Lyme Disease, Human Granulocytic Anaplasmosis & Babesiosis

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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*

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Tick Borne Diseases of the Upper Midwest

- LD
  - *Borrelia burgdorferi* sensu stricto
- HGA
  - *Anaplasma phagocytophilum*
- Babesosis
  - *Babesia microti*
- Tick paralysis
  - Tick neurotoxins
- Tularemia
  - *Francisella tularensis*

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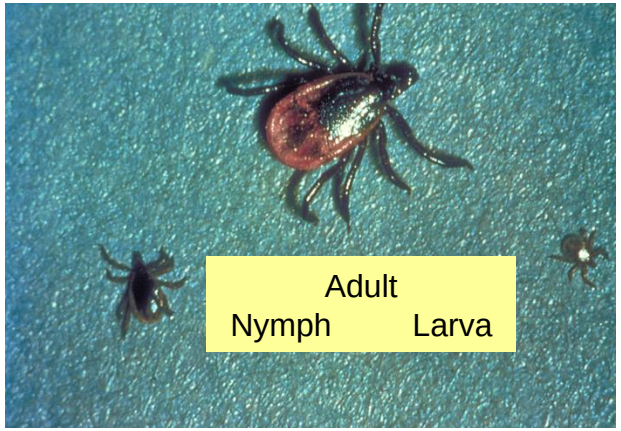
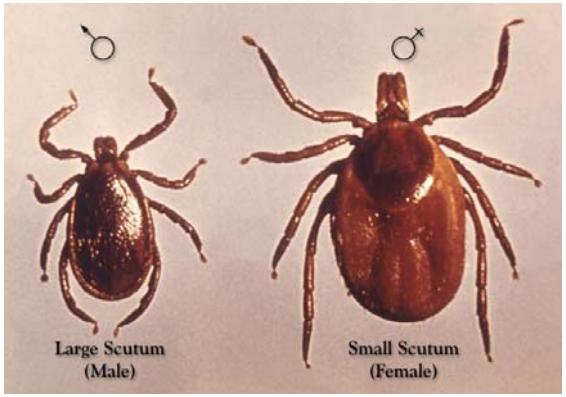
The Vector - *Ixodes scapularis*

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





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Images courtesy of CDC

Epidemiology

- LD, HGA, Babesosis are zoonotic diseases
  - Cycle between small and large mammal populations
  - Birds, reptiles also play roles
- Humans are effectively incidental, dead end hosts

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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

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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

LD

- 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

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1º 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^{o} 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

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“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)

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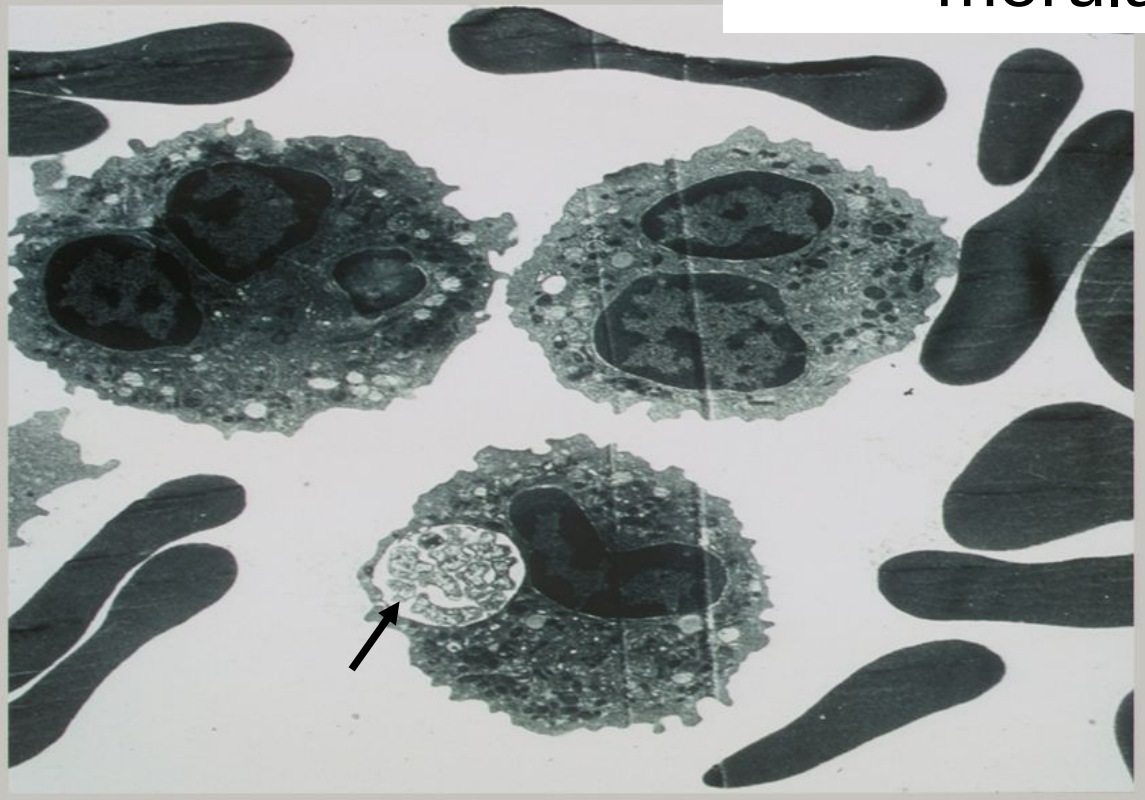
A. phagocytophilum morulae



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Photos courtesy of Jim Kazmierczak DVM
WI DPH

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*"

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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%)

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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

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Babesiosis

- Most cases asymptomatic
- Disease ranges from mild to fulminant (rare)
- Immunosuppression, advanced age, asplenia are predisposing factors
- Mortality $\leq$ 5% if untreated

Epidemiology

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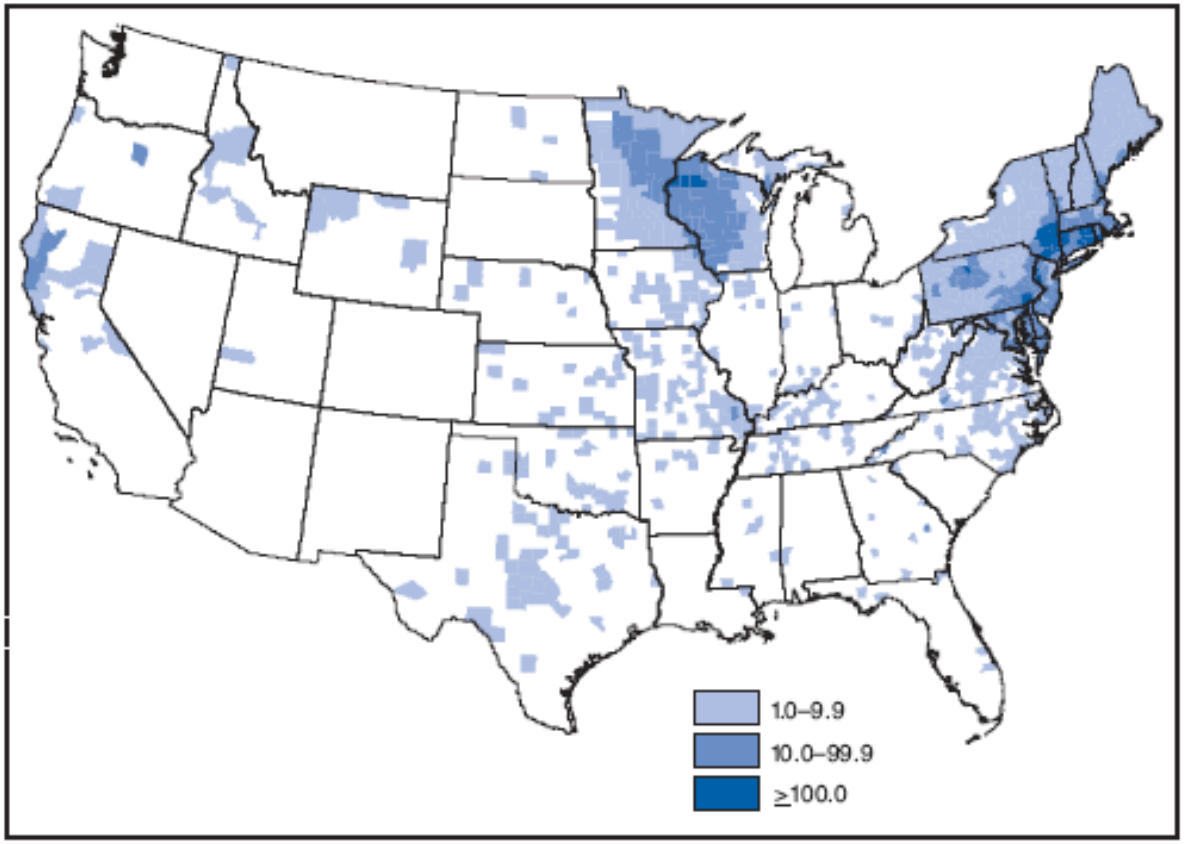
Average Annual Incidence of LD 1992-2006



* Per 100,000 population.

Image courtesy of CDC

Average Annual Incidence of HGA 2001-2002

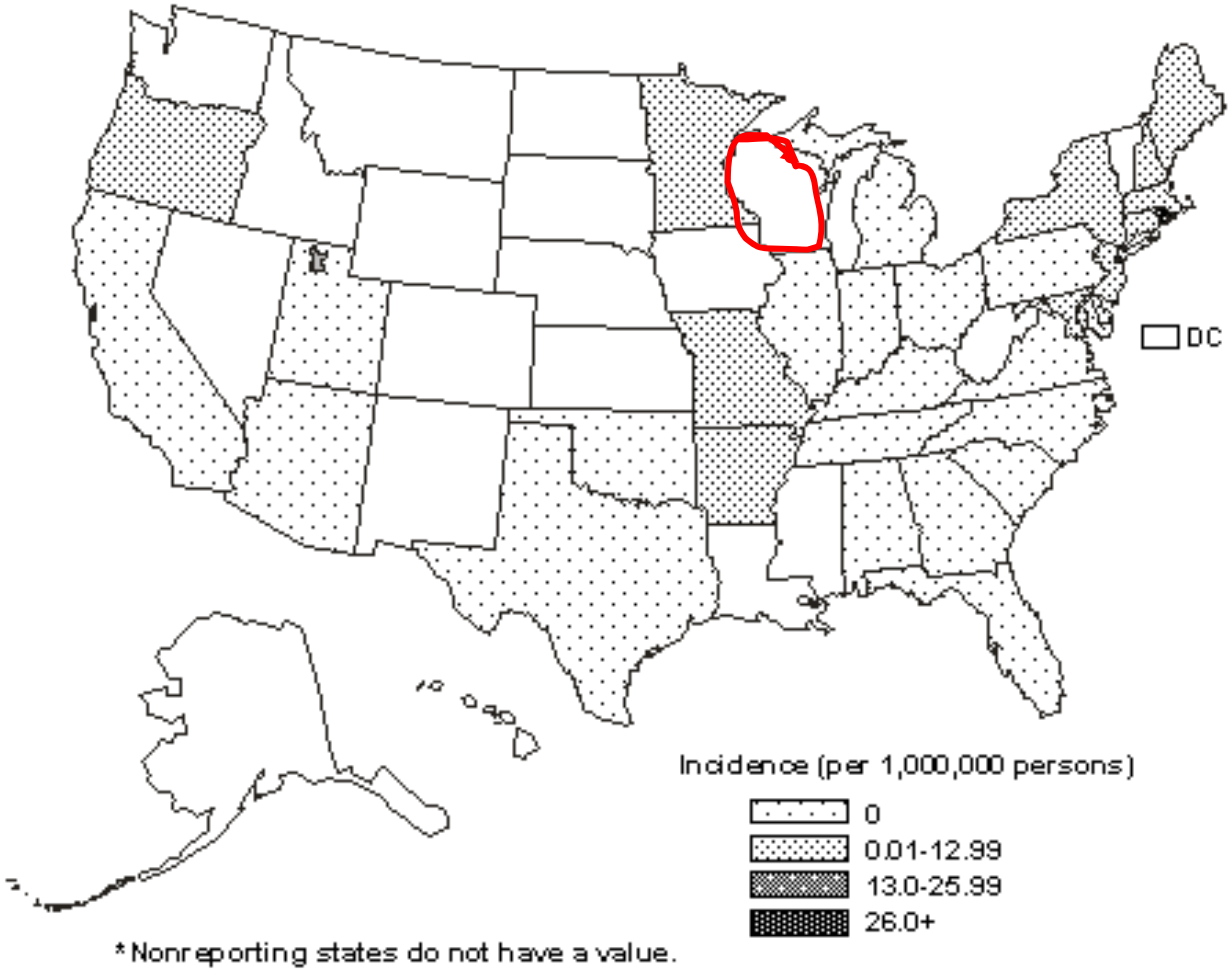


Image courtesy of CDC

LD Incidence, WI 2007
Cases per 100,000 population

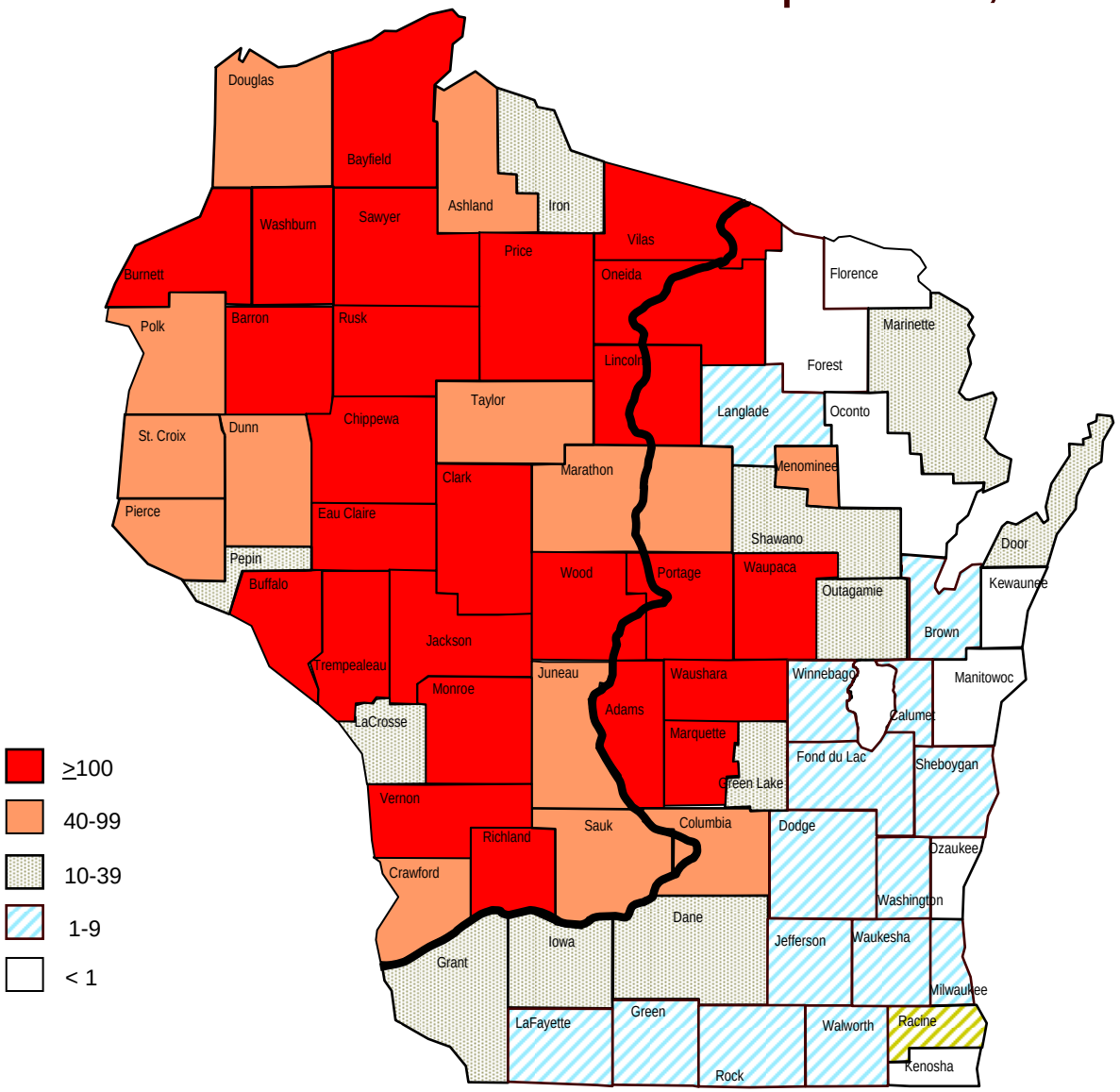
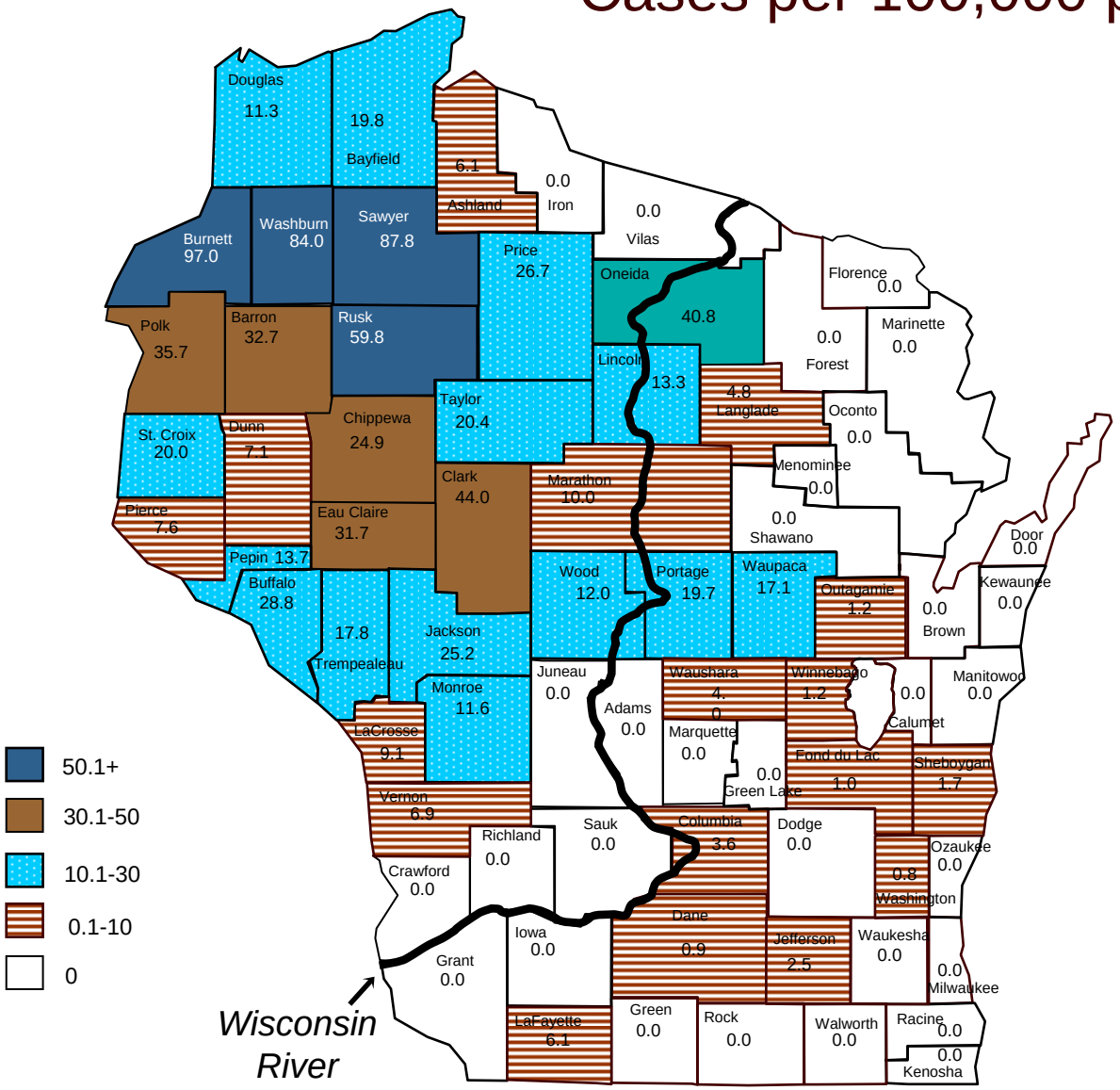


Image courtesy of WI DPH

HGA Incidence, WI 2007
Cases per 100,000 population



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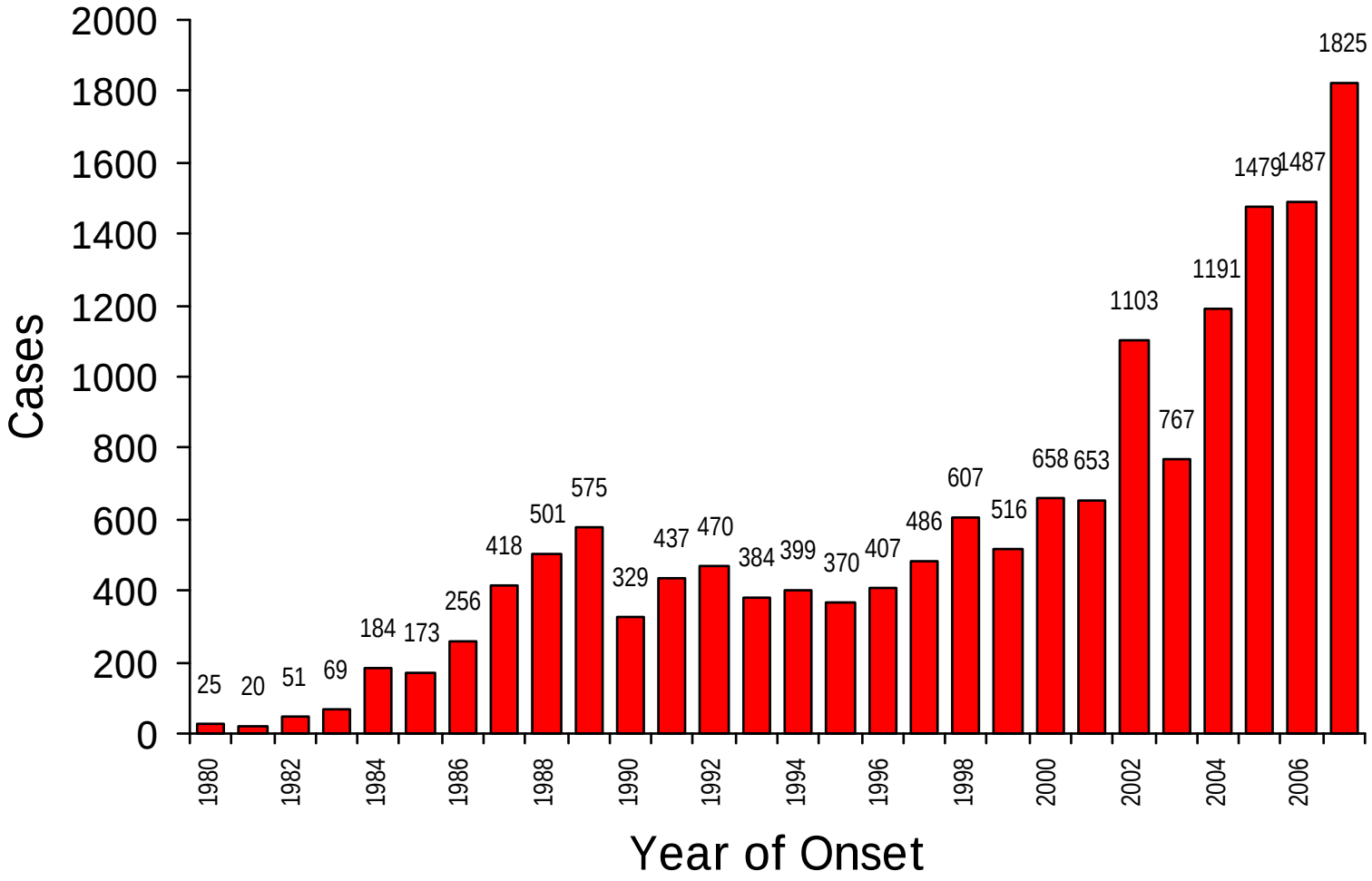
Reported LD Cases, WI

1980-2007



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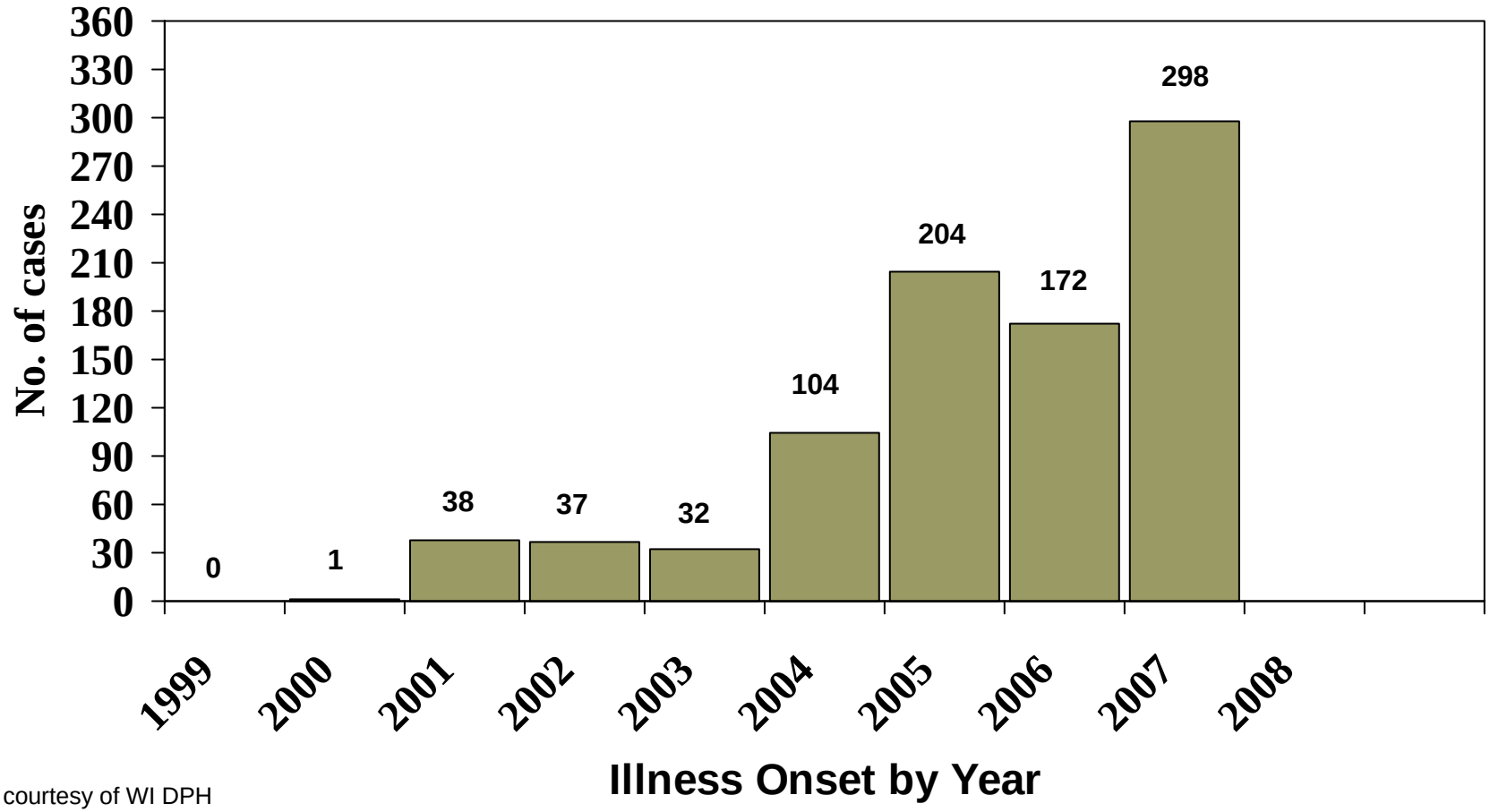
Reported HGA Cases, WI 1999-2007



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Reported Babesiosis, WI 1996-2008

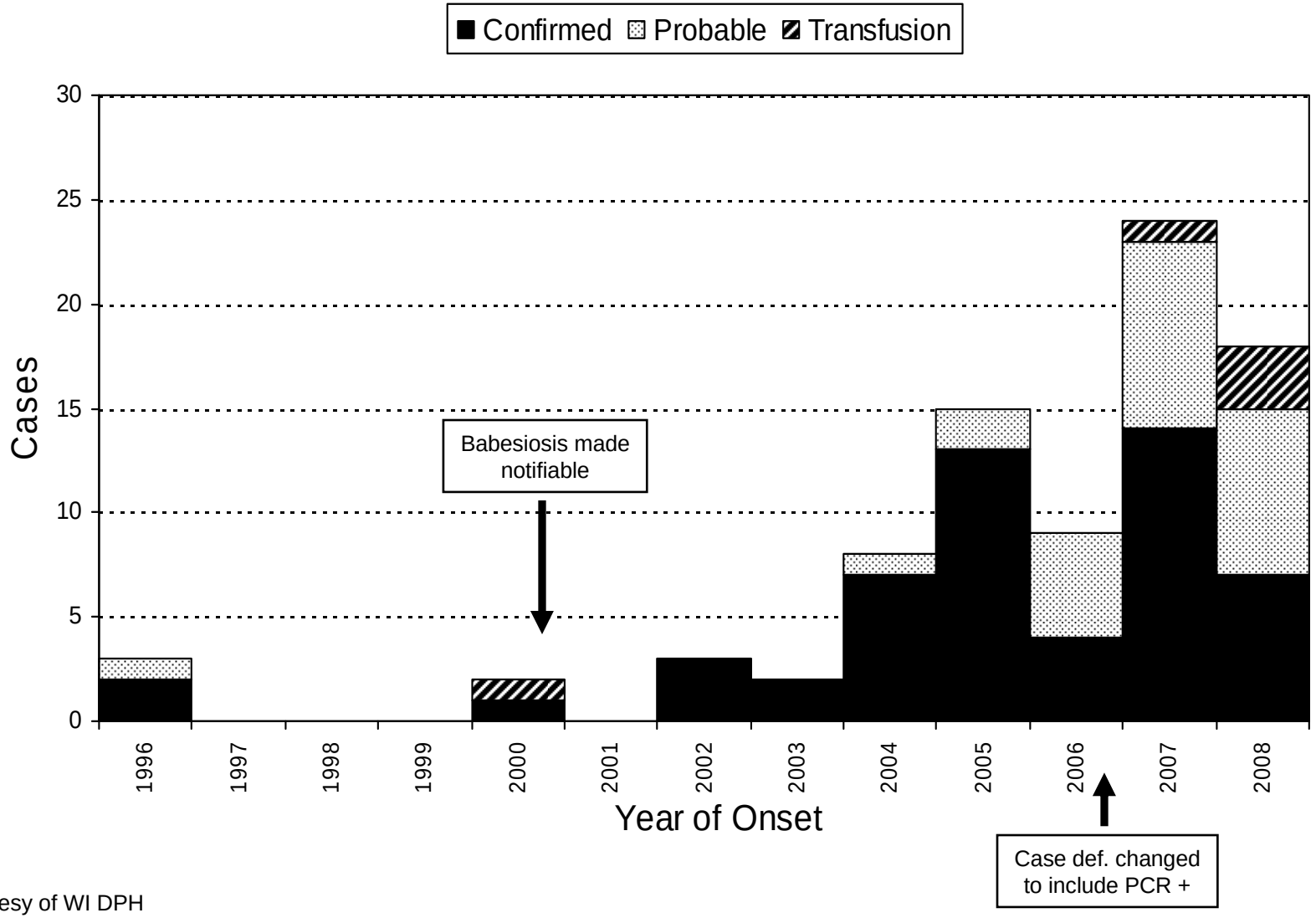
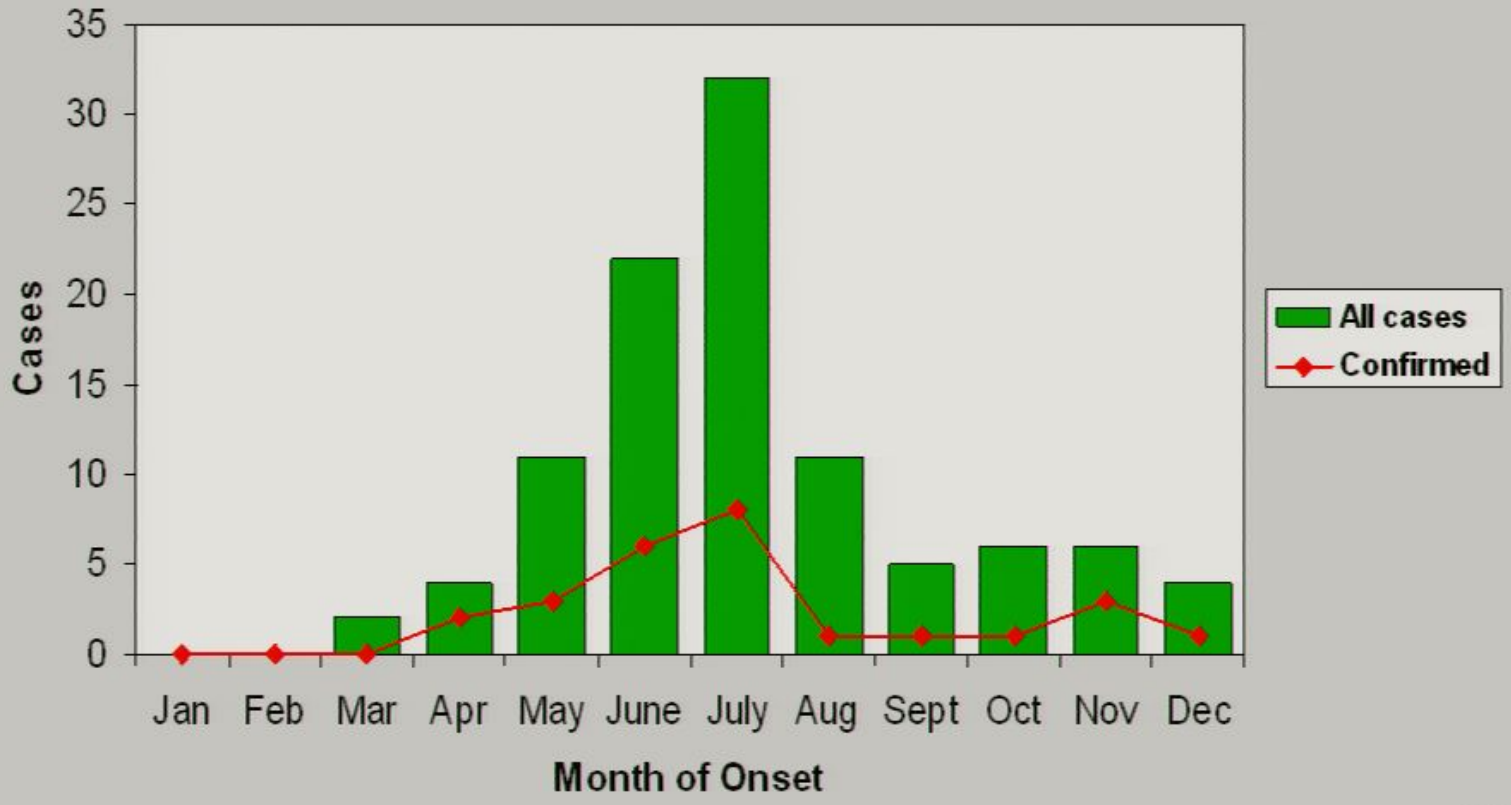


Image courtesy of WI DPH

Reported ehrlichiosis cases by month of onset, 2004
n = 103



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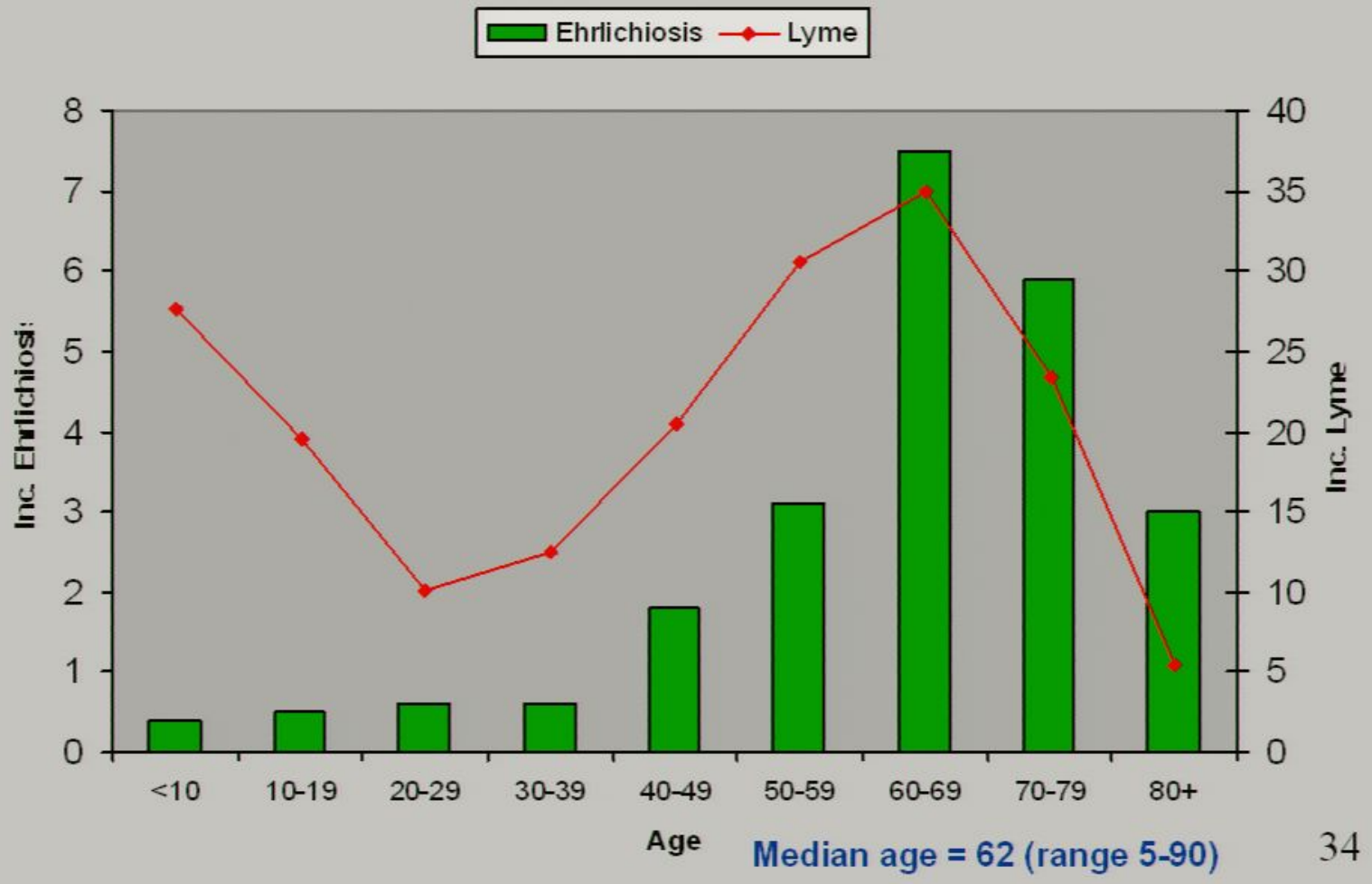
Age-Specific Incidence of Reported Ehrlichiosis, Wisconsin, 2004 (cases per 100,000 population)



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Diagnosis

Diagnosis of Tick-borne Diseases

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

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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”

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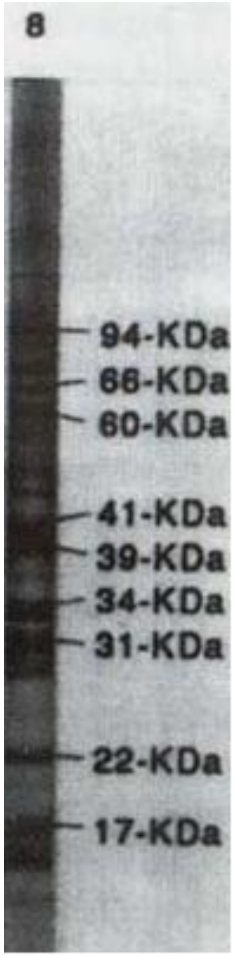
Western Blot - LD

- 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. 1o 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

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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

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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%)

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LD Diagnosis - Choosing a Reference Lab

- 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

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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)

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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

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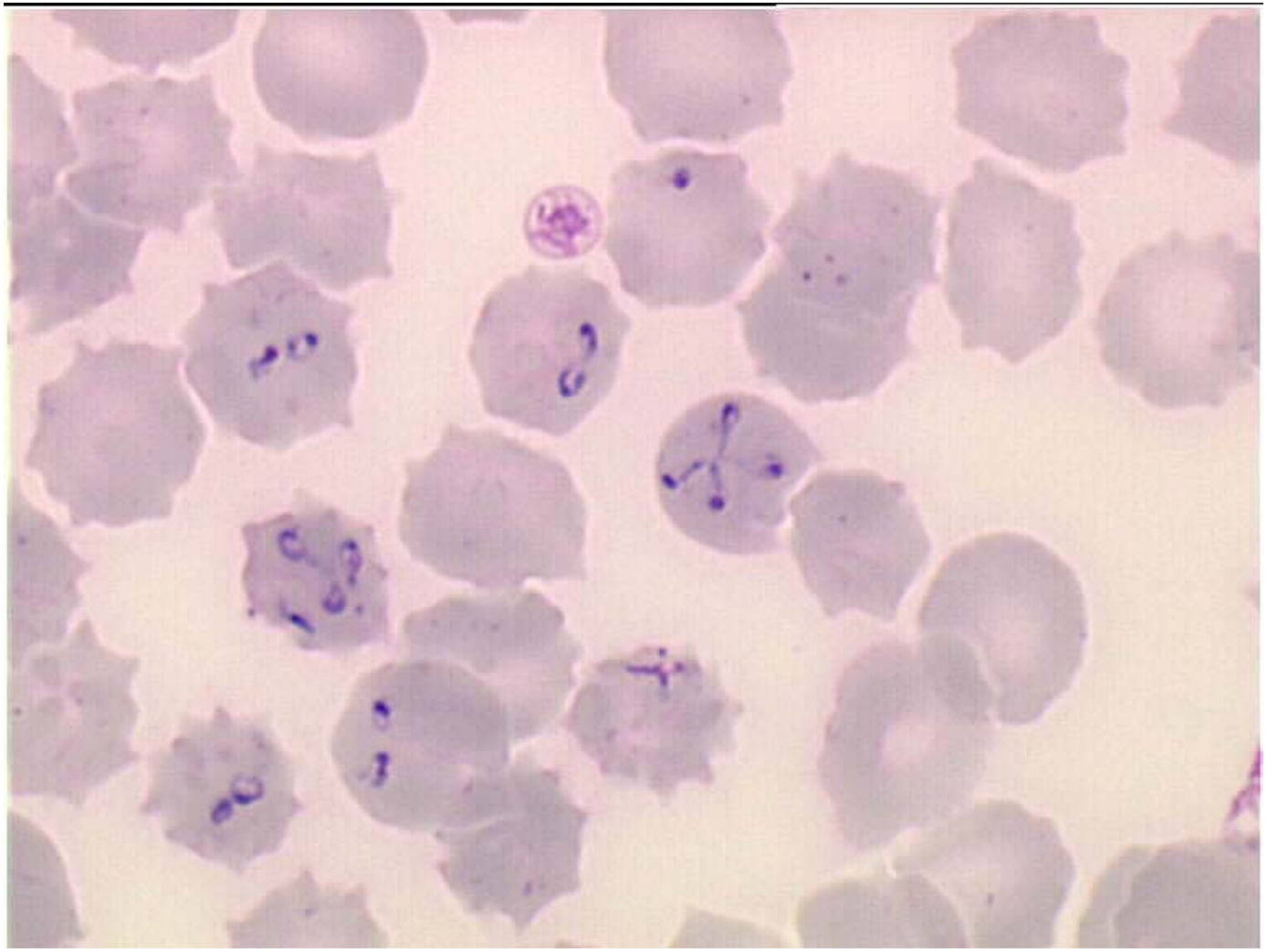
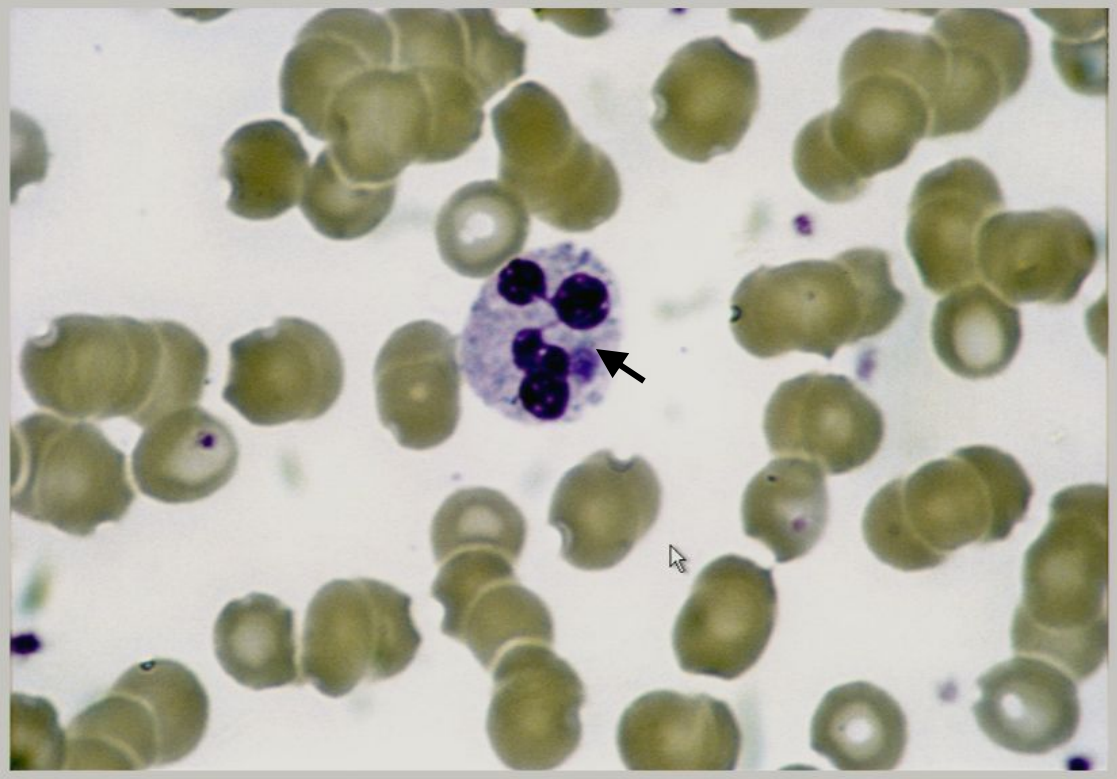
B. microti



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
      - ≥ 1:64 HGA
      - ≥ 1:32 babesiosis
    - IgM: any detectable level
  - Subject to challenges of all FA tests
    - Subjective, need FA ‘scope and trained microscopist
- Blood PCR

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Treatment

Treatment

- LD
  - **Doxycycline**, Ceftriaxone, Cefuroxime, Amoxicillin
- HGA
  - **Doxycycline**
- Babesiosis
  - Atovaquone+Azithromycin
  - Clindamycin+Quinine

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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?

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Selected References

1. Spach DH, et al. Tick-borne diseases in the United States. 1993. N Engl J Med 329:936
2. Nadelman, RB and GP Wormser. 2007. Reinfection in patients with Lyme Disease. Clin Inf Dis 45:1032
3. Feder, HM et al. A critical appraisal of “chronic Lyme Disease”. 2007. N Engl J Med 357:1422
4. Krause, PJ et al. Reinfection and relapse in early Lyme Disease. 2006. Am J Trop Med Hyg 75:1090
5. Feder HM et al. Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme Disease. 1992. Clin Inf Dis 15:788
6. Mitchell et al. Immunoserologic evidence of coinfection with *Borrelia burgdorferi, Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. 1996. J Clin Microbiol 34:724
7. CDC. Notice to readers recommendations for test performance and interpretation from the Second National Conference on serologic diagnosis of Lyme Disease. 1995. MMWR 44:590
8. Aguero-Rosenfeld, ME et al. Diagnosis of Lyme Borreliosis. 2005. Clin Microbiol Rev 18:484
9. Bacon, RM et al. Serodiagnosis of Lyme Disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-Tiered testing using whole-cell lysates. 2003. J Inf Dis 187:1187
10. Wormser GP et al. The clinical assessment, treatment, and prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: clincal practice guidelines by the Infectious Diseases Society of America. 2006. Clin Inf Dis 43:1098
11. Steere, AC. *Borrelia burgdorferi* (Lyme Disease, Lyme Borreliosis), pp 2504-2518. *In* Principles and Practice of Infectious Diseases, 5th ed. Mandell GL et al, *ed.* 2000. Churchill Livingstone, Philadelphia