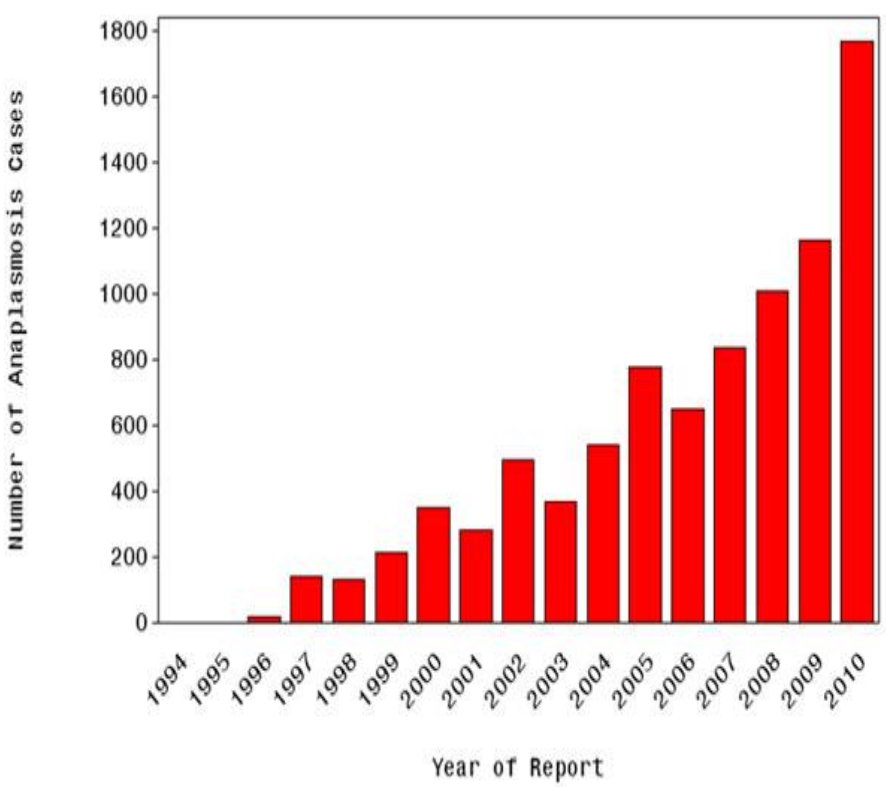
Human Granulocytic Anaplasmosis

- Causative agent of HGA is *Anaplasma phagocytophilum*
- First described in 1994, originally called HE
  - *Ehrlichia phagocytophilum*
- Obligate intracellular rickettsia
- Transmitted by *Ixodes scapularis*

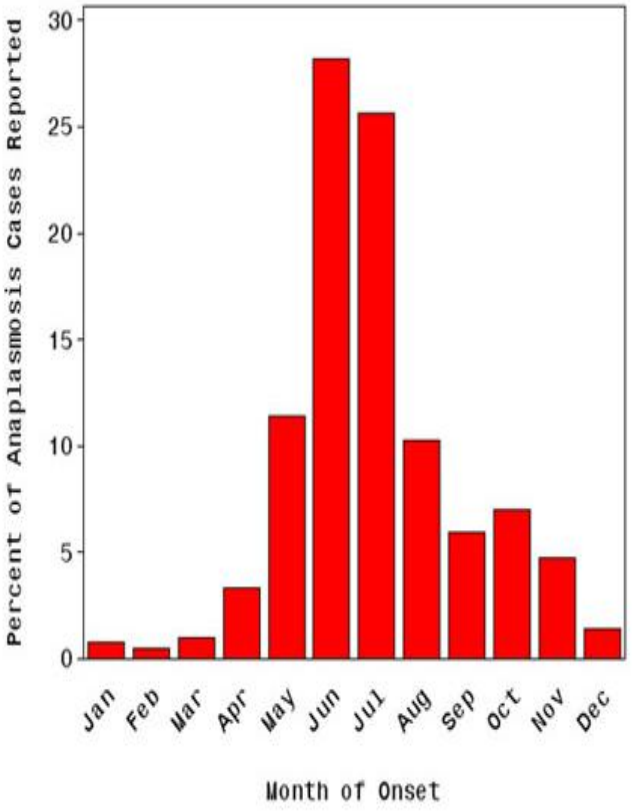


HGA Epidemiology

Increasing Incidence in US



Corresponds with Tick Season



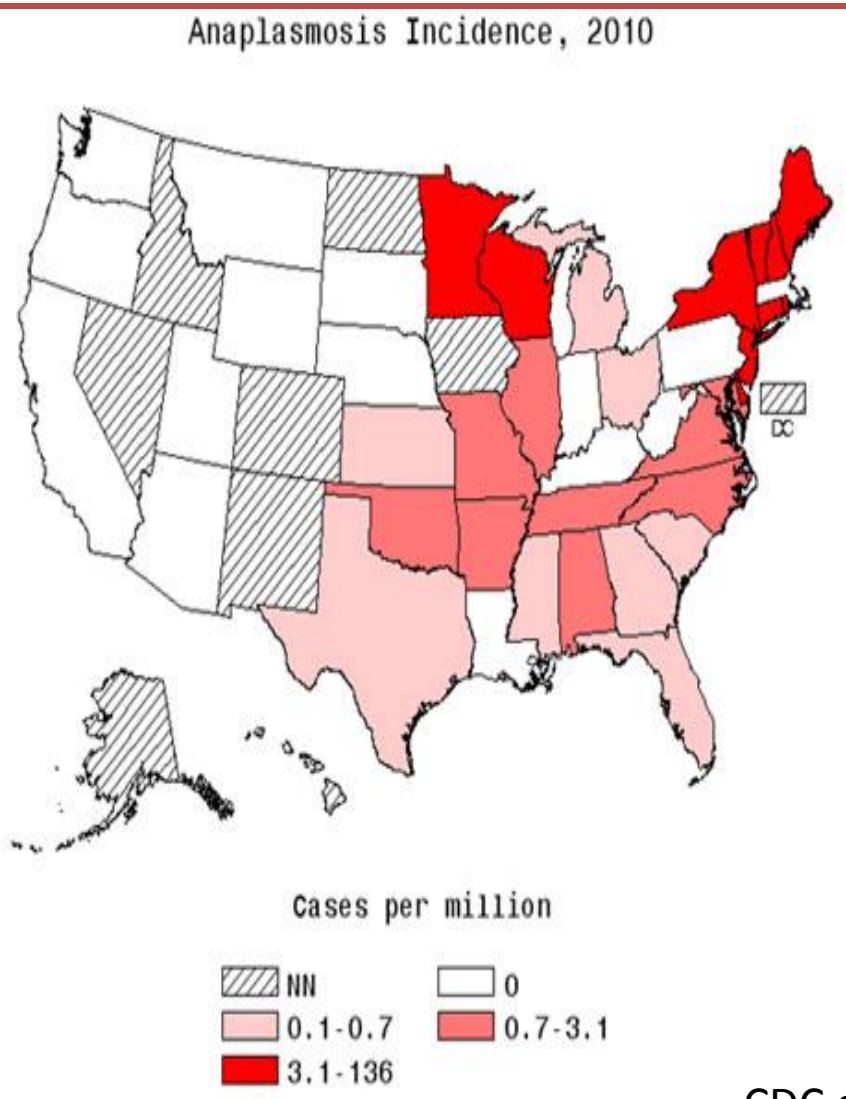
- Incidence in USA of 1.6 per million (2001-2002), and increasing

CDC.gov

HGA Epidemiology

Overlaps Lyme Endemic Areas

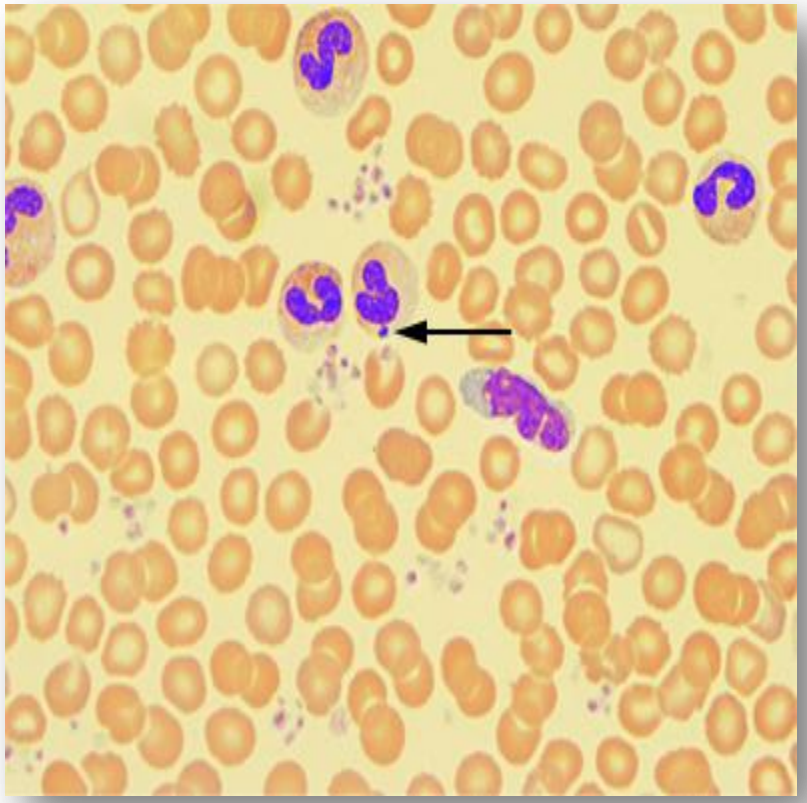
- Highest incidence
  - RI (36.5 cases/million)
  - Minnesota (3.9-12.3)
  - Connecticut (8.1-15.9)
  - Wisconsin (8.8-9.5)
  - NY (2.3-2.7)
  - Maryland (1.6)



CDC.gov

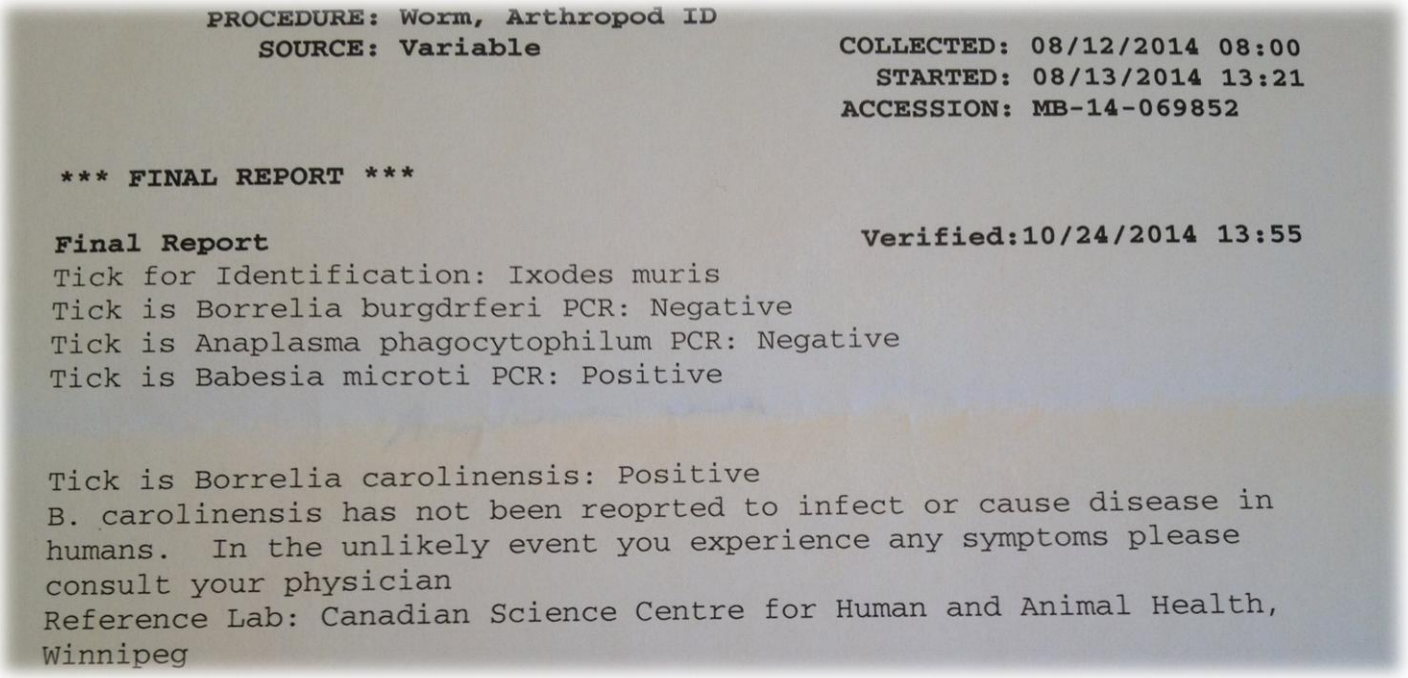
Diagnosis

- Not easily cultured
- Five accepted means of diagnosis:
  - Serology: sensitivity 94-100% (2-3 weeks after onset)
  - Buffy coat examination: morulae found in neutrophils of 20-80% of pts
  - PCR: not standardized: HGA sensitivity 60-70%
  - Immunohistochemical stains: bone marrow tissue, autopsy tissue
  - Clinical: most sensitive, recognition of endemic/epidemiologic risk factors, clinical presentation, and laboratory abnormalities



Case #4

- August 11, 2014
  - 43 yo female goes rasberry picking
  - The next day she finds a tick on her neck



PROCEDURE: Worm, Arthropod ID
SOURCE: Variable

COLLECTED: 08/12/2014 08:00
STARTED: 08/13/2014 13:21
ACCESSION: MB-14-069852

*** FINAL REPORT ***

Final Report
Verified:10/24/2014 13:55

Tick for Identification: Ixodes muris
Tick is Borrelia burgdrferi PCR: Negative
Tick is Anaplasma phagocytophilum PCR: Negative
Tick is Babesia microti PCR: Positive

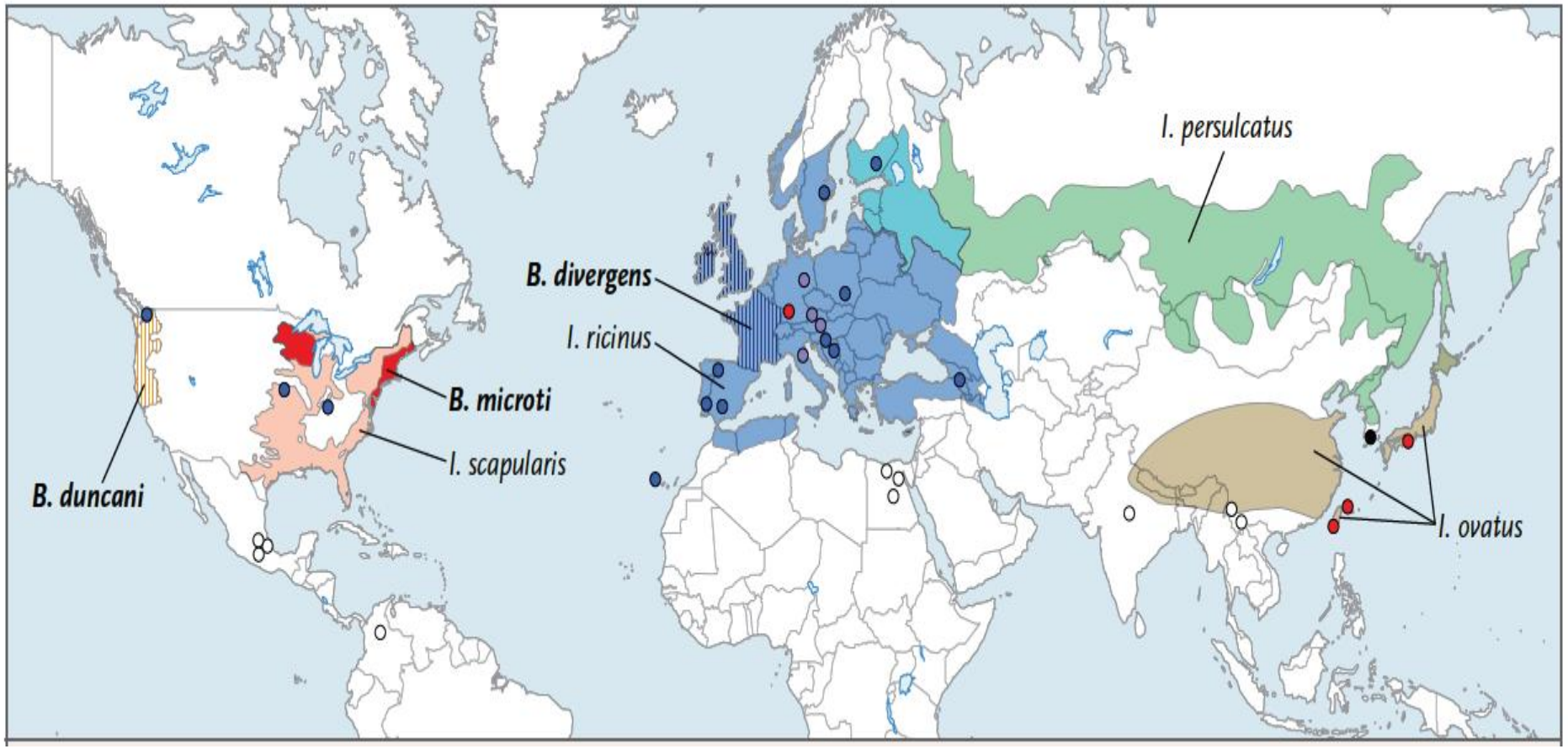
Tick is Borrelia carolinensis: Positive
B. carolinensis has not been reoprted to infect or cause disease in humans. In the unlikely event you experience any symptoms please consult your physician
Reference Lab: Canadian Science Centre for Human and Animal Health, Winnipeg

Babesiosis

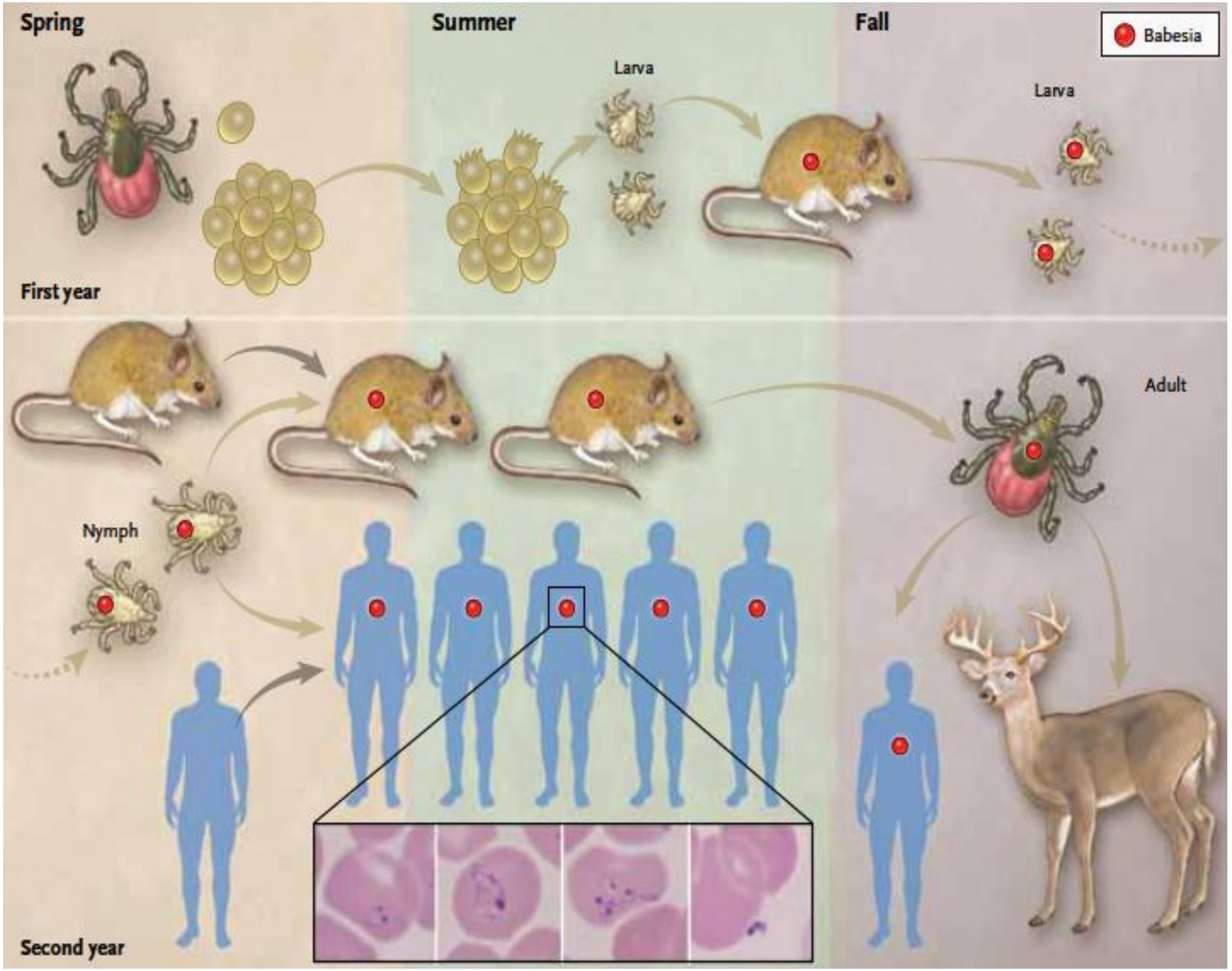
- Causative organism
  - *Babesia spp*
- Regional pathogen
  - *Babesia microti*
- Transmitted by *Ixodes scapularis*
- Discovered in 1888 by Victor Babes
- Originally seen in erythrocytes of cattle and sheep

http://www.kumc.edu/dc/pc/babes.jpg

Epidemiology



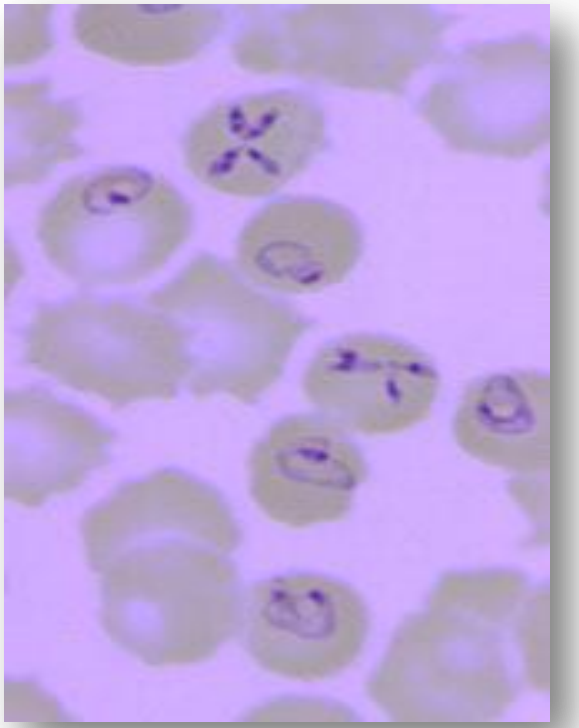
N Engl J Med 366:2397



N Engl J Med 366:2397

Presentation

- Fever, mild splenomegaly and hepatomegaly
- Haemolysis, thrombocytopenia, transaminitis
- Parasitemia seen on direct smear
- Most infections are self-limited or respond anti-infectives
  - Atovaquone, azithromycin, clindamycin, quinine



Diagnosis

- Microscopy
  - Definitive diagnosis on thin blood smear (multiple smears, multiple days)
- PCR
  - 18S rRNA has higher sensitivty than blood smear
  - RT-PCR can detect 0.0001% parasitemia
- Serology
  - Indirect immunofluorescent antibody testing (IFAT)
  - ABs may persist for years after infection is cleared

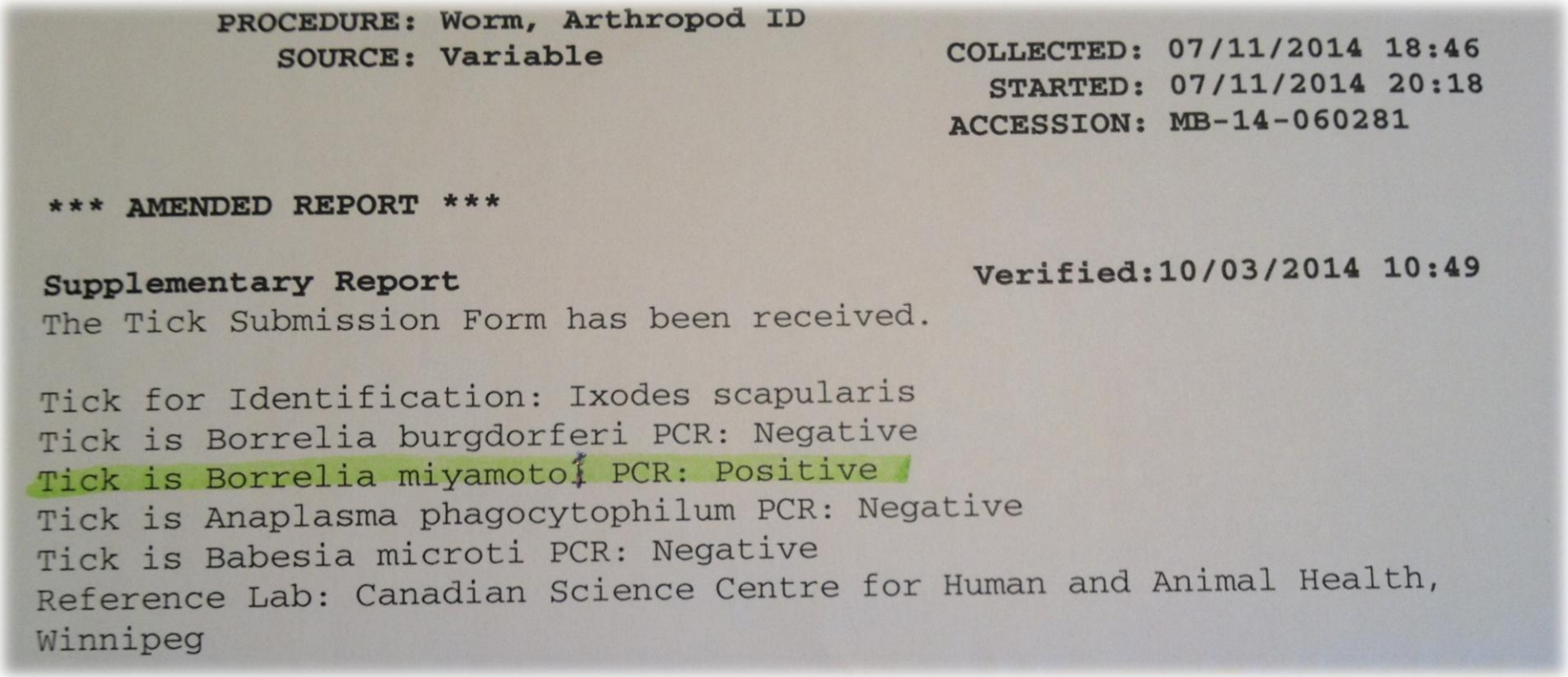
Case #5

41 yo male from Quispamsis

- July 4 - found a tick in his left groin
- July 9 - onset of fevers & drenching sweats
- July 12, 2014
  - Presents to SJRH with fever 39.1 °C
  - ALT 116 & bilirubin 36
  - Ceftriaxone 1g IV q24h
- July 13 - afebrile and requesting D/C home
  - Cefuroxime 500 mg PO bid x 14 days

Case #5

- July 11 - tick submitted for identification



PROCEDURE: Worm, Arthropod ID
SOURCE: Variable

COLLECTED: 07/11/2014 18:46
STARTED: 07/11/2014 20:18
ACCESSION: MB-14-060281

*** AMENDED REPORT ***

Supplementary Report
Verified:10/03/2014 10:49

The Tick Submission Form has been received.

Tick for Identification: Ixodes scapularis
Tick is Borrelia burgdorferi PCR: Negative
Tick is Borrelia miyamotoi PCR: Positive
Tick is Anaplasma phagocytophilum PCR: Negative
Tick is Babesia microti PCR: Negative
Reference Lab: Canadian Science Centre for Human and Animal Health, Winnipeg

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Meningoencephalitis from *Borrelia miyamotoi* in an Immunocompromised Patient

Joseph L. Gugliotta, M.D., Heidi K. Goethert, Sc.D., Victor P. Berardi, B.S., and Sam R. Telford III, Sc.D.

SUMMARY

Ixodes ticks serve as vectors for *Borrelia burgdorferi*, the agent of Lyme disease. Globally, these ticks often concurrently harbor *B. miyamotoi*, a spirochete that is classified within the relapsing-fever group of spirochetes. Although humans presumably are exposed to *B. miyamotoi*, there are limited data suggesting disease attributable to it. We report a case of progressive mental deterioration in an older, immunocompromised patient, and even though Koch's postulates were not met, we posit *B. miyamotoi* as the cause, owing to its direct detection in cerebrospinal fluid (CSF) with the use of microscopy and a polymerase-chain-reaction (PCR) assay. It is likely that *B. miyamotoi* is an underrecognized cause of disease, especially in sites where Lyme disease is endemic.

Guliotta. NEJM, 2013.

Borrelia miyamotoi in New Brunswick

Summary of blacklegged ticks infected or co-infected with *B. miyamotoi*					
Yr collected	**Identifier**	**Stage**	**Host**	**Province**	**Locality**
2014			Human	NB	St. Jacques
2013			Dog	NB	Quispamsis
2012	3323	Female	Dog	NB	Le-Goulet
2012	4985	Female	Cat and Dog	NB	Grand Manan
2012	4990	Female	Cat and Dog	NB	Grand Manan
2010	2554	Female	Human	NB	Precise location not available

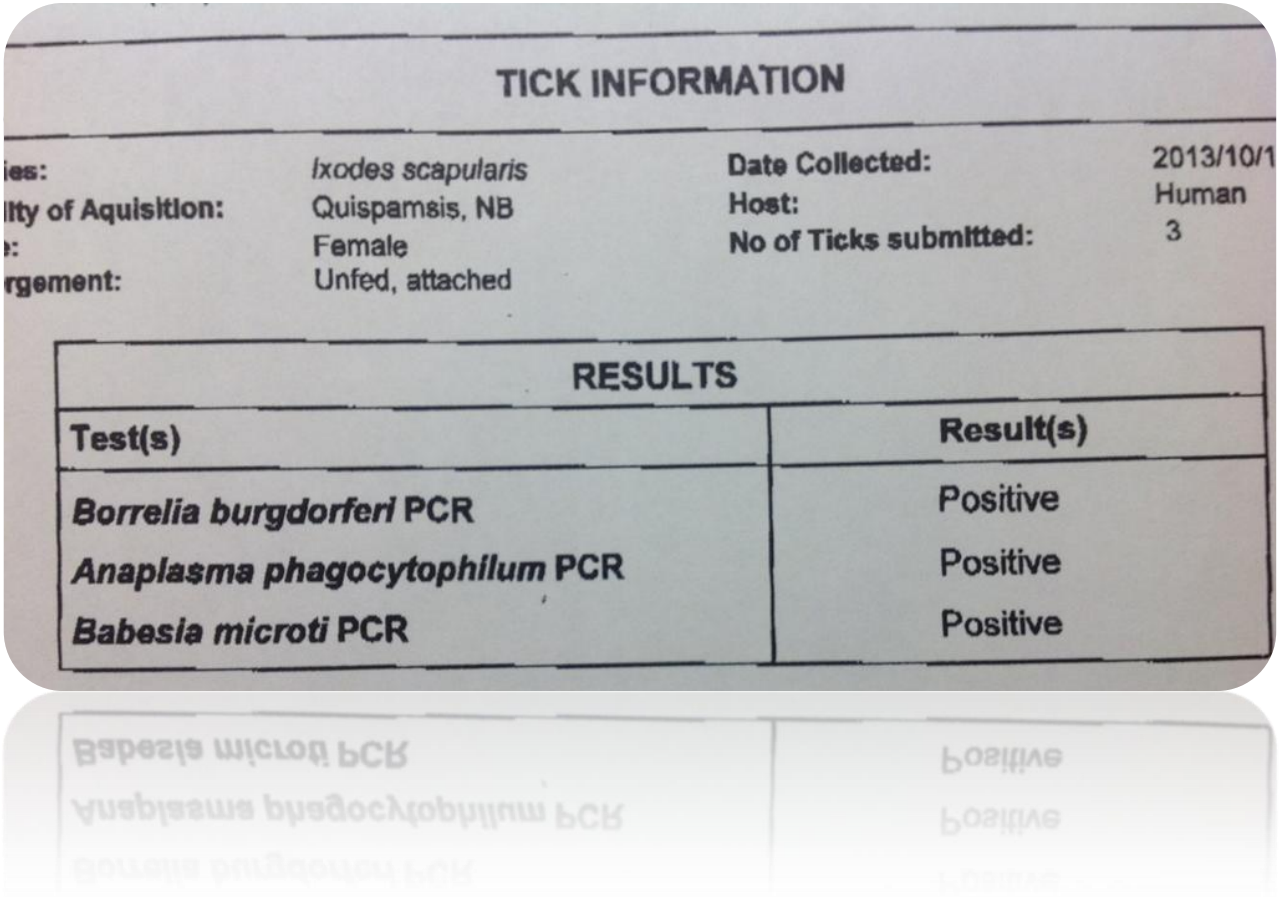
2 Additional human cases this summer 2014 – one with febrile illness.

R Lindsay & J Goltz.
Unpublished data.

Conclusions

- Increasing rates of tick-borne illness being observed in NB & NS
- Diagnostics via sensitive EIA and specific Western blot
- Potential for MALDI-TOF MS for tick identification and identifying infected ticks
- More than just B. burgdorferi to be aware of locally

Thanks



TICK INFORMATION

ies:	*Ixodes scapularis*	Date Collected:	2013/10/1
lity of Aquisition:	Quispamsis, NB	Host:	Human
e:	Female	No of Ticks submitted:	3
rgement:	Unfed, attached		

RESULTS

Test(s)	Result(s)
***Borrelia burgdorferi* PCR**	Positive
***Anaplasma phagocytophilum* PCR**	Positive
***Babesia microti* PCR**	Positive