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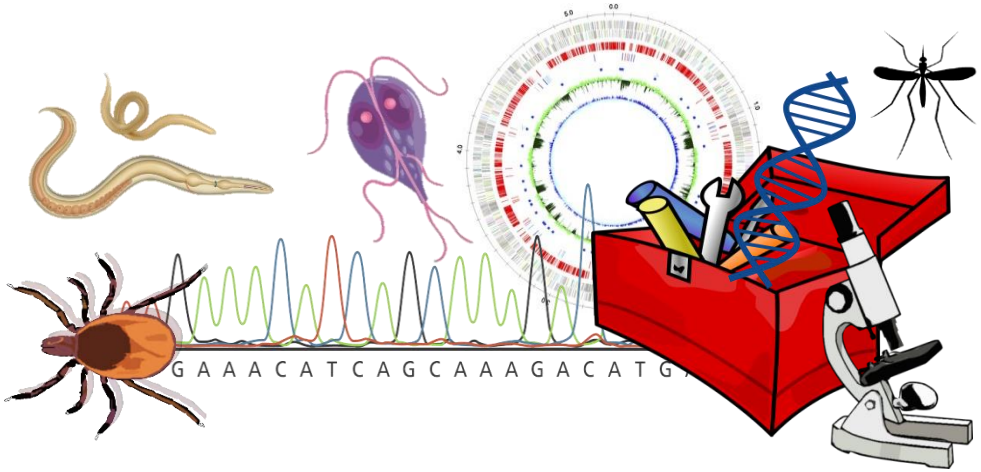
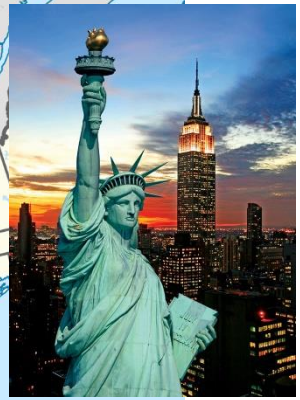


Development of molecular assays for parasitic and vector-borne diseases

Professor Bobbi S. Pritt, MD, MSc, DTMH
Chair, Division of Clinical Microbiology
Mayo Clinic, Minnesota, USA

Conflicts of Interest

- None



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Minnesota – the “Land of 10,000 Lakes”

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MAYO CLINIC
Ranked
#1
in the
Nation
U.S. News & World Report

Division of Clinical Microbiology

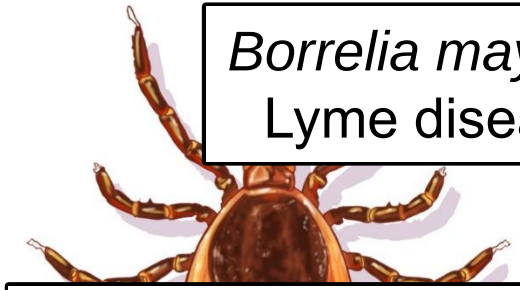
- International reference laboratory
- > 4 million tests annually
- Seven specialty laboratories
  - Bacteriology
  - Virology
  - Infectious Diseases Serology
  - Core Processing/Media Preparation
  - Mycology/Mycobacteriology
  - Parasitology/Vector-borne diseases
  - Clinical Microbiology Molecular lab

Vector-borne Diseases in the United States

Starting with tickborne diseases

Ixodes scapularis

Black-legged tick,
also known as deer tick
(female)



Borrelia mayonii
Lyme disease

Ehrlichia muris
erhlichiosis

Powassan virus
Disease (Lineage II:
deer tick virus)

Lyme disease
(*Borrelia burgdorferi*)

Babesiosis
(*Babesia* spp.)

Anaplasmosis
(*Anaplasma phagocytophilum*)

Borrelia miyamotoi
borreliosis

Eastern U.S. and
upper midwest

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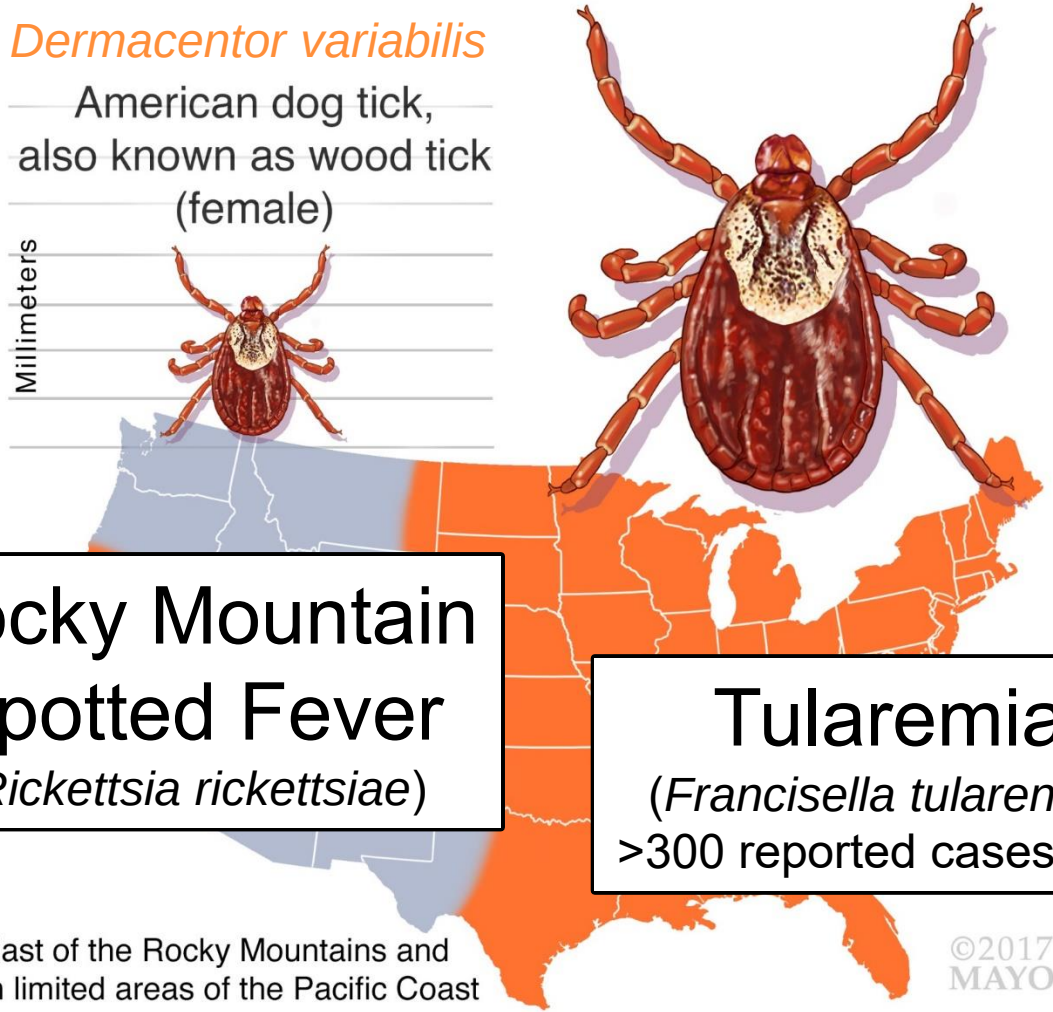
Dermacentor variabilis



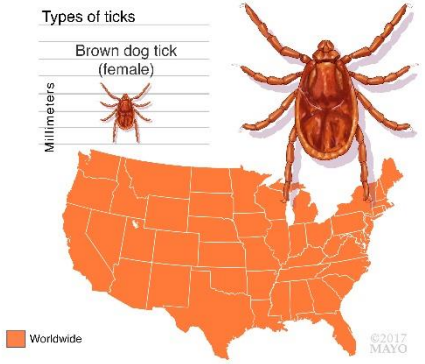
Rocky Mountain Spotted Fever

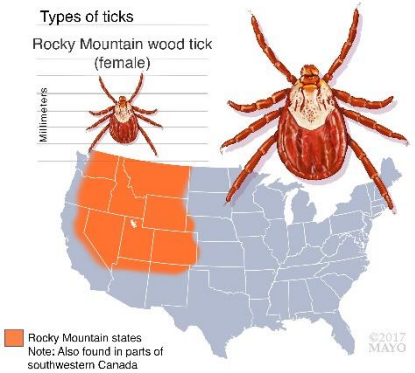
(*Rickettsia rickettsiae*)

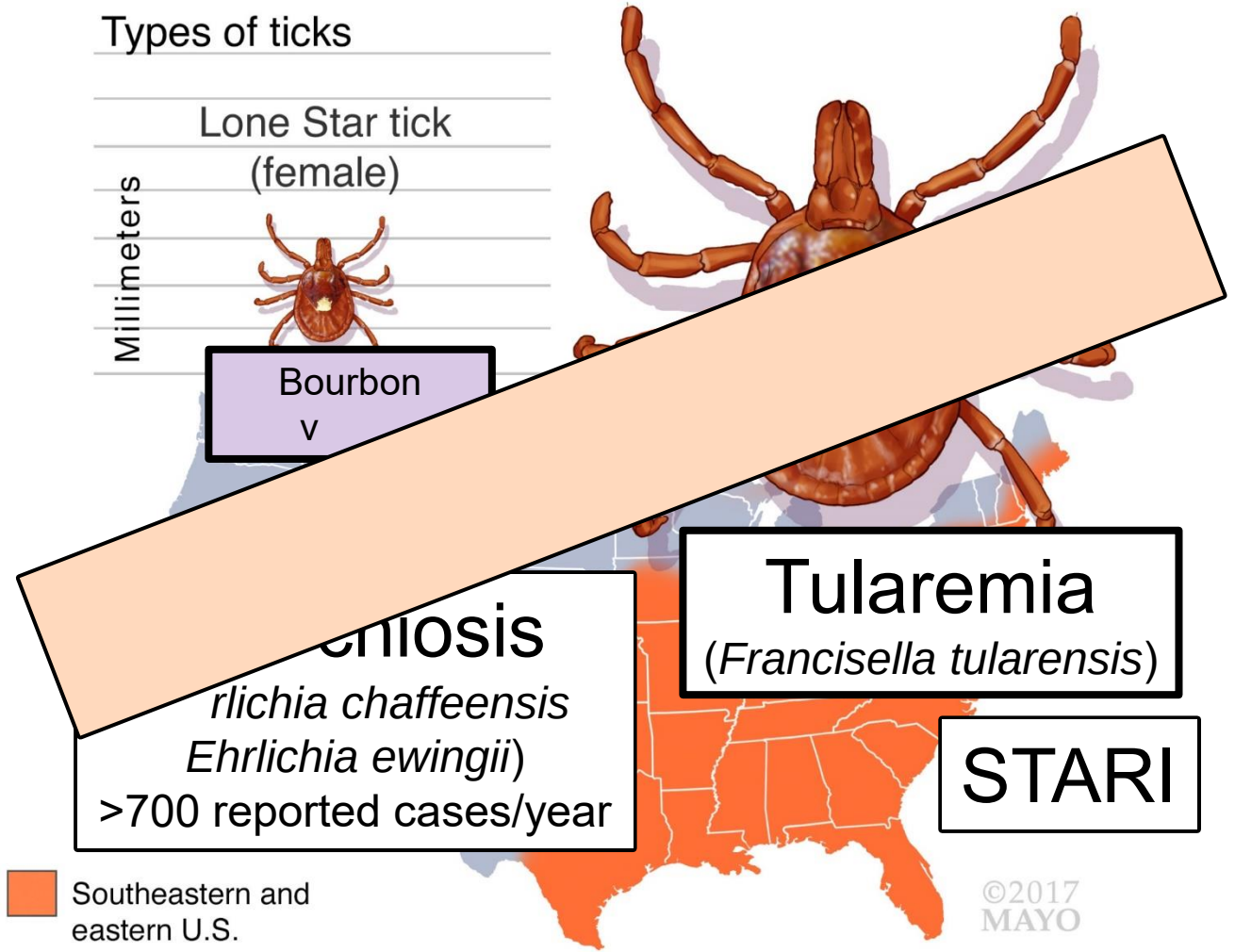
Tularemia

(*Francisella tularensis*)

>300 reported cases/year






Types of ticks
Lone Star tick
(female)
Millimeters
Bourbon
v
Tularemia
(Francisella tularensis)
STARI
rlichia chaffeensis
Ehrlichia ewingii)
>700 reported cases/year
Southeastern and
eastern U.S.
©2017
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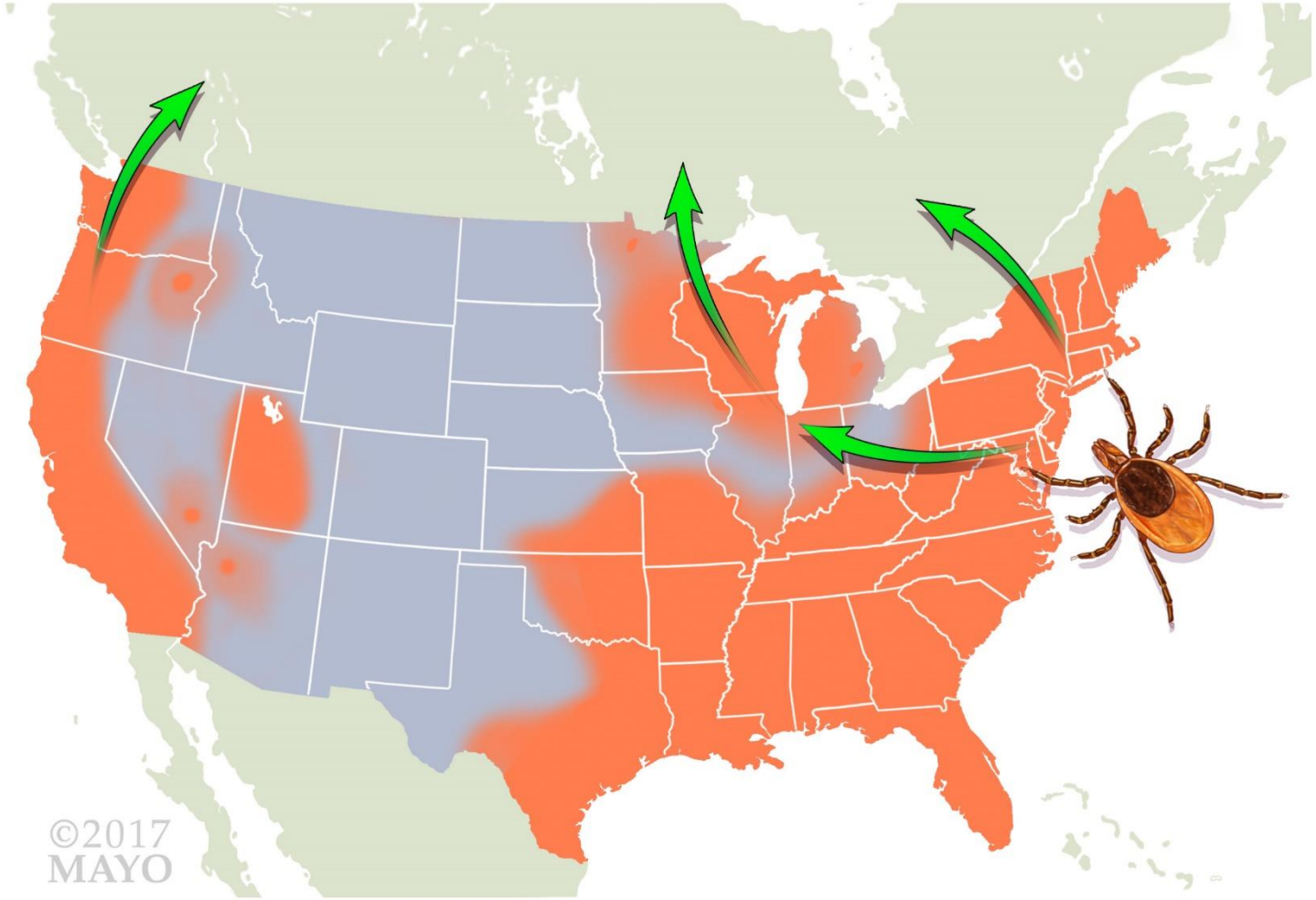
More trouble:
Increased disease incidence and expanded tick ranges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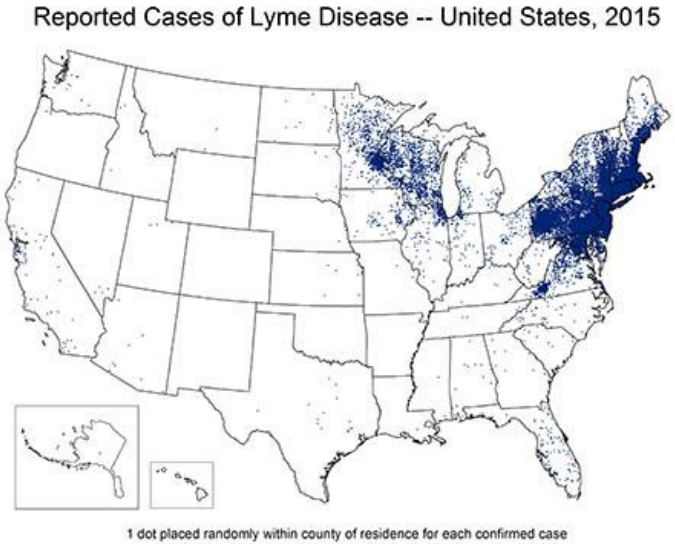
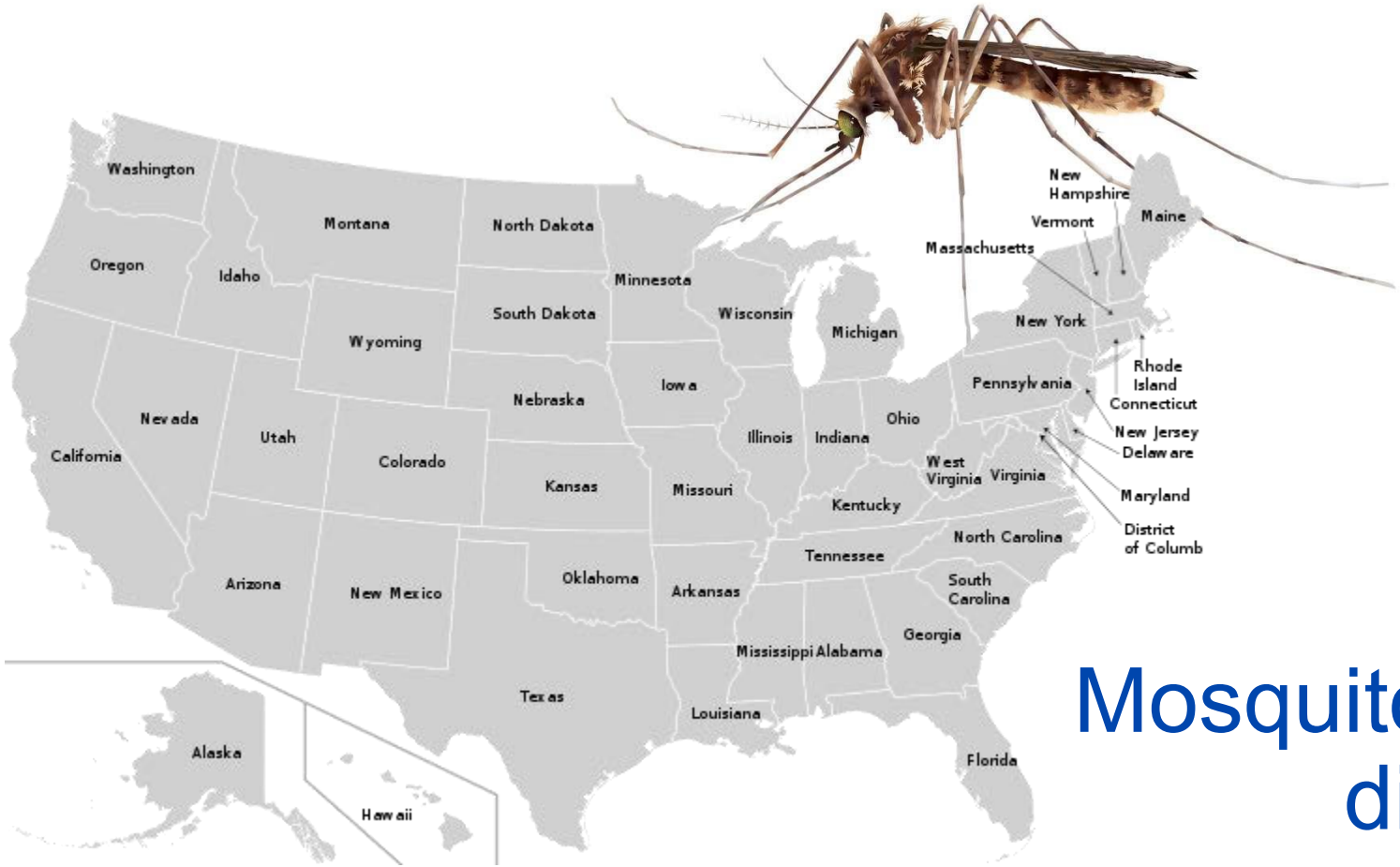
Lyme Disease in North America

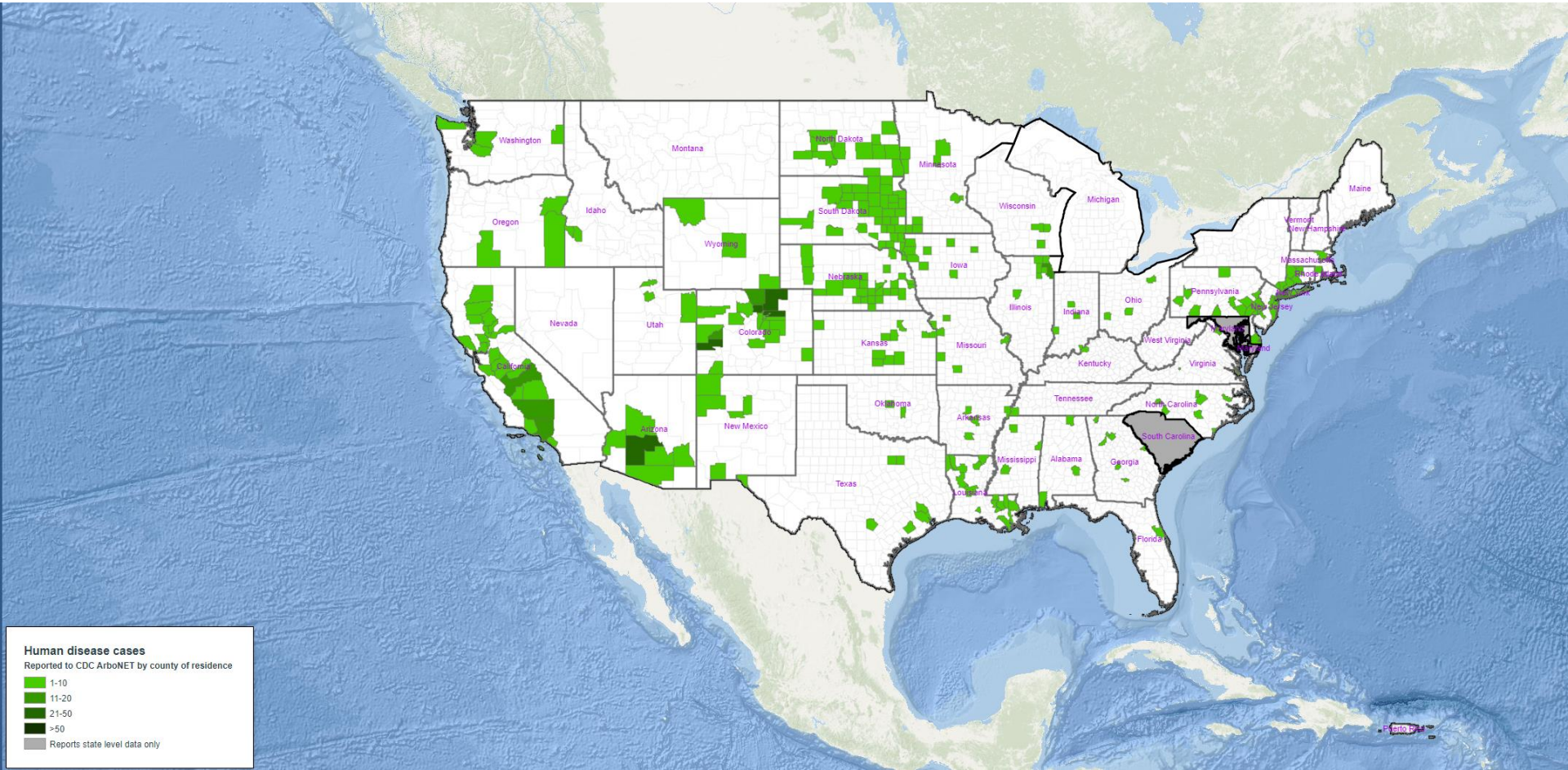


http://www.cdc.gov



Mosquito-borne diseases

West Nile Virus human cases - 2022



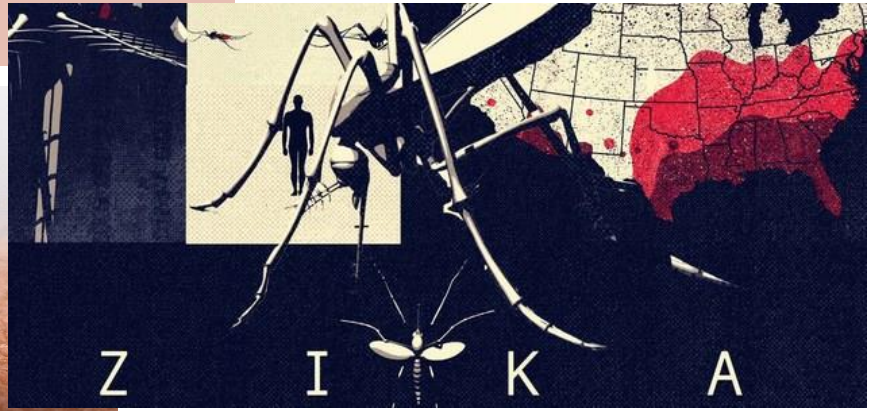
Other endemic mosquito-borne diseases

- St. Louis encephalitis virus
- Eastern Equine encephalitis virus
- Western Equine encephalitis virus
- LaCrosse encephalitis virus


MALARIA


DENGUE FEVER


CHIKUNGUNYA


ZIKA

Testing landscape in the United States for parasitic and vector-borne diseases

- There are no molecular assays approved by the food and drug administration for vector-borne diseases in symptomatic patients
  - Blood donor screening assay for *Babesia microti*
  - Tissue donor screening assay for WNV
- Only a few FDA-approved assays for parasites:
  - Gastrointestinal protozoa, *Trichomonas vaginalis*

This profile rapidly and accurately detects 22 common gastrointestinal pathogens, including viruses, bacteria and parasites that cause infectious diarrhea:

Bacteria:

- *Campylobacter*
- *Clostridium difficile* toxin A/B
- *Plesiomonas shigelloides*
- *Salmonella*
- *Yersinia enterocolitica*
- Vibrio, including *Vibrio cholerae*
- Enteroaggregative *E. coli* (EAEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC) lt/st
- Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, including *E. coli* O157
- *E. coli* O157
- *Shigella*/Enteroinvasive *E. coli* (EIEC)

Parasites:

- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

Viruses:

- Adenovirus F 40/41
- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus

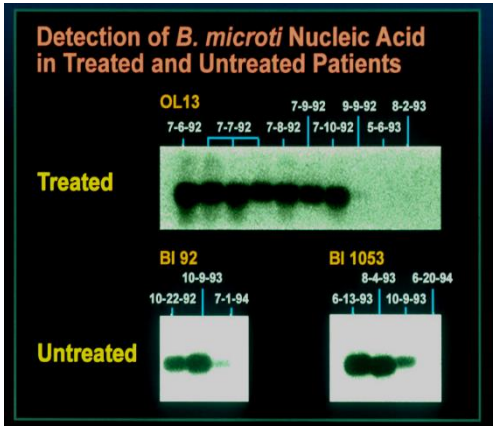
Testing landscape in the United States for parasitic and vector-borne diseases, cont.

- Due to the lack of FDA-approved tests, laboratories must create their own tests (Laboratory-Developed Tests = LDTs)
- Some labs will also purchase test kits that are not FDA-approved and validate them like LDTs
- The FDA is hoping to regulate LDTs in the near future, which will have a large impact on labs

Vector-borne disease laboratory testing at Mayo Clinic

1990s: Conventional Molecular testing began

David Persing, MD, PhD



CAP TODAY

COLLEGE OF AMERICAN PATHOLOGISTS

May 1994 Pathology/Laboratory Medicine/Laboratory Management Vol. 8 No. 5

PCR: More pathogens than dreamed of

William A. Check, PhD

In May 1981, public health officials first became aware of an outbreak of opportunistic infections and cancers in gay men. It took two years from that time until the isolation of the retrovirus, human immunodeficiency virus-1, that is the etiologic agent of AIDS.

In May 1993, public health officials first became aware of an outbreak of fatal pulmonary disease in the southwestern United States. Within two months the outbreak had been attributed to a member of the hantavirus family, a variant of the prototype Hantan virus that causes Korean hemorrhagic fever.

In each of these instances the new agent was related to a known and cultivated pathogen. Why the dramatic difference in identification time? According to David H. Persing, MD, PhD, consultant in the Divisions of Experimental Pathology, Clinical Microbiology, and Infectious Diseases at the Mayo Clinic, this telescoping in isolation time reflects the power of the molecular laboratory technique, polymerase chain reaction, or PCR.

"The difference here is the presence or absence of PCR in the discovery stages," Dr. Persing says. "Much of the work on HIV was done before PCR was widely available. In the hantavirus investigation PCR was one of the first tools to be used."

In this respect the story of the new hantavirus, now called Muerto Canyon virus, typifies a half-dozen infectious agents that have been identified in the last three to four years. None of these new and emerging pathogens can be grown in culture (or at best from only a small minority of infected patients), and their identification was possible almost solely because of PCR.

Hantavirus typifies the emerging pathogens in another way, in Dr. Persing's view. "I see it as representative of a legion of microorganisms that we simply don't know exist yet and that may represent a threat to human health," he says.

Burt Anderson, PhD, a microbiologist in the Viral and Rickettsial Zoonoses Branch at the Centers for Disease Control and Prevention, seconds Dr. Persing's interpretation. "We are entering a new age

continued on page 12

David Persing, MD, PhD, with research technologist Jennifer Magera (right), infectious disease fellow Cassandra Harrison, MD (rear, left), and research technologist Dana Mathiesen, uses a thermal cycler (lower left) to carry out the polymerase chain reaction.

Joseph Kane and Mary Frantz

Direct-billing mandate looking ever more likely

Carl Graziano

Efforts by the College and other laboratory groups to see a direct-billing mandate extended to all public and private payers has paid dividends recently in Congress, with major direct-billing legislation being introduced in one key health care committee and included in reform legislation passed by another.

Rep. Jim Slattery (D-Kan.), a member of the House Committee on Energy and Commerce, introduced in March his "Ethics in Billing Act," which would require

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Tick-borne pathogen PCR began in 1990s

Conventional PCR-EIA Tests offered:

- *Anaplasma phagocytophilum* - 1996
  (formerly *Ehrlichia phagocytophila)*
- *Babesia microti* - 1996
- *Borrelia burgdorferi* - 1994

	1996	1997	1998	2002
PCR, Lyme disease	119	49	20	3230
PCR, *Ehrlichia* agent	48	30	32	336
PCR, *Babesia microti*	17	7	12	418

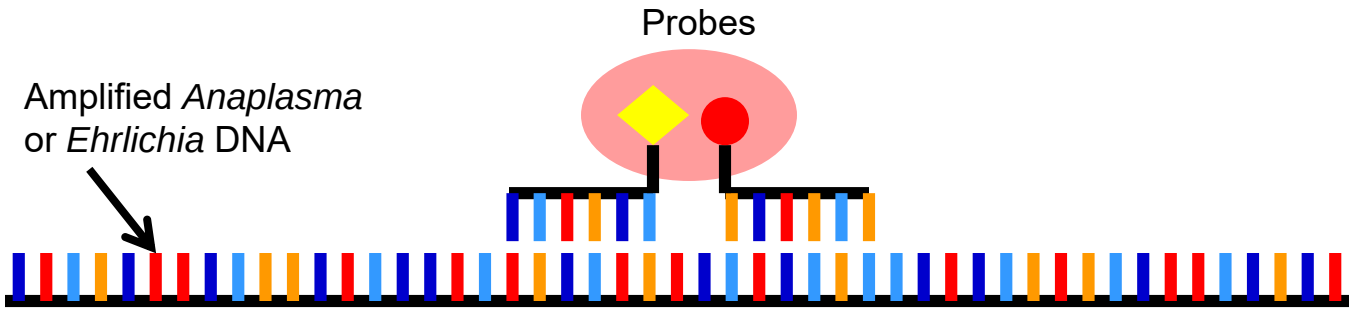
Introduction of real-time PCR

- Overseen by Dr. Frank Cockerill
- Assays included:
  - *Plasmodium* species (malaria)
  - *Babesia* spp.
  - *Anaplasma phagocytophilum*
  - *Ehrlichia* spp.
  - *Toxoplasma gondii*
  - And many others…

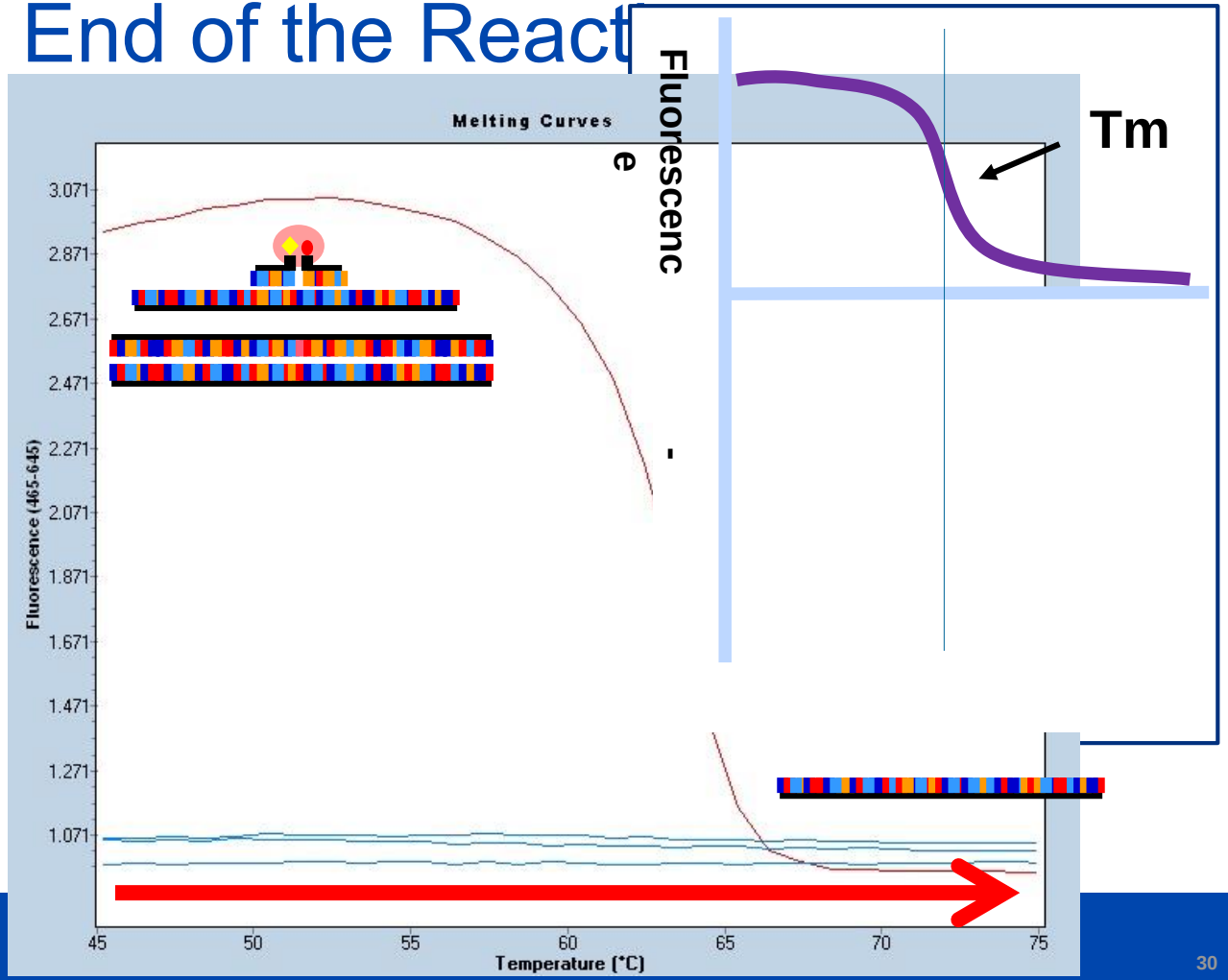
Frank Cockerill, MD

Mayo Clinic *Ehrlichia/Anaplasma* Real time PCR assay

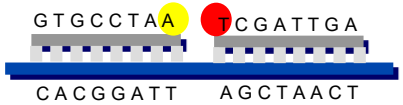
- Targets the *gro*EL heat shock operon
- Uses specific primers to amplify target DNA
- Uses FRET hybridization probes to detect the amplified DNA
  - When the dyes are close to one another, a fluorescent signal is produced

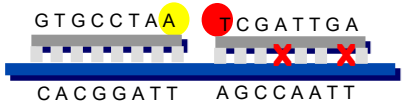


End of the React

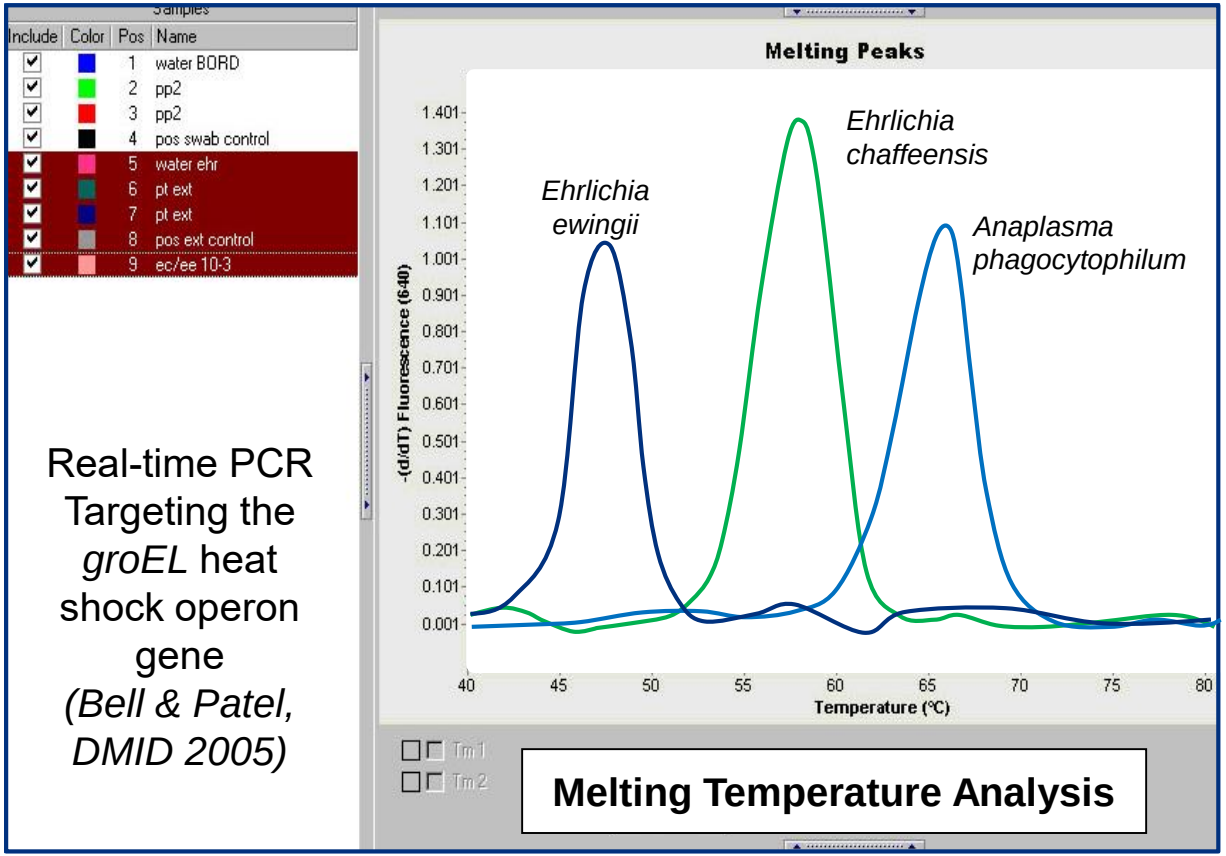


A single base change can shift the Tm



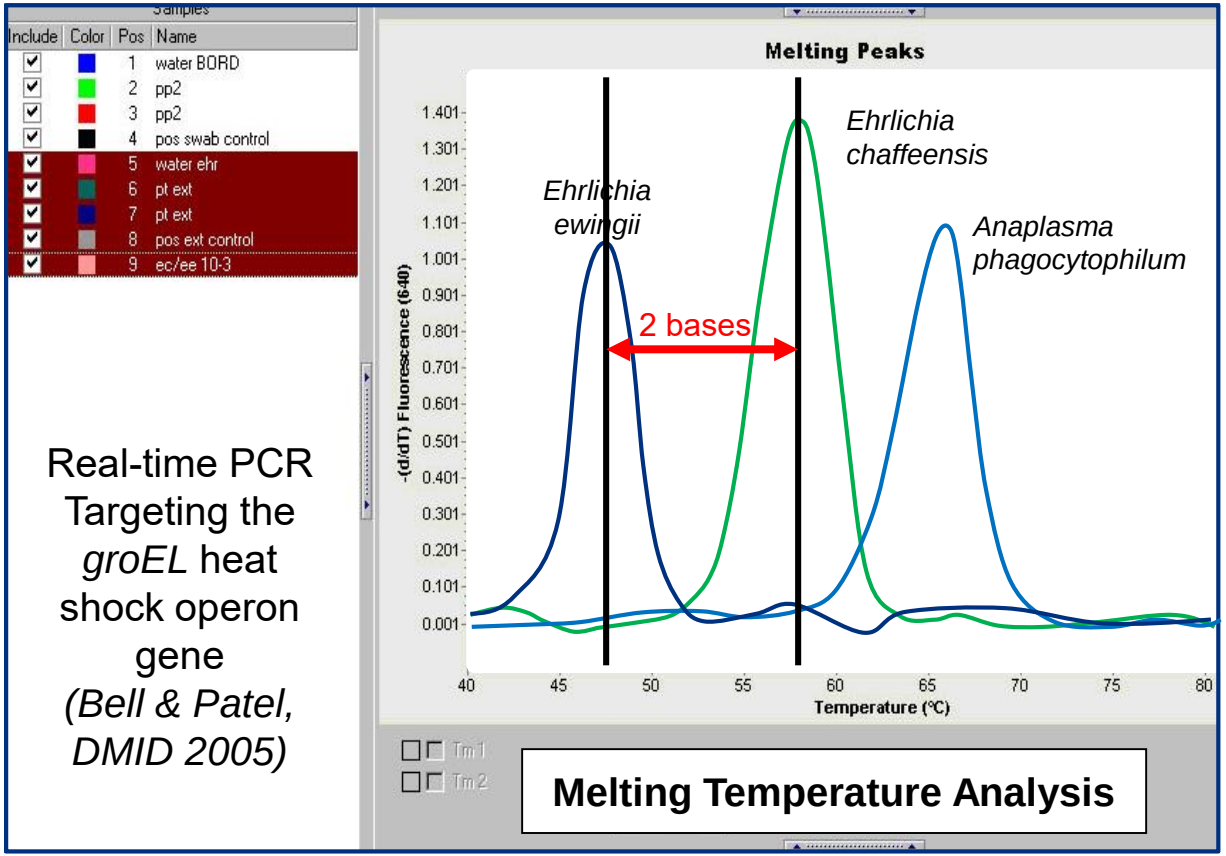


Anaplasma/Ehrlichia PCR – FRET hybridization probes



Real-time PCR Targeting the *groEL* heat shock operon gene *(Bell & Patel, DMID 2005)*

Anaplasma/Ehrlichia PCR – FRET probes

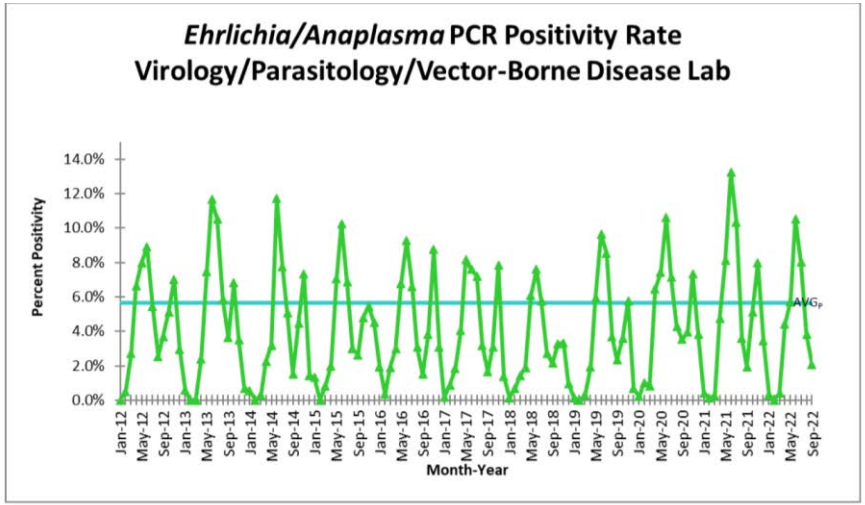


Real-time PCR Targeting the *groEL* heat shock operon gene *(Bell & Patel, DMID 2005)*

Ehrlichia/Anaplasma PCR volumes

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Tests performed	275	520	779	1224	1909	2198	4365	7349	7723	13639	9592	13516	22995

2021
42,000

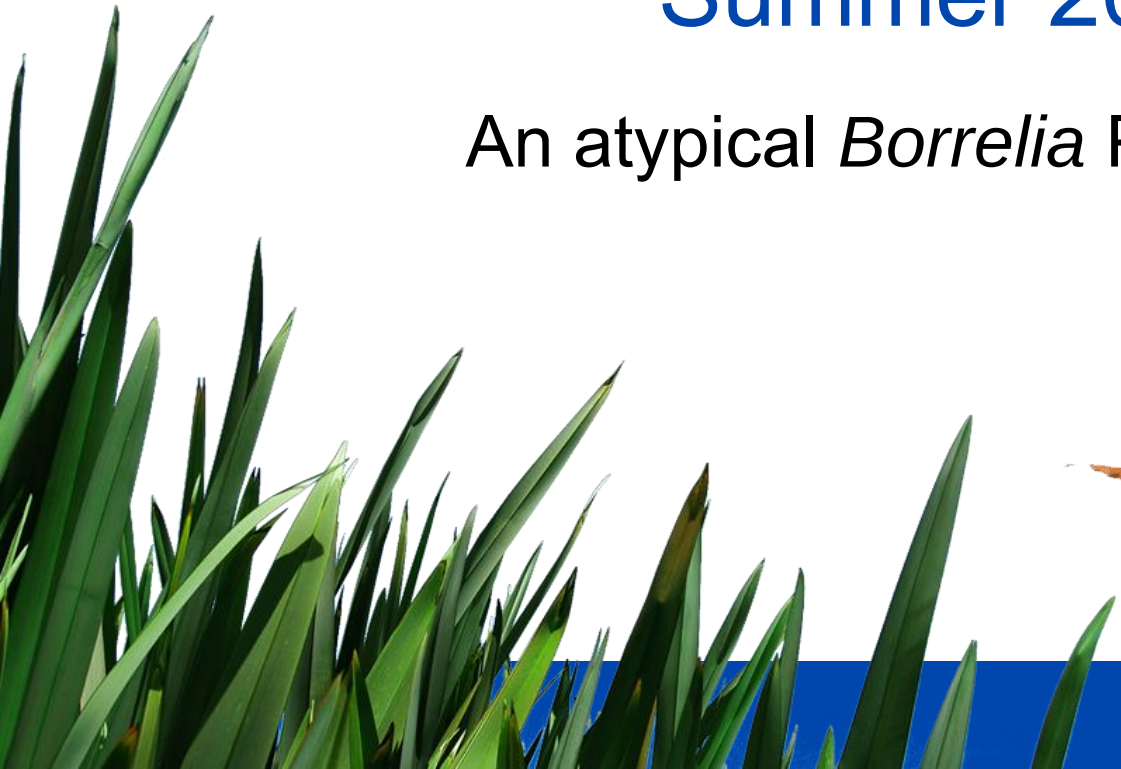


New Vector-Borne Diseases Service Line Created at Mayo Clinic

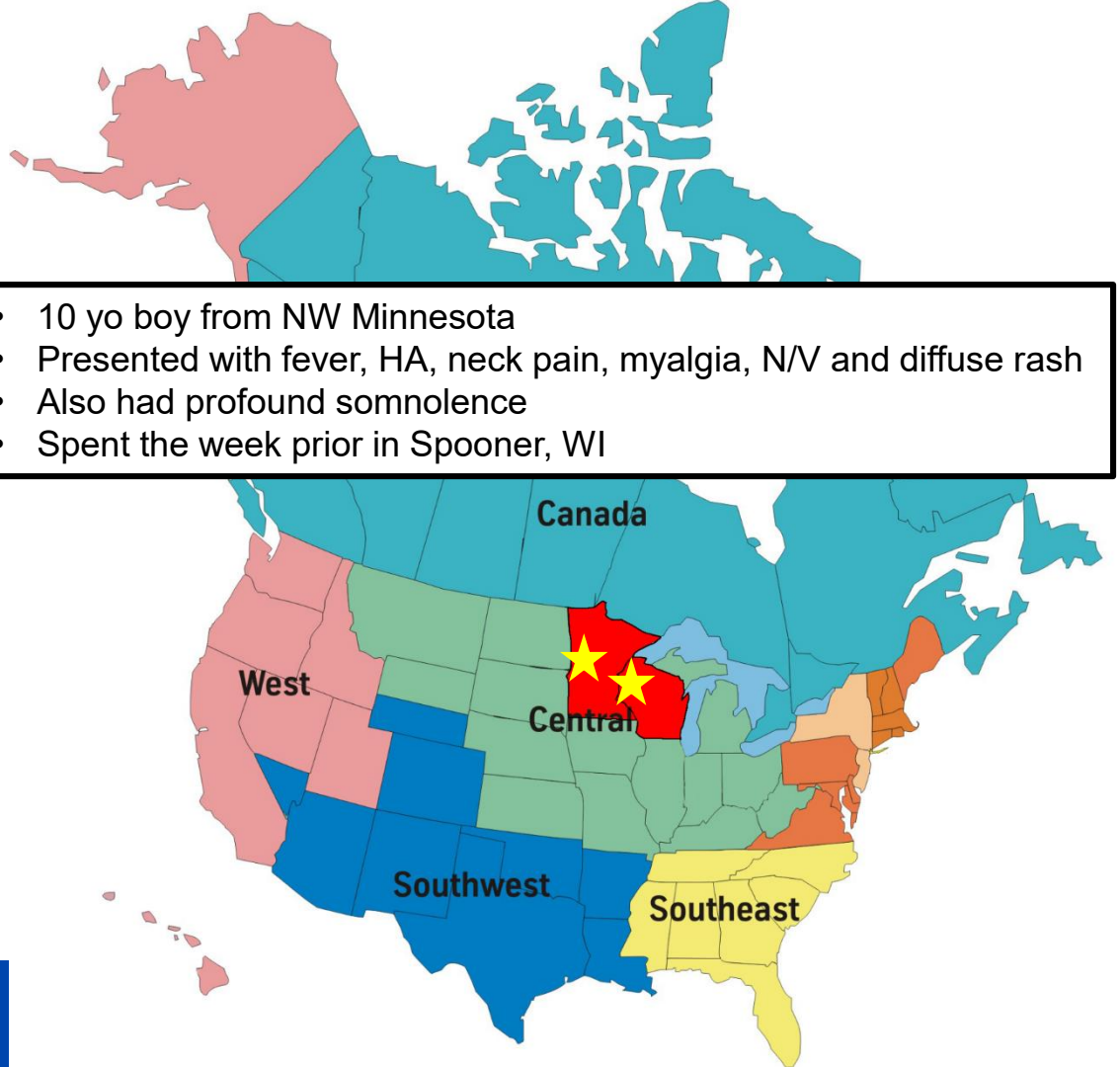
By **April Josselyn** • September 27, 2016

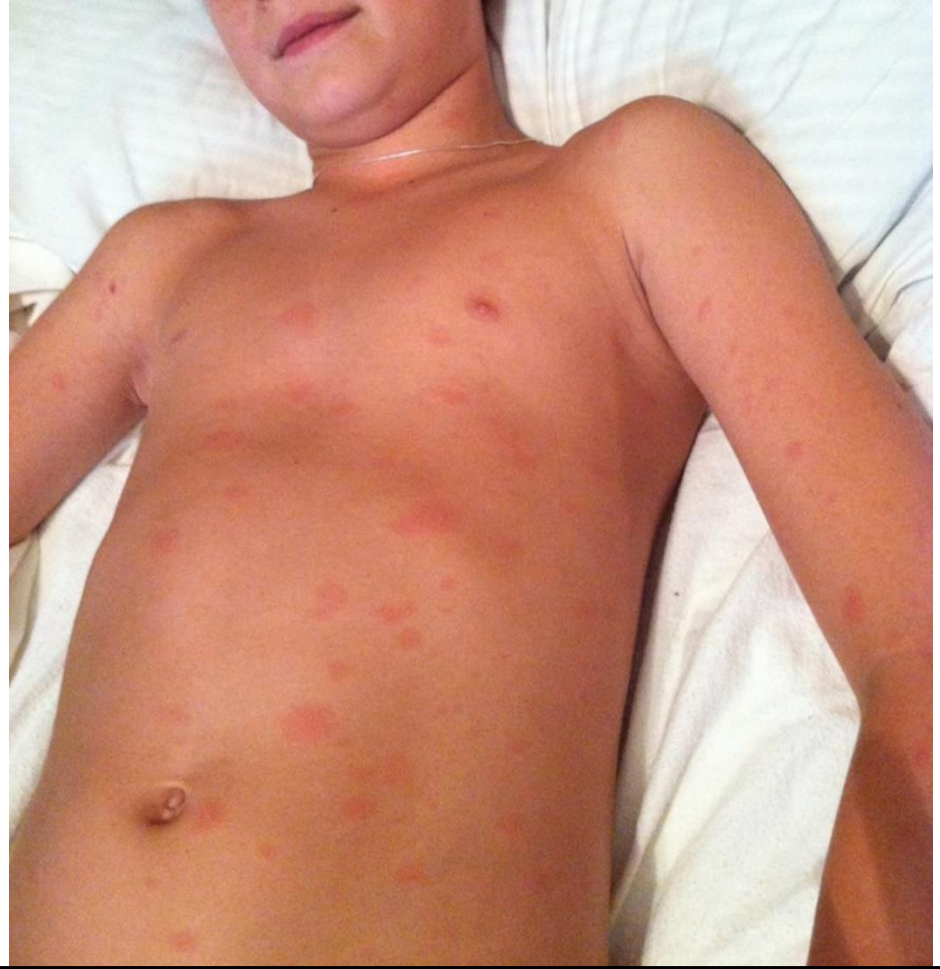
Summer 2013

An atypical *Borrelia* PCR result

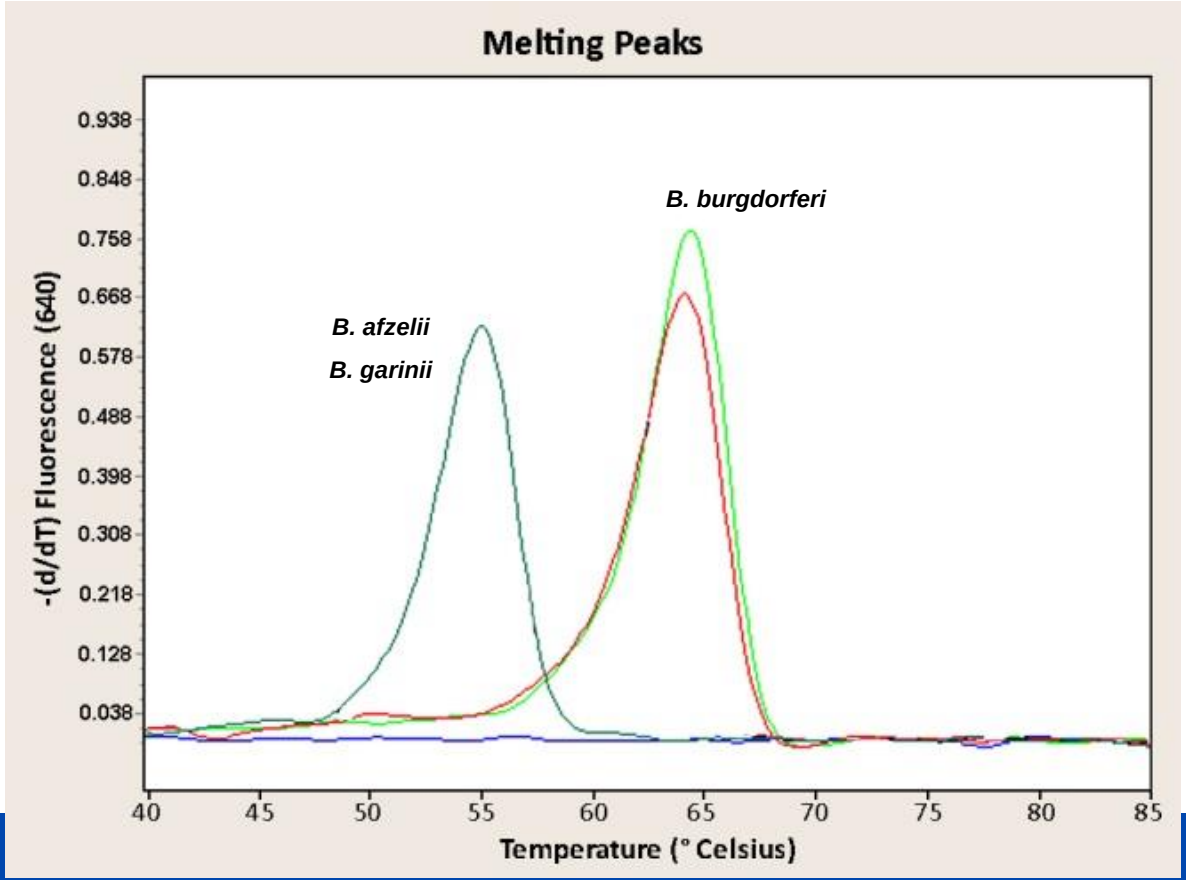


- 10 yo boy from NW Minnesota
- Presented with fever, HA, neck pain, myalgia, N/V and diffuse rash
- Also had profound somnolence
- Spent the week prior in Spooner, WI





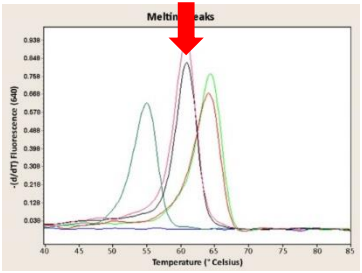
Lyme PCR - EDTA whole blood specimen



Patient History, continued

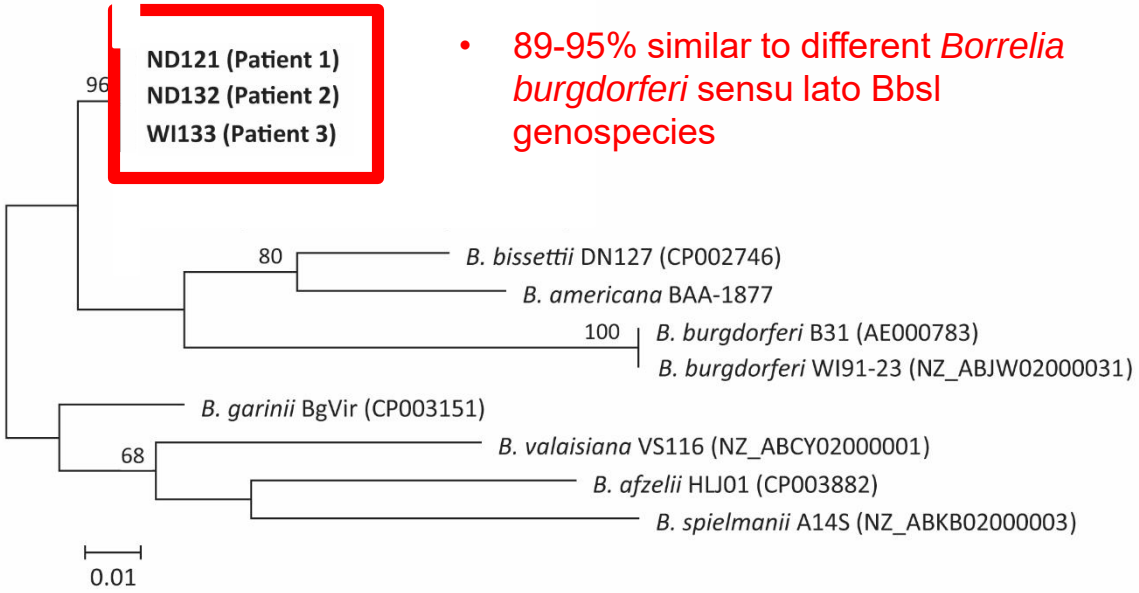
- Patient was hospitalized for 4 days
- Treated with ceftriaxone (1d), followed by 21 d of amoxicillin
- Complete recovery

More cases identified



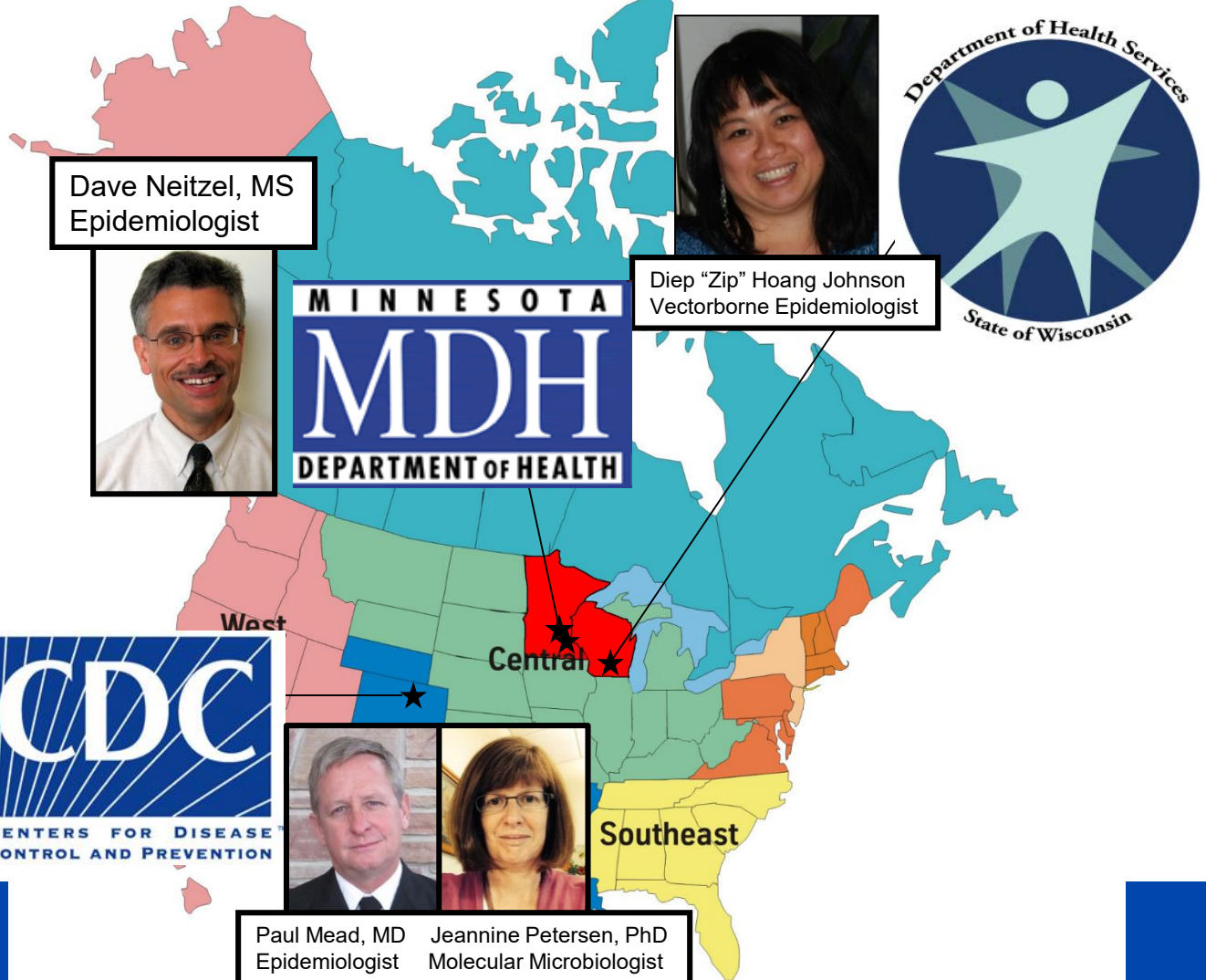
- PCR positive in whole blood:
  - July 2013 – 11 yo male from WI
  - Retrospective review: July 2012 – 65 yo male from ND (exposure in MN)
- Synovial fluid specimen from another Mayo Clinic site in Wisconsin
  - June 2013 – 21 yo woman from WI

Plasminogen binding protein gene (*oppA2*) – 149 bp



- 89-95% similar to different *Borrelia burgdorferi* sensu lato Bbsl genospecies

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


Dave Neitzel, MS
Epidemiologist
Diep “Zip” Hoang Johnson
Vectorborne Epidemiologist
MINNESOTA
MDH
DEPARTMENT OF HEALTH
Department of Health Services
State of Wisconsin
CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION
West
Central
Southeast
Paul Mead, MD
Epidemiologist
Jeannine Petersen, PhD
Molecular Microbiologist

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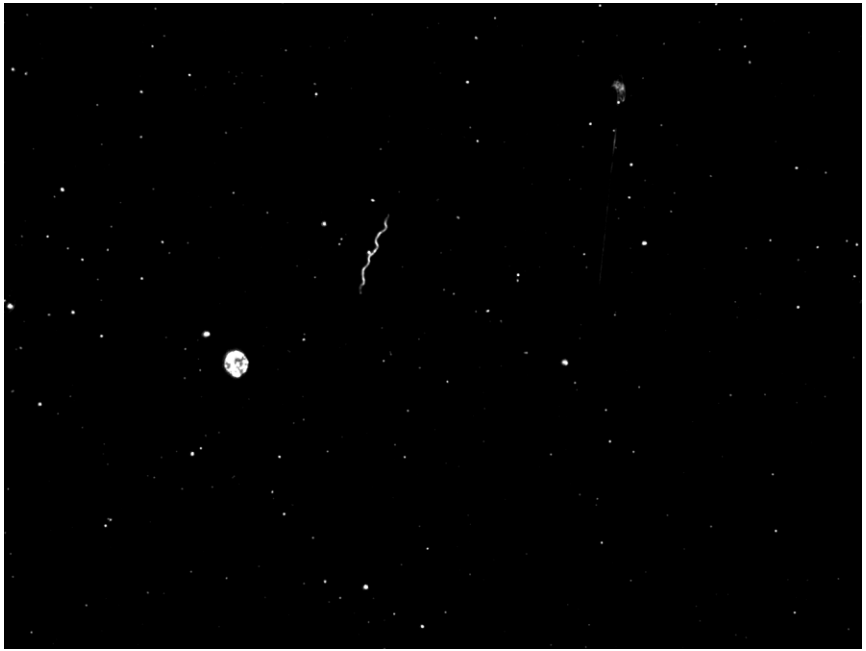
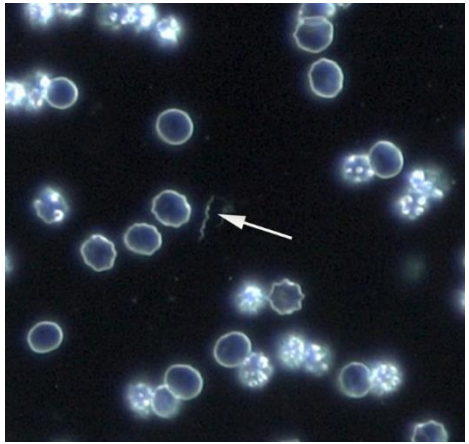
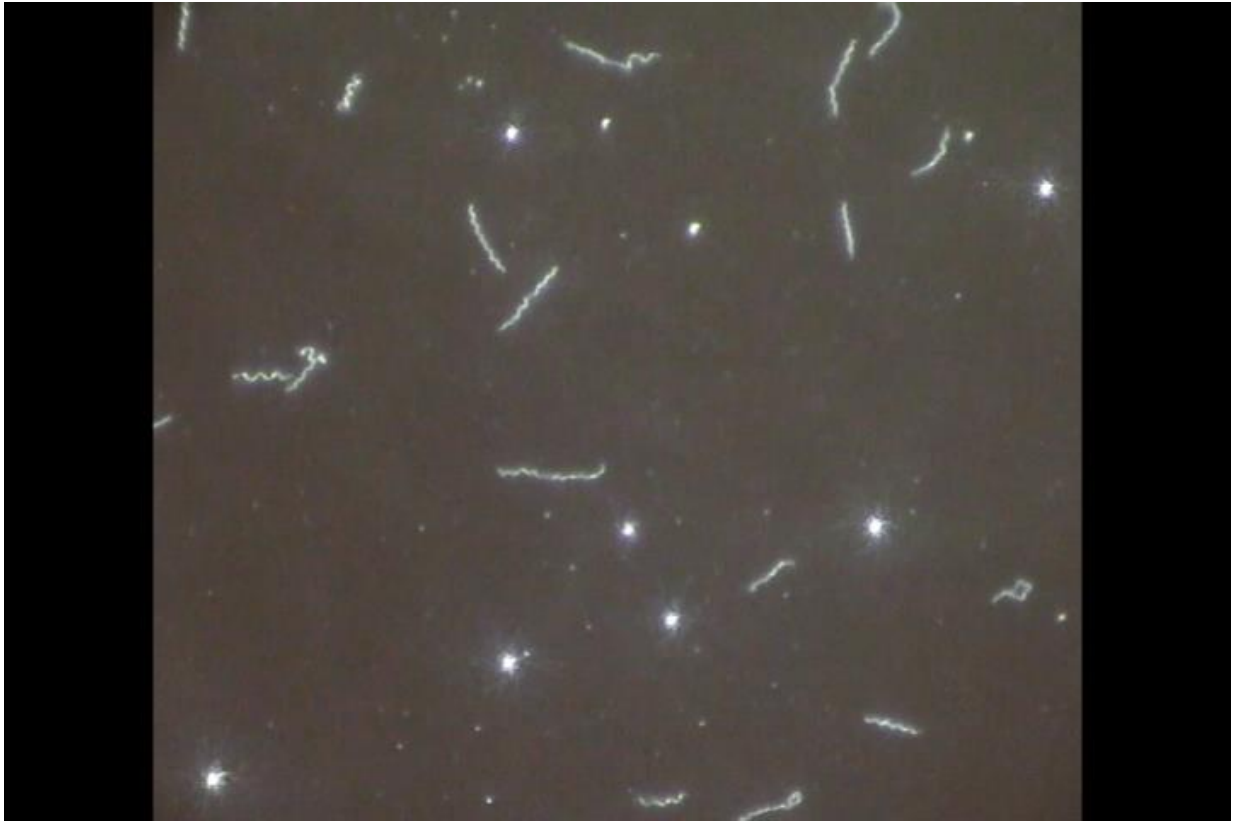
Blood from 2 patients sent for culture and sequencing



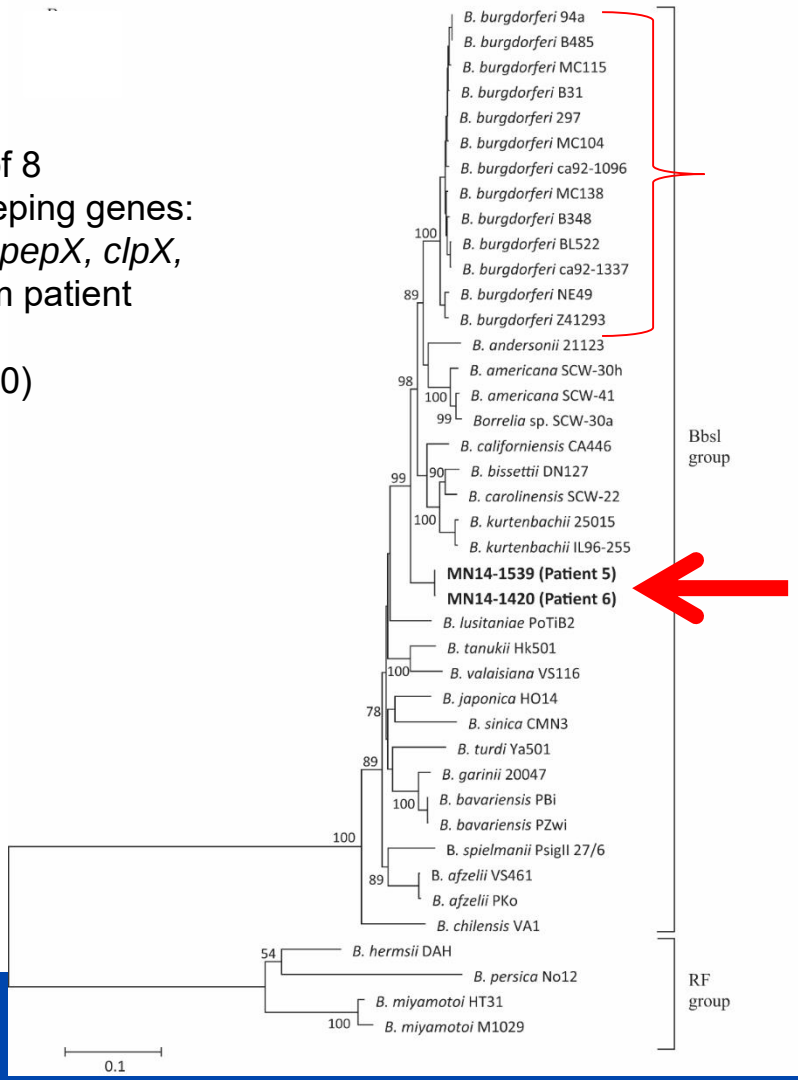


Spirochetes visualized in blood from 1 patient (2/70 hpf of blood, diluted 1:10) = ~85,000 spirochetes/mL

Cultures positive for blood from 2 patients



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

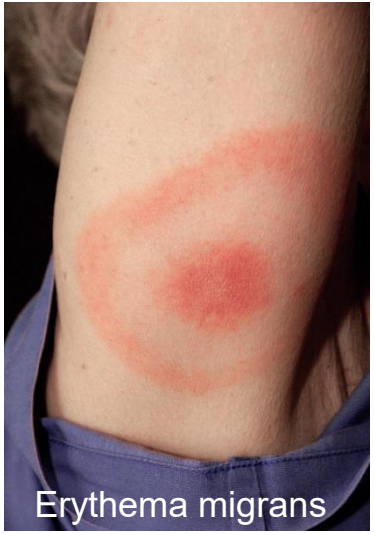


Multi-Locus Sequence Analysis (MLSA)

- 8 gene MLSA performed
  - Previously used for defining *Borrelia burgdorferi* sensu lato (Bbsl) genospecies
  - Confirmed that this is a novel Bbsl genospecies
    - *Therefore, it is in the Lyme disease causing group of pathogens*
  - Named: *Borrelia mayonii*

Key Findings - *B. mayonii*

- Most patients lack the classic erythema migrans (EM) rash seen with *B. burgdorferi* infection
- Reacts with *B. burgdorferi* serologic tests
  - Serology is the test of choice for *B. burgdorferi* infection
- Only found in the upper midwest
- May have a propensity for causing neurologic manifestations
- Seen in peripheral blood!
- High DNA loads in peripheral blood
  - Therefore, PCR of whole blood is considered the best test for diagnosis (a game changer!)


Erythema migrans

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, Jeff rey P Davis, Elizabeth Schiff man, Lynne M Sloan, Martin E Schriefer, Adam J Replogle, 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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Eau Claire–Luther Campus

THE UNIVERSITY of WISCONSIN MADISON

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA
CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

MINNESOTA MDH DEPARTMENT OF HEALTH

Department of Health Services
State of Wisconsin

UNIVERSITY OF MINNESOTA

Epidemic Intelligence Service (EIS)
Boots-on-the-ground disease detectives

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.
Ne
Ne
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. Eremeeva, M.D., Ph.D., Sc.D.

Ehrlichia muris eauclairensis

N Engl J Med 2011;365:422-429

TICKNET

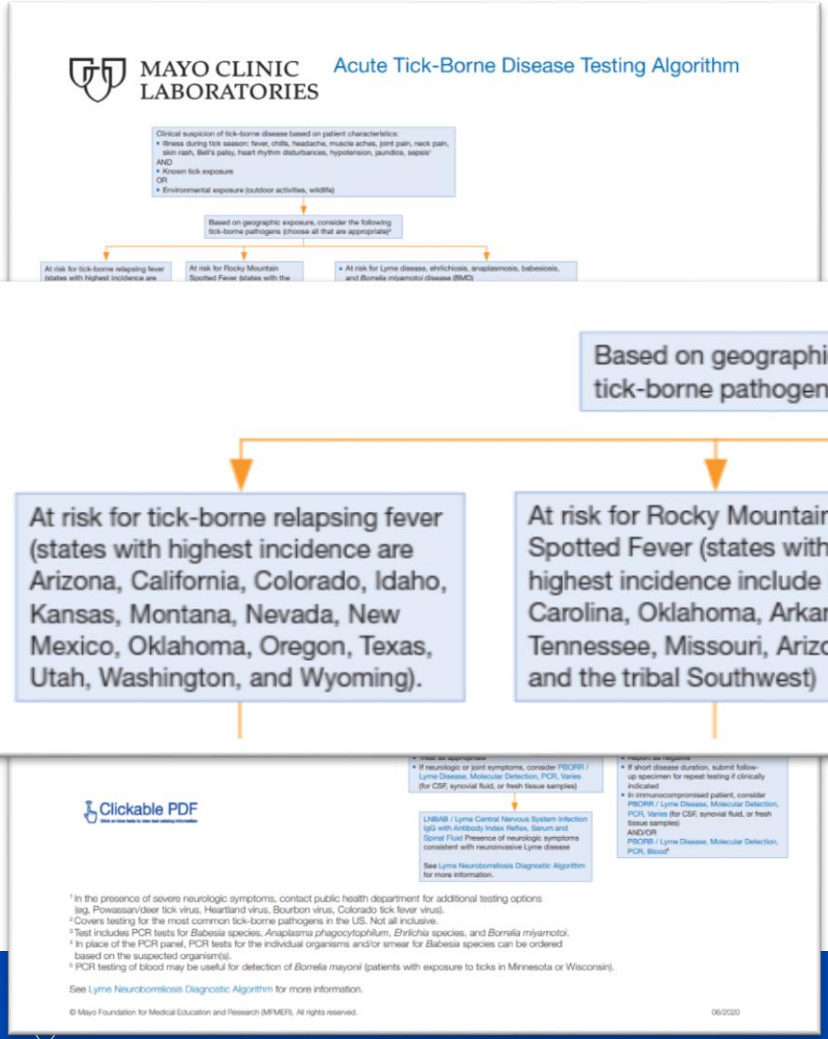
- A PROSPECTIVE, Collaborative Public Health Approach to Tickborne Disease Surveillance and Research
- Established in 2007
- Provides funding to state and local health departments to support surveillance activities
- Mayo Clinic has contributed >30,000 specimens from patients who are suspected of having a tickborne disease
- Tested for bacterial pathogens by targeted (16S rRNA gene) next-generation sequencing (NGS)

Recent publications

- Kingry et al. Targeted Metagenomics for Clinical Detection and Discovery of Bacterial Tick-Borne Pathogens
  - Results of 16S V1-V2 rRNA gene-based metagenomics on >13,000 residual specimens
  - 881 specimens were positive for TBP
  - Also 2 new possible tick-borne pathogens! (in addition to *B. johnsonii*)
    - *Anaplasma* sp. and *Rickettsia* sp.
  - *Also: Neorickettsia risticii, R. typhi, Coxiella burnetii, Francisella tularensis, Leptospira* spp.
- Rodino et al. JCM 2021 Detection of Tick-Borne Bacteria from Whole Blood Using 16S Ribosomal RNA Gene PCR Followed by Next-Generation Sequencing

Broad range Bacterial PCR/Sequencing

- Targets the 16S rRNA gene found in all bacteria
- Allows for detection of all bacteria in a specimen
- More time consuming and expensive than PCR
- **Targeted and shotgun metagenomic testing is likely to be a first-line test in the near future.

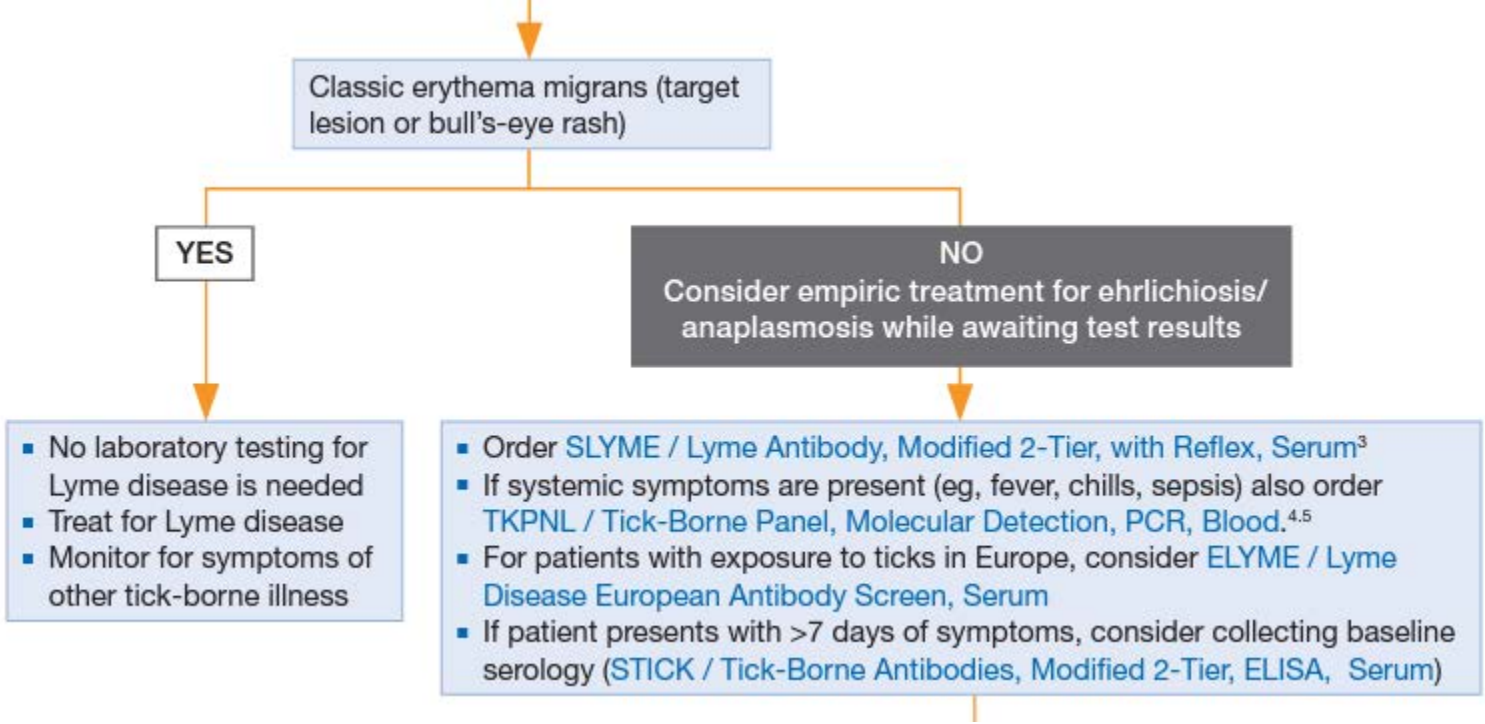


Based on geographic exposure, consider the following tick-borne pathogens (choose all that are appropriate)[2]

At risk for tick-borne relapsing fever (states with highest incidence are Arizona, California, Colorado, Idaho, Kansas, Montana, Nevada, New Mexico, Oklahoma, Oregon, Texas, Utah, Washington, and Wyoming).

At risk for Rocky Mountain Spotted Fever (states with the highest incidence include North Carolina, Oklahoma, Arkansas, Tennessee, Missouri, Arizona, and the tribal Southwest)

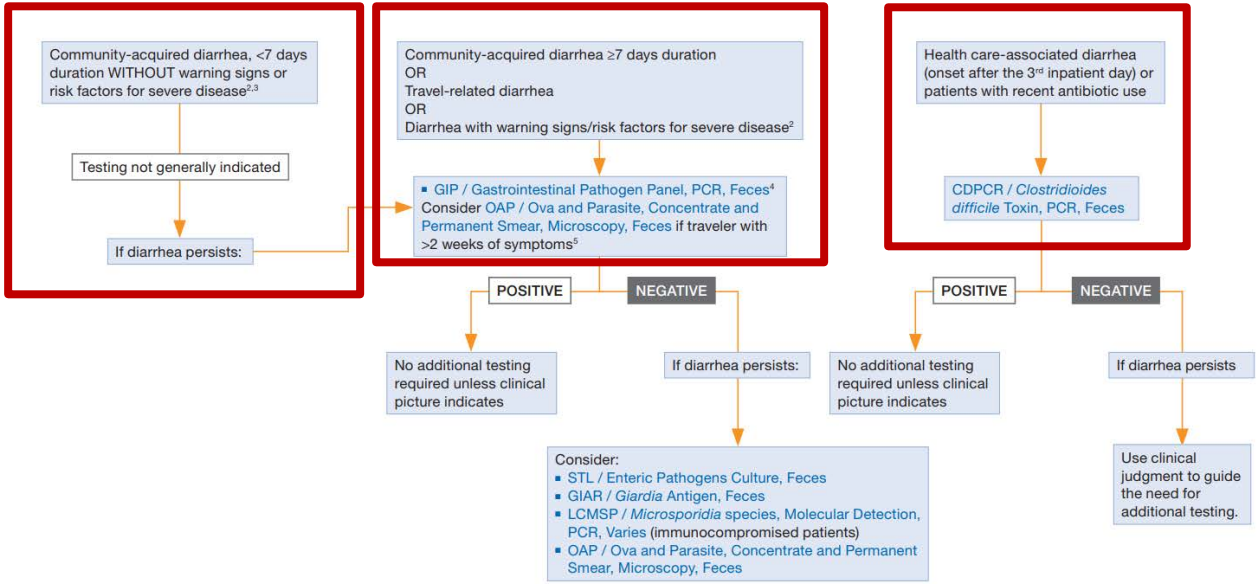
- At risk for Lyme disease, ehrlichiosis, anaplasmosis, babesiosis, and *Borrelia miyamotoi* disease (BMD)
- Endemic areas for Lyme disease, anaplasmosis, babesiosis, and BMD include the Northeastern and Upper Midwestern United States, into Canada
- Ehrlichiosis is most frequently reported from the Southeastern and South Central United States



If the Tick-Borne disease molecular panel is negative, consider the 16S rRNA broad range bacterial PCR on peripheral blood

MAYO CLINIC LABORATORIES

Laboratory Testing for Infectious Causes of Diarrhea[1]

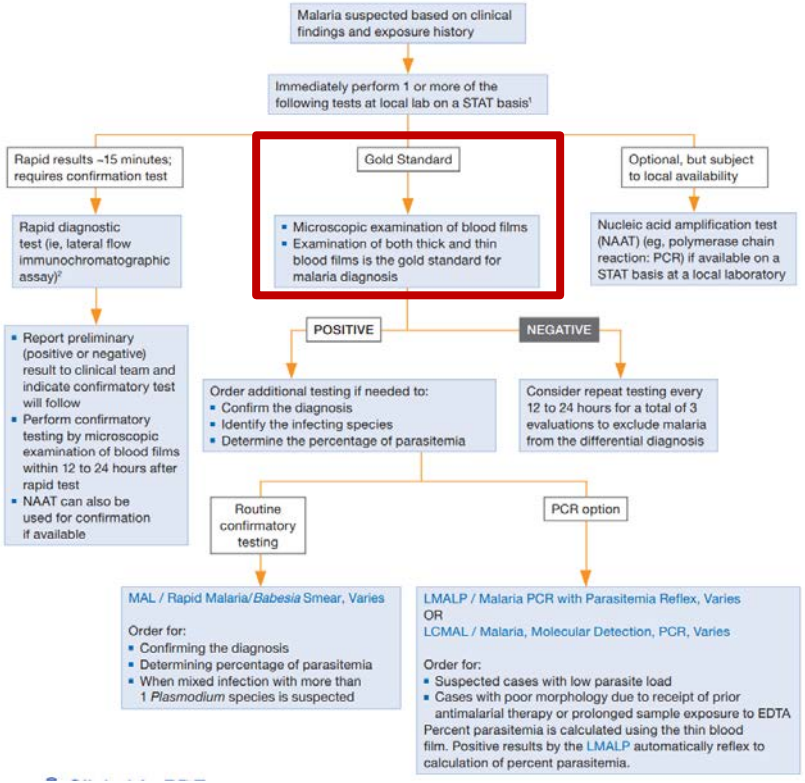


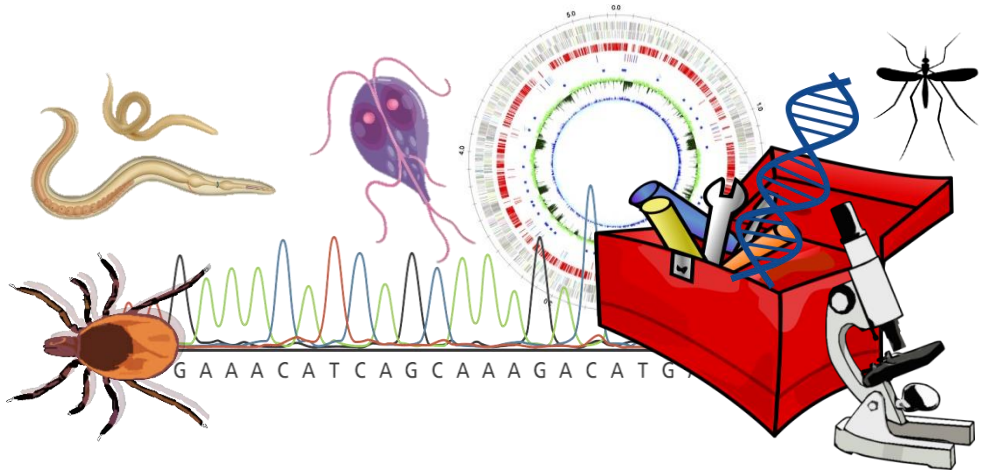
[1] This panel should NOT be used for chronic diarrhea.
[2] Warning signs and risk factors for severe disease include fever, bloody diarrhea, dysentery, severe abdominal pain, dehydration, hospitalization, and immunocompromised state.
[3] During the summer, consider ordering STFRP / Shiga Toxin, Molecular Detection, PCR, Feces on children with diarrhea even if they don't have frankly bloody diarrhea, are not toxic-appearing, and diarrhea has been present <7 days.
[4] GI Pathogen Panel tests for common bacterial, viral, and parasitic causes of diarrhea
[5] Submit 3 stool collected on separate days for maximum sensitivity

Note: In outbreak scenarios with a known organism, consider ordering a specific test for that organism (CYCL / Cyclospora Stain, Feces; CRYPS / *Cryptosporidium* Antigen, Feces; GIAR / Giardia Antigen, Feces; bacterial stool culture)

MAYO CLINIC LABORATORIES

Malaria Laboratory Testing Algorithm





Questions?