Lyme Disease, Human Granulocytic Anaplasmosis & Babesiosis

Thomas Novicki Ph.D. D(ABMM)
Marshfield Labs (A division of Marshfield Clinic)
Marshfield WI
Major Tick-Borne Diseases of the USA and Their Tick Vectors

- Lyme Disease (LD)
- Human Granulocytic Anaplasmosis (HGA)
- Tularemia
- Ehrlichiosis
- Relapsing Fever
- Rocky Mtn Spotted Fever
- Colorado Tick Fever
- Babesiosis
- Tick Paralysis

- *Ixodes*
- *Dermacentor*
- *Amblyomma*
- *Ornithodoros*
Tick-Borne Diseases of the Upper Midwest

- **LD**
  - *Borrelia burgdorferi* sensu stricto

- **HGA**
  - *Anaplasma phagocytophilum*

- **Babesiosis**
  - *Babesia microti*

- **Tick paralysis**
  - Tick neurotoxins

- **Tularemia**
  - *Francisella tularensis*
The Vector - *Ixodes scapularis*

**Primary Hosts**

- **Larva:** white footed mouse, other small mammals
- **Nymph:** small rodents, humans *i.e. The principle vector for human LD*
- **Adult:** white tail deer, and sometimes humans
- **Wild animals remain asymptomatic**

Images courtesy of CDC
Epidemiology

- LD, HGA, Babesiosis are zoonotic diseases
  - Cycle between small and large mammal populations
  - Birds, reptiles also play roles

- Humans are effectively incidental, dead end hosts
I. *Scapularis* - Feeding

- Female is the predominant feeder, source of disease
- Tick remains attached 3-7 days if not disrupted
- Main blood meal, “the big sip” occurs in final 4hrs
- Transmission occurs
The Etiological Agents and their Diseases
LD

- *Borrelia burgdorferi*, a spiral bacterium related to *Treponema* (syphilis, yaws, pinta)

- Three species of *B. burgdorferi* sensu lato
  - *B. burgdorferi* sensu stricto
  - *B. afzelii*
  - *B. garinii*

- Geographic differences
  - N. America: *B. burgdorferi* sensu stricto only
  - Europe: all three species
  - Disease spectrum in Europe differs
Early, Local (days-weeks post tick bite)

- Primary erythema migrans (EM) at site of bite
  - Papule, expanding in annular rings
  - 80-90% of patients exhibit EM

- Early signs of dissemination may also occur
  - Fever
  - Malaise/myalgia
  - Headache/stiff neck
  - Migratory arthralgias
  - Local lymphadenopathy
1° EM

- Classic EM form, but may be more diffuse, less annular
- Has central *punctum*, site of tick bite
- And, 10-20% have no EM lesion
- Bottom Line: not always easy to diagnose!

Image courtesy of CDC
LD

- Early, Disseminated (weeks-months post bite)
  - Multiple 2° EM lesions (no punctum)
  - Lyme carditis
  - Neuroborreliosis
    - Meningitis
    - Cranial neuritis
    - Myelitis
    - Encephalitis
  - Lymphocytoma (cutaneous B-cell pseudolymphoma), achrodermatitis chronica atrophicans
    - Primarily seen in Europe, is rare in here
LD

- Late, Disseminated (months-years post bite)
  - Migrating arthritis, esp. knees
  - Various chronic neuropathies
“Chronic” LD

- Is there disease beyond late LD?
  - Post-Lyme Disease Syndrome
    - Small proportion of patients living in endemic areas, who are diagnosed by validated lab methods, and complete approved treatment continue to show some residual symptoms
    - Symptoms usually mild, abate over time
    - Immune-related?
Chronic LD

- Many other cases do not fit these criteria - “Chronic LD”
  - No lab evidence of infection, or “evidence” by poorly validated methods
  - Live outside of endemic areas
  - No Hx of tick bite, EM
  - Vague symptoms - fatigue, aches, night sweats, etc
  - Can result in much money spent, questionable treatments
HGA

- Anaplasma (Ehrlichia) phagocytophilum

- Order Rickettsiales

- 1st described in 1994 (Chen et al JCM 32:589)
  - 6 pts. MN & WI
  - 33% mortality
  - Granulocytes of one pt. had cytoplasmic inclusions reminiscent of Ehrlichia chaffeensis monocytic inclusions (morulae)
A. *phagocytophilum* morulae
HGA

- 16S rRNA sequence analysis:
  - > 99.8% homologous to the animal pathogens *E. phagocytophila* and *E. equi*
  - only 92.5% homologous to *E. chaffeensis*

- “Agent of human granulocytic ehrlichiosis”

- After several reclassifications, now known as “*Anaplasma phagocytophilum*”
HGA

- Common Symptoms
  - Fever
  - Headache
  - Malaise/myalgia
  - Thrombocytopenia, neutropenia
  - ↑ hepatic transaminases
  - Rash is rare: compare to Rocky Mountain Spotted Fever (*Rickettsia rickettsii*) where rash is common

- Usually self-limited, but fatalities occur (<1%)
Babesiosis

Approximately 100 species of *Babesia*

Human agents

- Upper Midwest, Northeast USA: *B. microti*
- West Coast: CA1, WA1
- Europe: *B. divergens*
Babesiosis

- Unicellular protozoan that parasitizes RBCs

- Distant relative of *Plasmodium* (malaria)
  - *Babesia* differs from *Plasmodium*:
    - Vector = tick, not mosquito
    - No hepatic forms
    - No schizonts
    - Has extracellular forms
Babesiosis

- 1st human case reported Nantucket RI 1969

- Similarities to *Plasmodium* extend to symptoms:
  - Relapsing fever
  - Hemolytic anemia/jaundice
  - Fatigue
  - Chills, sweats
  - Headache
  - Myalgia/arthralgia
  - Anorexia
Babesiosis

- Most cases asymptomatic
- Disease ranges from mild to fulminant (rare)
- Immunosuppression, advanced age, asplenia are predisposing factors
- Mortality $\leq 5\%$ if untreated
Epidemiology
Average Annual Incidence of LD 1992-2006

* Per 100,000 population.

Image courtesy of CDC
Average Annual Incidence of HGA 2001-2002

*Nonreporting states do not have a value.*

Image courtesy of CDC
LD Incidence, WI 2007
Cases per 100,000 population

Image courtesy of WI DPH
HGA Incidence, WI 2007
Cases per 100,000 population

Image courtesy of WI DPH
Reported Babesiosis, WI 1996-2008

- Confirmed
- Probable
- Transfusion

Babesiosis made notifiable
Case def. changed to include PCR +

Image courtesy of WI DPH
Reported ehrlichioses cases by month of onset, 2004
n = 103

Image courtesy of WI DPH
Age-Specific Incidence of Reported Ehrlichiosis, Wisconsin, 2004 (cases per 100,000 population)

Median age = 62 (range 5-90)

Image courtesy of WI DPH
Diagnosis
Diagnosis of Tick-borne Diseases

- Serology: the cornerstone of lab diagnosis
  - Fluorescent antibody staining
  - EIA
  - Western blot
Western Blot

1. Antigens of an organism are separated by size by gel electrophoresis, blotted onto a nitrocellulose strip

2. Pt. serum is applied to the strip: any antibody(ies) present bind to the immobilized antigen(s); other serum components are then washed off

3. Antigen/Antibody complex bands are visualized by immunochemistry

4. Number and size of visible bands enumerated by eye or computerized blot scanner
Western Blot

- Newer technique “paints” various purified antigens onto strips at the “correct” positions

- Also known as “immunoblots”
Western Blot - LD

- IgM & IgG Blots add specificity over EIA and FA

- Must look at **intensity**, **size** and **number** of bands

- CDC interpretive criteria
  Positive =
  - IgG ≥ 5/10 **significant** bands
  - IgM ≥ 2/3 **significant** bands
Diagnosis - LD

- CDC Two-tier algorithm
  - Screen with an EIA or IFA
  - Confirm Positive and Equivocal screens with immunoblot (IB)
    - IgM & IgG in 1st month of disease (i.e. 1st EM present)
    - IgG only thereafter
  - 38%-100% Sensitive, 99% Specific (Bacon et al 2003 J Infect Dis 187:1187)

- CDC: “A clinical diagnosis” in the end
Diagnosis - LD

- Serological caveats
  - Sensitivity of two-tier serological algorithm increases with length of untreated disease
  - Early therapy blunts immune response
  - IgM persists for > 1 yr – do not test IgM after 1mo

- No data supports repeat sero-testing during treatment, or in suspected reoccurrence
LD Diagnosis - New Fronts

- FDA cleared product scans blots, performs software analysis, and archives strip images

- Painted immunoblot strips may soon be available, allowing for more uniformity, ease of reading

- EIAs using purified VLSE and C6 antigens
  - promise better performance
  - may eliminate/reduce need for WB
Diagnosis - LD

- Culture
  - Skin Bx: Reserve for very early, unusual EM
  - Recovery from other sources is poor
  - Takes 1-2 weeks or more
  - Not readily available

- PCR
  - Most sensitive on synovial fluid (83%) and CSF (73%)
LD specialty labs have arisen in response to “chronic” LD. Can often be found on the Web.

Often do not follow the CDC two-tier serological method, do not use FDA-cleared lab tests, use FDA-cleared tests “off label”, or use incompletely validated tests.

Your physicians or patients may ask you to use one of these labs.
LD Diagnosis - Choosing a Reference Lab

What can you do?

- When searching for a reference lab, ask:
  - Are they accredited? (Joint Commission, CAP, CLIA)
  - Does the lab use
    - FDA-cleared tests? If so, are they used “on label”?
    - The CDC 2-Tier LD algorithm?
    - Non-FDA cleared tests? If so, how validated? Data published in peer-reviewed journals?
  - Do the same physicians that run the lab also provide clinical services? (Potential conflict of interest)
Diagnosis: HGA, Babesiosis

- Blood smear
  - Thin smear fresh whole blood stained with Wright or Giemsa

- Carefully observe for characteristic forms
  - Ring and tetrad forms of *Babesia*
    - Multiply infected RBCs
    - Extracellular forms
    - Extreme size variation
  - Granulocyte morulae of *A. phagocytophilum*
    - Azure, stippled in appearance
B. microti
A. *phagocytophilum* morulae

Photos courtesy of Jim Kazmierczak DVM
WI DPH
Diagnosis: HGA, Babesiosis

- **Serology**
  - Indirect Fluorescent Antibody (IFA)
    - IgG: 4X rise in acute & convalescent titers, or
      - $\geq 1:64$ HGA
      - $\geq 1:32$ babesiosis
    - IgM: any detectable level
  - Subject to challenges of all FA tests
    - Subjective, need FA ‘scope and trained microscopist

- **Blood PCR**
Treatment
Treatment

- LD
  - **Doxycycline**, Ceftriaxone, Cefuroxime, Amoxicillin

- HGA
  - **Doxycycline**

- Babesiosis
  - Atovaquone+Azithromycin
  - Clindamycin+Quinine
Treatment – LD

- Treatment resistant/recurrent Lyme rarely occurs when appropriately treated

- Reinfection is now recognized, usually in patients previously treated in early disease

- Co-infection does occur: incidence is not clear
Questions?
Selected References