Expanding Diversity and Distribution of Tickborne Diseases

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The Basics of Tickborne Diseases

- All known tickborne infectious diseases are diseases of animals that can be transmitted to humans via a tick vector (e.g., zoonoses)
  - Ticks can maintain the pathogens through transmission to their offspring
  - Ticks can acquire infection through feeding on infectious hosts
- Humans are incidental hosts, infected by the bite of infected ticks
## Ticks Can Transmit Diverse Types of Bacteria in the United States

<table>
<thead>
<tr>
<th>Bacterial Diseases (9)</th>
<th>Pathogens (14)</th>
<th>Tick Genera (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasmosis</td>
<td><em>Anaplasma phagocytophilum</em></td>
<td><em>Ixodes</em> spp.</td>
</tr>
<tr>
<td><strong>Borrelia miyamotoi disease</strong></td>
<td><em>Borrelia miyamotoi</em></td>
<td><em>Ixodes</em> spp.</td>
</tr>
<tr>
<td>Ehrlichiosis</td>
<td><em>Ehrlichia chaffeensis</em> <em>Ehrlichia ewingii</em> <em>Ehrlichia muris eauclairensis</em></td>
<td><em>Amblyomma</em> spp. <em>Ixodes</em> spp.</td>
</tr>
<tr>
<td>Lyme disease</td>
<td><em>Borrelia burgdorferi</em> <em>Borrelia mayonii</em></td>
<td><em>Ixodes</em> spp.</td>
</tr>
<tr>
<td><strong>Rickettsia parkeri rickettsiosis</strong></td>
<td><em>Rickettsia parkeri</em></td>
<td><em>Amblyomma</em> spp.</td>
</tr>
<tr>
<td>Rocky Mountain spotted fever</td>
<td><em>Rickettsia rickettsii</em></td>
<td><em>Dermacentor</em> spp. <em>Rhipicephalus</em> spp.</td>
</tr>
<tr>
<td>Pacific Coast tick fever</td>
<td><em>Rickettsia philipii</em></td>
<td><em>Dermacentor</em> spp.</td>
</tr>
<tr>
<td>Relapsing fever</td>
<td><em>Borrelia hermsii</em> <em>Borrelia parkeri</em> <em>Borrelia turicatae</em></td>
<td><em>Ornithodoros</em> spp.</td>
</tr>
<tr>
<td>Tularemia</td>
<td><em>Francisella tularensis</em></td>
<td><em>Amblyomma</em> spp. <em>Dermacentor</em> spp.</td>
</tr>
</tbody>
</table>

Other Types of Pathogens Ticks Can Transmit

<table>
<thead>
<tr>
<th>Diseases (4)</th>
<th>Pathogens (4)</th>
<th>Tick Genera (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado tick fever</td>
<td>Colorado tick fever virus (Coltivirus)</td>
<td>Dermacentor spp.</td>
</tr>
<tr>
<td>Heartland virus disease</td>
<td>Heartland virus (Phlebovirus)</td>
<td>Amblyomma spp.</td>
</tr>
<tr>
<td>Powassan encephalitis</td>
<td>Powassan virus (Flavivirus)</td>
<td>Ixodes spp.</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Babesiosis</td>
<td>Babesia microti</td>
<td>Ixodes spp.</td>
</tr>
</tbody>
</table>

Overview of Trends

- Majority of vector-borne diseases in the U.S. are tickborne diseases
- Increasing number of tickborne disease cases over time
- Expanding geographic range of tickborne cases
- Growing number of tickborne agents recognized to cause human disease
Cases of Nationally Notifiable Vector-borne Diseases Reported in the U.S., 2014

Lyme disease (69%)

Other tickborne diseases (25%)

Mosquito-borne diseases (6%)

N= 48,519 cases

Three Species Cause Majority of Human Diseases

- *Ixodes scapularis* (Blacklegged tick)
- *Amblyomma americanum* (Lone star tick)
- *Dermacentor variabilis* (American dog tick)
Distribution of *Ixodes scapularis* (Blacklegged Tick)

Transmits agents that cause:

- Anaplasmosis
- Babesiosis
- *Borrelia miyamotoi* disease
- Ehrlichiosis
- Lyme disease
- Powassan encephalitis

cdc.gov/ticks/geographic_distribution.html
Distribution of *Amblyomma americanum* (Lone star tick)

Transmits agents that cause:
- Ehrlichiosis
- Tularemia
- Heartland virus disease

Adult female  
Adult male  
Nymph  
Larva

cdc.gov/ticks/geographic_distribution.html
Distribution of *Dermacentor variabilis* (American Dog Tick)

Transmits agents that cause:
- Rocky Mountain spotted fever
- Tularemia

Adult female

Adult male

Nymph

Larva

https://www.cdc.gov/ticks/geographic_distribution.html
Geographic Distribution of Nationally Notifiable Tickborne Diseases, 2015

- Lyme disease
- Anaplasmosis
- Babesiosis
- Ehrlichiosis
- Rocky Mountain Spotted Fever
- Tularemia

Each dot represents a reported case in the county of residence.
Cases of Lyme Disease Have Increased

Annual Reported Cases of Lyme Disease, 1992–2015

Reported Cases

Year

 CDC.gov/mmwr/mmwr_nd/index.html
 CDC.gov/mmwr/volumes/65/wr/pdfs/mm6546.pdf
Other Nationally Notifiable Tickborne Diseases Have Also Increased

Annual Reported Cases of Three Selected Tickborne Diseases, 2000–2015

- Anaplasmosis
- Ehrlichioses
- Spotted fever group rickettsioses

Reported Cases

Year


cdc.gov/mmwr/mmwr_nd/index.html
cdc.gov/mmwr/volumes/65/wr/pdfs/mm6546.pdf
Expanding Numbers and Geographic Distribution of Lyme Disease Cases Mirrors Other Tickborne Diseases

[cdc.gov/lyme/stats/index.html](https://www.cdc.gov/lyme/stats/index.html)
Discovery of Tickborne Pathogens as Causes of Human Disease By Year, 1909–1959

Year represents when tickborne pathogen was recognized as cause of human disease.
Discovery of Tickborne Pathogens Has Accelerated, 1960–2016

Year represents when tickborne pathogen was recognized as cause of human disease.
Explanation of Increasing Cases and Geographic Spread

- Improved diagnostics and clinical recognition
- Range expansion and population increases of ticks
- Lack of effective prevention strategies

A blood-fed *Amblyomma americanum*, “Lone star tick”
Geographic Expansion of Ticks
Locations Where *Ixodes scapularis* Recorded

Established: ≥6 or more ticks or ≥1 life stage recorded in a single year

Reported: <6 individuals of a single life stage recorded in a single year

Geographic Expansion of Vectors Matches Increases in Tickborne Disease Cases


- Established as of 1996
- Reported as of 2015
- Established as of 2015

Time Period When County First Reached High Incidence of Lyme Disease

- 1993–1997
- 1998–2002
- 2003–2007
- 2008–2012

Established defined as: 6 or more ticks; or 1 or more; Reported defined as: 6 or more ticks or >1 life stage recorded in a single year

Prevention in the Absence of Vaccines

- Increasing human contact with ticks
- No human vaccines to prevent tickborne diseases in the U.S.
  - No single effective, widely accepted method of preventing tickborne diseases
- Prevention strategies include
  - Personal protection
  - Environmental modification
  - Tick suppression
Avoid tick habitat

Repel ticks

- Use 20–30% DEET on exposed skin and clothing
- Wear permethrin-treated clothing
Prevention Through Personal Protection

- **Find and remove ticks from your body**
  - Bathe or shower as soon as possible after coming indoors
  - Every day check for and remove ticks on body, pets and outdoor gear

- **Tumble dry clothing**
  - Tumble dry clothes in a dryer on high heat for 10 minutes
  - If the clothes are damp, additional time may be needed
  - If the clothes require washing first, use hot water
    - Cold and medium temperature water will not kill ticks effectively

[cdc.gov/ticks/avoid/on_people.html](http://cdc.gov/ticks/avoid/on_people.html)
Prevention Through Environmental Modification and Tick Suppression

- Reduce the numbers of host-seeking ticks
  - Landscape management
  - Kill host-seeking ticks with acaricides or biological agents

Host reduction or exclusion
  ● Install deer-proof fencing

Reduce the numbers of ticks on hosts
  ● Acaricide treatment of rodents or deer

Emerging Prevention Strategies

- Reduce the number of infected hosts
- Rodent-targeted methods to reduce infection in ticks
  - Vaccines
  - Antibiotics

Attracted by the bait inside, rodents enter. Once inside, the rodent consumes the bait containing an oral vaccine or antibiotic.

Looking Forward

- Recognition of more tickborne diseases and tickborne agents
Looking Forward

- Recognition of more tickborne diseases and tickborne agents
- Continuing range expansion of ticks and associated tickborne diseases
Looking Forward

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- Continuing range expansion of ticks and associated tickborne diseases
- Importance of co-infections
  - Single species can carry multiple disease agents
Looking Forward

- Recognition of more tickborne diseases and tickborne agents
- Continuing range expansion of ticks and associated tickborne diseases
- Importance of co-infections
  - Single species can carry multiple disease agents
- Need for effective, widely-acceptable approaches to prevent tickborne diseases
Tickborne Spotted Fevers – Old and New

Christopher D. Paddock, MD
Medical Officer, Rickettsial Zoonoses Branch
Division of Vector-Borne Diseases
National Center for Emerging and Zoonotic Infectious Diseases
A Long Time Ago, in A Valley Far, Far Away...

Image courtesy of Pete Ramberg
Rocky Mountain Spotted Fever
The First Recognized Tickborne Disease of Humans in the U.S.

Years | Cases | Deaths | CFR
--- | --- | --- | ---
1880–1889 | 19 | 15 | 79%
1890–1899 | 99 | 70 | 71%
1900–1909 | 225 | 128 | 57%

1880–1909 | 343 | 213 | 62%

CFR: Case fatality rate
RMSF: Rocky Mountain spotted fever

Why is Rocky Mountain Spotted Fever So Deadly?

*Rickettsia rickettsii* bacteria infecting endothelial cells of small blood vessel

Petechial rash involving sole

Histologic section of lung from patient with fatal RMSF
The Favorable Impact of Doxycycline Therapy on Survival

- Discovery of tetracycline-class drugs resulted in dramatic reduction in case-fatality rates
- Doxycycline is the drug of choice for patients of all ages with RMSF and other tickborne rickettsial diseases
- Doxycycline should be initiated immediately if RMSF is suspected
  - Delay of therapy (> 5 days) is the most important predictor of fatal outcome

RMSF: Rocky Mountain spotted fever


Incidence By County, 2008–2012
Rate per million person-years

- Not notifiable
- 0
- >0–<5
- 5–<15
- 15–<30
- 30+

Dermacentor variabilis,
the American dog tick
The Brown Dog Tick Emerges as an Unexpected Vector of Rocky Mountain Spotted Fever (RMSF) in Arizona

- Not considered important in the epidemiology of RMSF in the U.S. until 2004
- Cause of epidemic levels of RMSF in several American Indian communities in Arizona
- Outbreaks precipitated by large populations of free-roaming, tick-infested dogs

Community-Based Intervention Successfully Reduces the Number of Ticks and Cases of RMSF

- Collaborative endeavor among tribal partners, IHS, private sector, Arizona DOH, USDA, Inter Tribal Council of Arizona, and CDC
  - Yards treated with acaricide spray
  - Tick collars placed on dogs
- Dramatic reduction in ticks on dogs and in the environment
  - Ticks found on <1% of treated dogs vs. 64% of untreated dogs
- 43% reduction in cases of RMSF

DHS: Department of Health Services
RMSF: Rocky Mountain spotted fever

IHS: Indian Health Service
USDA: United States Department of Agriculture
A Second Pathogen Emerges from the Past

1939

**OBSERVATIONS ON AN INFECTIOUS AGENT FROM AMBLYOMMA MACULATUM**

By R. R. Parker, Director, Rocky Mountain Laboratory, Glen M. Kohls, Assistant Entomologist, United States Public Health Service, George W. Cox, Executive Officer, Texas State Department of Public Health, and Gordon E. Davis, Bacteriologist, United States Public Health Service

2004

**Rickettsia parkeri: A Newly Recognized Cause of Spotted Fever Rickettsiosis in the United States**

Christopher D. Paddock,1 John W. Sumner,1 James A. Comer,1 Sherif R. Zaki,1 Cynthia S. Goldsmith,1 Jerome Goddard,2 Susan L. McAllan,3 Cynthia L. Temmenga,4 and Christopher A. Ohl5

1Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 2Mississippi Department of Health, Jackson, Mississippi; 3Infectious Diseases Section, Tulane University Health Sciences Center, New Orleans, Louisiana; 4Division of Infectious Diseases, Portsmouth Naval Medical Center, Portsmouth, Virginia; and 5Section on Infectious Diseases, Wake Forest University School of Medicine, Winston-Salem, North Carolina

(See the editorial commentary by Reeuw on pages 812–3)

Rashes of Rocky Mountain Spotted Fever (RMSF) versus *Rickettsia parkeri* rickettsiosis
Distinctive Eschars of *Rickettsia parkeri* Rickettsiosisis

### RMSF versus *Rickettsia parkeri* rickettsiosis

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>RMSF (n = 398)</th>
<th><em>R. parkeri</em> rickettsiosis (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>Headache</td>
<td>80%</td>
<td>86%</td>
</tr>
<tr>
<td>Any rash</td>
<td>92%</td>
<td>90%</td>
</tr>
<tr>
<td>Petechial rash</td>
<td>52%</td>
<td>14%</td>
</tr>
<tr>
<td>Pustular/vesicular rash</td>
<td>---</td>
<td>33%</td>
</tr>
<tr>
<td>Eschar</td>
<td>---</td>
<td>95%</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>66%</td>
<td>10%</td>
</tr>
<tr>
<td>Coma/seizure</td>
<td>27%</td>
<td>---</td>
</tr>
<tr>
<td>Death</td>
<td>8%</td>
<td>---</td>
</tr>
</tbody>
</table>

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Rickettsia parkeri rickettsiosis: How Much is Out There?

- 5 cases identified by one clinician in Mississippi during 2007–2012
- 5 cases identified at one urgent care practice in Georgia during 2012–2014

Notes from the Field

Rickettsia parkeri Rickettsiosis — Georgia, 2012–2014
Anne Straily, DVM1-2; Amanda Feldpausch, MPH3; Carl Ulbrich, DO1; Kiersten Sehle1; Shannon Casillas, MPH4; Sherif R. Zaki, MD, PhD5; Amy M. Denison, PhD5; Mariah Cordis, MS2; Julie Gabsi, DVM3; Christopher D. Paddock, MD6

During 2012–2014, five cases of Rickettsia parkeri rickettsiosis were identified by a single urgent care practice in Georgia, located approximately 40 miles southwest of Atlanta. Symptom onset occurred during June–October, and all patients had a known tick bite. Patients ranged in age from 27 to 78 years (median = 53 years), and all were male. The most commonly reported initial signs were erythema (n = 3) and swelling (n = 2) at the site of the bite. Two patients reported fever and a third patient reported a rash and lymphadenopathy without fever. Other symptoms included myalgia (n = 3), chills (n = 3), fatigue (n = 2), arthralgia (n = 2), and headache (n = 2). Eschar biopsy specimens were collected from each patient using a 4-mm or 5-mm punch and placed in 10% neutral buffered formalin or

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Location, years</th>
<th>No. (% infected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermacentor variabilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. rickettsii</em></td>
<td>North Carolina, 1982</td>
<td>2,123 (0.05)</td>
</tr>
<tr>
<td></td>
<td>Ohio, 1984–1989</td>
<td>12,631 (0.06)</td>
</tr>
<tr>
<td></td>
<td>Maryland, 2002</td>
<td>392 (0)</td>
</tr>
<tr>
<td></td>
<td>Tennessee, 2007–2008</td>
<td>555 (0)</td>
</tr>
<tr>
<td>Amblyomma maculatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. parkeri</em></td>
<td>Florida, 2005–2007</td>
<td>128 (22)</td>
</tr>
<tr>
<td></td>
<td>Mississippi, 2008–2012</td>
<td>698 (15)</td>
</tr>
<tr>
<td></td>
<td>North Carolina, 2009–2010</td>
<td>101 (31)</td>
</tr>
<tr>
<td></td>
<td>Virginia, 2010–2011</td>
<td>293 (53)</td>
</tr>
</tbody>
</table>

Conclusions

- Etiologic spectrum of tickborne rickettsioses in the U.S. has expanded during the past 15 years

- Rocky Mountain spotted fever and *R. parkeri* rickettsiosis share many clinical features but differ considerably in severity

- Doxycycline is the drug of choice for all tickborne rickettsioses and in patients of all ages
  - Therapy should be initiated immediately, based on a presumptive diagnosis

Tickborne Viruses: Emerging Public Health Concern

Gregory D. Ebel, ScD
Associate Professor and Director
Arthropod-borne and Infectious Diseases Laboratories
Colorado State University
# Worldwide Ticks Are Vectors For Diverse Array of Viral Pathogens and Diseases

<table>
<thead>
<tr>
<th>Disease and Etiologic Agent</th>
<th>Signs and Symptoms</th>
<th>Geographic Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colorado Tick Fever</strong></td>
<td>Flu-like symptoms (FLS), headache, rash</td>
<td>Western U.S.</td>
</tr>
<tr>
<td><strong>Crimean-Congo Hemorrhagic Fever</strong></td>
<td>FLS, hemorrhagic fever (HF)</td>
<td>Asia, Africa, and Europe</td>
</tr>
<tr>
<td>CCHF virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Omsk Hemorrhagic Fever</strong></td>
<td>FLS, HF</td>
<td>Southwestern Russia</td>
</tr>
<tr>
<td>Omsk hemorrhagic fever virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kyasanur Forest Disease</strong></td>
<td>FLS, HF</td>
<td>Southern India, Saudi Arabia (aka Alkhurma disease in Saudi Arabia)</td>
</tr>
<tr>
<td>Kyasanur forest disease virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tick-Borne Encephalitis</strong></td>
<td>FLS, encephalitis, HF</td>
<td>Temperate regions of Europe and northern Asia</td>
</tr>
<tr>
<td>Tick-borne encephalitis virus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ticks Are Highly Efficient Vectors

- **Abundance**
  - One fully engorged female *Ixodid* yields several thousand offspring

- **Host preference**
  - At least two stages of feeding (usually larvae and nymphs) on the same host

A female *Ixodes scapularis* (Blacklegged tick) engorged with a host blood meal
Ticks Are Highly Efficient Vectors

- **Longevity of ticks**
  - Once infected with a pathogen, ticks are likely to survive and to transmit pathogen to new host
    - Survival of pathogen’s extrinsic incubation period

- **Modification of the site of attachment**
  - Pharmacology of tick saliva

- **Favorable gut environment**
  - Intracellular digestion of bloodmeal
Emerging Tickborne Viruses in the United States

- **Powassan virus** (*Flaviviridae: Flavivirus*)
  - Isolated 1958, Powassan, Ontario

- **Sole North American representative of the tickborne encephalitis complex of the Flaviviruses**

- **Severe acute disease**
  - 10.5% case-fatality rate

- **Long-term sequelae**
  - 47% of cases experience hemiplegia, wasting, severe headaches, etc.

- **Transmission depends on ecological factors that are not well understood**

References:

Deer tick-associated viruses appear to pose greatest public health risk.
Infection Among Deer Is Increasing

Serological Prevalence of POWV in Hunter-killed Deer, 1979–2010

POWV: Powassan virus
Clinical Features of Powassan Virus Disease Consistent Through Time

Demographic, Clinical Features and Outcomes of 8 Patients with Powassan Virus Encephalitis, 2013–2015

<table>
<thead>
<tr>
<th>Patient Age and Gender</th>
<th>Exposure</th>
<th>Fever</th>
<th>Rash</th>
<th>Gastro-intestinal</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>82 M</td>
<td>Outdoors</td>
<td>Y</td>
<td>N</td>
<td>Vomiting</td>
<td>Death</td>
</tr>
<tr>
<td>74 M</td>
<td>Outdoors, gardening</td>
<td>Y</td>
<td>N</td>
<td>None</td>
<td>Residual Deficits</td>
</tr>
<tr>
<td>21 M</td>
<td>Outdoors</td>
<td>Y</td>
<td>Y</td>
<td>Vomiting</td>
<td>Improved</td>
</tr>
<tr>
<td>67 M</td>
<td>Outdoors</td>
<td>Y</td>
<td>Y</td>
<td>Vomiting, diarrhea</td>
<td>Improved</td>
</tr>
<tr>
<td>65 F</td>
<td>Tick bite</td>
<td>Y</td>
<td>N</td>
<td>Vomiting</td>
<td>Improved</td>
</tr>
<tr>
<td>52 M</td>
<td>Tick bite</td>
<td>Y</td>
<td>N</td>
<td>None</td>
<td>Residual Deficits</td>
</tr>
<tr>
<td>49 M</td>
<td>Tick bite</td>
<td>Y</td>
<td>N</td>
<td>None</td>
<td>Death</td>
</tr>
<tr>
<td>44 M</td>
<td>Outdoors, hunting</td>
<td>N</td>
<td>Y</td>
<td>None</td>
<td>Improved</td>
</tr>
</tbody>
</table>

Number and Geographic Distribution of Reported Powassan Virus Cases, 2006-2015

Powassan Virus Neuroinvasive Disease Cases Reported by Year, 2006–2015

Powassan Virus Neuroinvasive Disease Cases Reported by State, 2006–2015

Powassan Virus Causes Neurological Damage Both Directly and via Inflammatory Processes

Inflammatory changes within the perivascular and parenchymal portions of the brain

Direct neuronal injury of the Purkinje cells

Discovery of New Tickborne Viruses Is Ongoing

- **Heartland virus** (*Bunyaviridae: Phlebovirus*)
  - First 2 cases in Missouri, 2009
  - Additional 6 confirmed cases in Tennessee and Missouri, 2012–2013
  - CDC continues to investigate additional cases
  - Few fatalities
  - Lone star tick
    - *(Amblyomma americanum)*
  - Wildlife serologic studies suggest widespread distribution of virus

Discovery of Another Virus Likely to Be Tickborne

➢ **Bourbon virus**  
(*Orthomyxoviridae: Thogotovirus*)

- First case in Kansas, 2015
- Likely tickborne
  - *In vitro* studies support
- Fatal outcome

Numerous extracellular Bourbon virus virions with slices through strands of viral nucleocapsids

Knowledge Gaps and Unmet Needs

- As new viruses emerge, so do questions and concerns
- What determines human risk and likelihood of emergence as human threat? How best to intervene?

Forest cover in the U.S., East of the Mississippi, from colonization to the present
Reforestation Drives Emergence
Ecology: How Do Tickborne Arboviruses Perpetuate in Nature?

- Modeling and experimental studies show multiple transmission routes required
- Model parameters obtainable and transmission cycles can be studied
- Niche complexity

How Do Ticks, Hosts, and Pathogens Interact?

- What factors influence tick vector competence at the molecular level?
- Most work covers tick salivary secretions
  - Hundreds of protein and non-protein molecules
    - Affect hemostasis, inflammation, i.e., host immune response to tick feeding
    - Effect of pathogens poorly understood

Figuring Out Function of Tick Genes

- **Tick genome sequence**
  - Extremely large
  - Most genes duplicated
  - Metagenomics also contributes to key phenotypes

Distribution of assembled contigs from cDNA library from the salivary glands of adult female *Hyalomma marginatum rufipes* ticks

Exciting Time to Study Tickborne Diseases

- Ecology of ticks and their hosts
- New sequencing and computational tools
- In vitro and in vivo systems to study tickborne viruses
Barriers to Rapid Advances in Tickborne Virus Research

**Technical**
- Containment and regulatory restrictions
- System complexity
- Long tick life cycle
  - One tick lifecycle per grant or funding cycle

**Environmental**
- Tickborne viruses emerge slowly relative to mosquito-borne viruses
- Significance, impact, innovation

**Few researchers active**

*Note tick in tweezers*
 Tick borne viruses are emerging health concerns
 Interesting, relevant and feasible questions present opportunities to the field
 Difficult technical and environmental barriers currently impede progress
Advances in Diagnosing Tickborne Diseases

Bobbi S. Pritt, MD, MSc, DTM&H
Director, Clinical Parasitology Laboratory
Co-Director, Vector-borne Diseases Laboratory Services
Professor of Pathology and Laboratory Medicine
Division of Clinical Microbiology, Mayo Clinic
Primary Methods for Diagnosis of Tickborne Diseases

- Clinical evaluation
- Tickborne diseases must be considered in differential diagnosis
  - Order correct laboratory tests
  - Begin empirical antimicrobial therapy if indicated
  - Especially if rickettsiosis, ehrlichiosis, or anaplasmosis is suspected
    - Infections may be rapidly fatal
    - Need to be treated quickly, often before test results are available

Rash of Rocky Mountain spotted fever

Eschar of *Rickettsia parkeri* rickettsiosis

Tickborne Diseases of the United States. 2016. CDC, Atlanta, GA.
www.cdc.gov/ticks/diseases/
Indirect Laboratory Methods: Serology

**Principle:**
- Detection of the host’s immune response to organisms
- IgM or IgG class host antibodies in serum

**Uses:**
- Method of choice for diagnosing many tickborne diseases
  - *Rickettsia, Ehrlichia* and *Anaplasma* species, tickborne viruses
- Testing for tickborne viruses uses laboratory-developed methods
  - Available primarily through state public health laboratories or CDC
- Not primary diagnostic choice for babesiosis
- Sensitivity varies by the time that the specimen is obtained during the course of infection

Tickborne Diseases of the United states. 2016. CDC, Atlanta, GA.
www.cdc.gov/ticks/diseases/
Serology: IgM and IgG Patterns

<table>
<thead>
<tr>
<th>Infection</th>
<th>Days</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td></td>
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<td></td>
<td>14</td>
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<td></td>
<td>21</td>
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<tr>
<td></td>
<td>~180</td>
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</table>
Direct Laboratory Methods for Diagnosis of Tickborne Diseases

- Microscopy
  - Anaplasma phagocytophilum morula
  - Relapsing fever Borrelia spp. spirochetes
  - Babesia spp. parasites within red blood cells

- Nucleic acid amplification tests (e.g., polymerase chain reaction)
- Culture not routinely used, except for tularemia

Tickborne Diseases of the United States. 2016. CDC, Atlanta, GA.
www.cdc.gov/ticks/diseases/
Direct Methods - General Pattern

<table>
<thead>
<tr>
<th>Infection</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>~180</th>
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<tr>
<td>IgM</td>
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<tr>
<td>IgG</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Onset of symptoms</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titer/quantity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA or RNA</td>
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</tbody>
</table>
Nucleic Acid Amplification Tests – What Can They Do?

- One of the earliest methods for detecting some organisms
- Insensitive if nucleic acid is no longer detectable by the time the patient presents for evaluation
- Especially useful for *Anaplasma phagocytophilum*, *Ehrlichia* species and *Babesia* species (whole blood specimens)
  - These organisms are usually present in high amounts during the symptomatic stage of infection
- Variety of nucleic acid amplification methods available
  - None are FDA-approved/cleared

Tickborne Diseases of the United States. 2016. CDC, Atlanta, GA.  
www.cdc.gov/ticks/diseases/
Polymerase Chain Reaction

- **Fluorescent probes are used to detect DNA as it is amplified**
  - Can be highly sensitive and specific
  - Semiquantitative detection

- **Melting curve analysis**
  - Uses nonspecific DNA-binding dyes or specific probes
  - Additional benefit of detecting mutations (and even new organisms)

Tickborne Diseases of the United states. 2016. CDC, Atlanta, GA.
www.cdc.gov/ticks/diseases/
Example: Melting Temperature Analysis with Fluorescence Resonance Energy Transfer (FRET) Hybridization Probes

Real-time PCR targeting *groEL* (gene encoding the heat shock operon) of *Ehrlichia* spp.

Example: Melting Temperature Analysis with Fluorescence Resonance Energy Transfer (FRET) Hybridization Probes

Real-time PCR targeting \textit{groEL} (gene encoding the heat shock operon) of \textit{Ehrlichia} spp.

Previous name: \textit{Ehrlichia muris}-like agent (EMLA)

New accepted name: \textit{Ehrlichia muris eauclairensis}

This same method allowed for detection of \textit{Borrelia mayonii}


Multiplex molecular panels
- Specific primers/probes to detect multiple bacterial, viral, and parasitic pathogens in a single test
  - Panels for tickborne disease under development
- Only detects targeted organisms, non-targeted organisms will be missed

Broad range sequencing
- Targeted gene amplification with subsequent sequence identification
  - Common targets: 16S rRNA gene (bacteria), internal transcribed spacer (ITS) region (fungi)
  - No equivalent for viruses; instead, groups of closely related viruses can be targeted (e.g. flavivirus)
Next Steps in Testing

**Metagenomics**

- Amplification all nucleic acid in a specimen: bacterial, fungal, viral, parasitic and human
- Extensive pre- and post-processing steps used to select for targets of interest and remove nonrelevant nucleic acid
- Currently very expensive and time consuming; this is bound to change in the future!
Recommended Reading

Resources for Tickborne Diseases

cdc.gov/lyme/resources/tickbornediseases.pdf

cdc.gov/mobile/applications/mobileframework/tickborne-diseases.html

www.cdc.gov/ticks
Emerging Tickborne Diseases