Rickettsioses

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Rickettsial Diseases

General comments

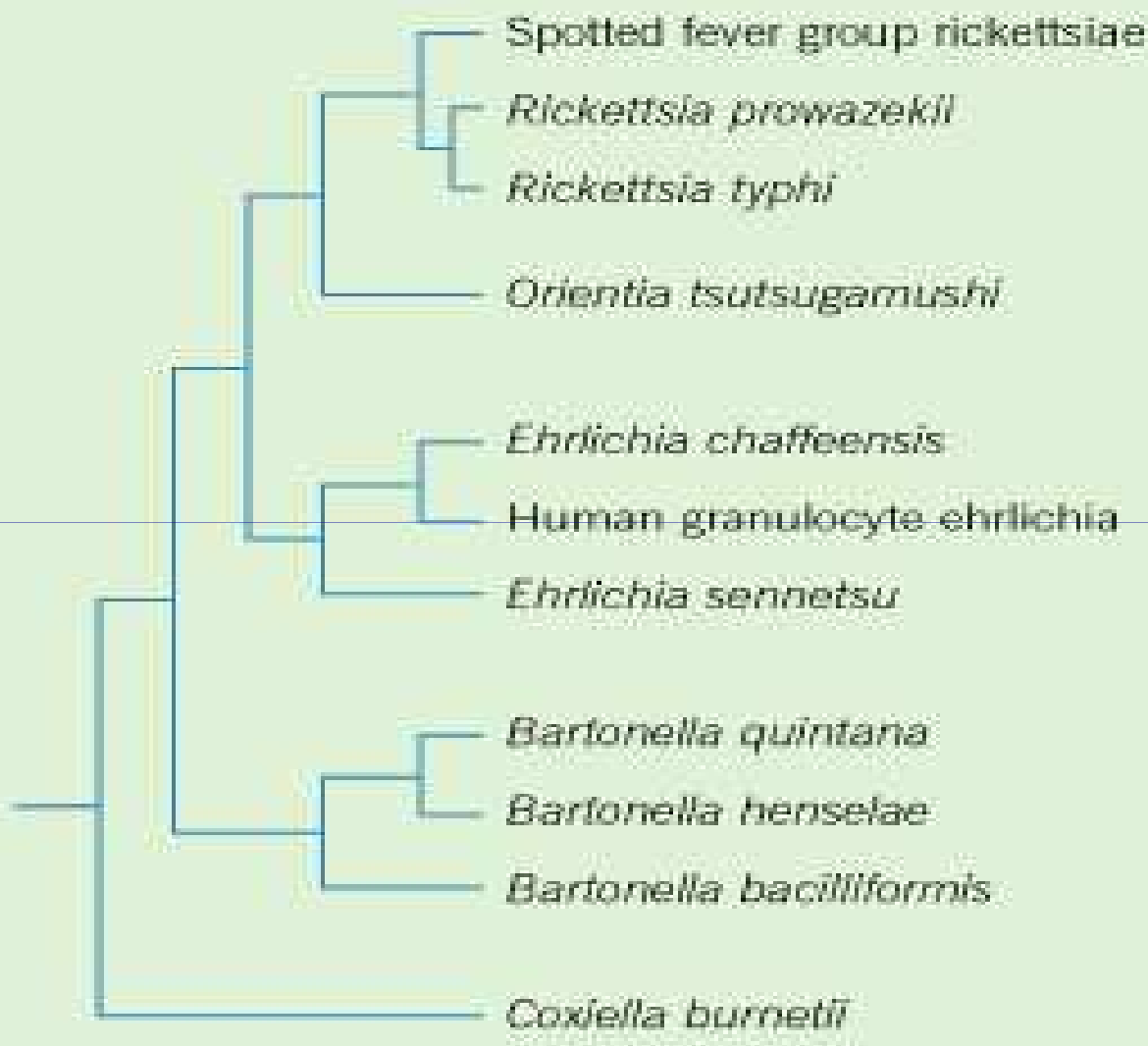
- Rickettsias as a group have a worldwide distribution
- Many new rickettsial diseases were discovered in recent years, and the list is growing
- Rickettsias are associated with variety of different vectors and hosts
- Most types of rickettsiosis are geographic area-specific

Rickettsia and *Rickettsia*-like Pathogens

Phylogenetic trees based on molecular taxonomic methods show three major groups of rickettsias:

- *Rickettsia, Ehrlichia, Anaplasma*
- *Bartonella*
- *Coxiella*

PHYLOGENETIC RELATIONSHIPS BETWEEN RICKETTSI BASED ON 16S rRNA GENE SEQUENCES



Rickettsial Diseases
Epidemiology

- Most rickettsial diseases have arthropod vectors.
- Humans are incidental hosts, except for epidemic typhus (humans principal reservoir).
- Ricketsial life cycles typically involve arthropod and mammalian reservoirs, and animal-to-human or vector-to-human transmission occurs as a result of environmental or occupational exposure.

Rickettsial Diseases
General Characteristics 1

- **Resemble bacteria/grow only in living cells (NOT viruses)**
- **Parasites of arthropods = lice, fleas, ticks, mites - infect humans via bite**
- **Similar in size and shape - nonmotile coccobacillary forms**
- **Most produce agglutinating antibody**

Rickettsial Diseases
General Characteristics 2

- Rickettsial diseases are clinically non-specific with many overlapping signs and symptoms
- Rickettsiosis in humans may be:
  Subclinical
  Mild, self-limited
  Severe, life-threatening
- Confirmation of rickettsiosis cases requires consideration of ***clinical***, and ***epidemiological***, and ***laboratory*** data

Rickettsial Diseases
Pathogenesis

- **Arthropod bite**
- **Invade endothelial cells/vascular**
- **Destroy endothelial cells**
- **Inflammatory cells accumulate/blood leakage:** rash
- **Released organisms reinfect**

Rickettsial Diseases

Pathogenesis

- The disease is caused by the bite of an arthropod (flea, tick, mite, louse).
- As the insect bites it defecates on the skin surface; the irritated area causes the patient to rub the area, forcing the fecal organisms into the bite wound.
- Here they multiply in cells that line blood vessels = endothelial cells; this eventually causes the leakage of blood into tissue which is displayed as a rash.

Rickettsial Infections
Common Features

- Fever, rash, headache, myalgias, and RT symptoms
- Local primary eschars (spotted fever group)
- Systemic capillary and small vessel endothelial damage
- Can become life threatening rapidly

Signs of Infection

- **Fever, chills**
- **Severe headache**
- **4th-6th day later = skin rash = lasts throughout course of disease**
- **EXCEPTION: Q-fever = no rash**

Genus *Rickettsia*

- Spotted fever group
- Typhus group
- Scrub typhus group
  R.tsutsugamushi has been reclassified into a new genus, ***Orientia***

Spotted Fever Group Rickettsias (SFG)

- **About 30 different rickettsias in the group; Worldwide distribution**
- **At least 9 different rickettsias in USA.**
- **At least 3 of them cause human disease:**
  R. rickettsii **cause of RMSF (tick vector)**
  R. felis **cause of "Flea typhus" (flea vector)**

  R. akari **cause of "Rickettsial pox" (house mouse mite vector); also associated with IV drug abuse**

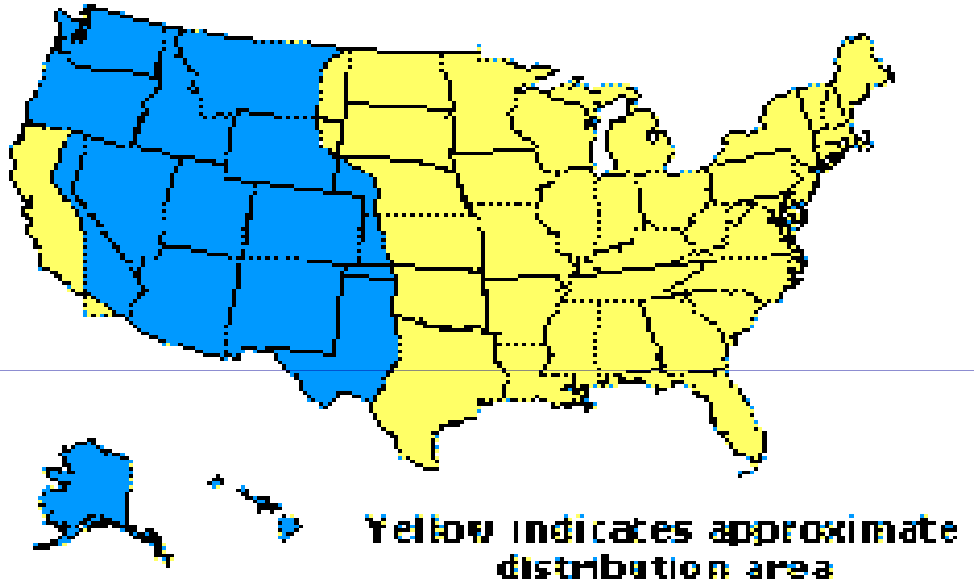
Spotted Fever Group

- *Rickettsia rickettsii* = spotted fever Rocky Mountain
  - Tick bite (*Ixodes spp*)

Rocky Mountains wood tick

Figure 7. Approximate distribution of the American dog tick

American dog tick (*Dermacentor variabilis*)



Rocky Mountain wood tick (*Dermacentor andersoni*) is found in the Rocky Mountain states and in southwestern Canada. The life cycle of this tick may require up to 2 to 3 years for completion. Adult ticks feed primarily on large mammals. Larvae and nymphs feed on small rodents.

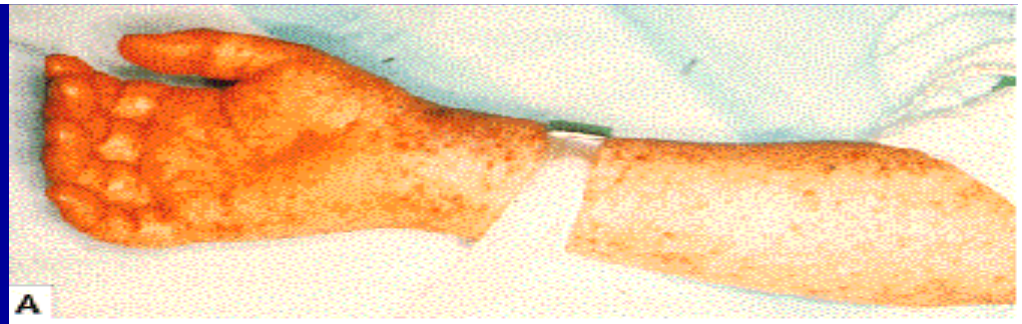
Compliments of the CDC

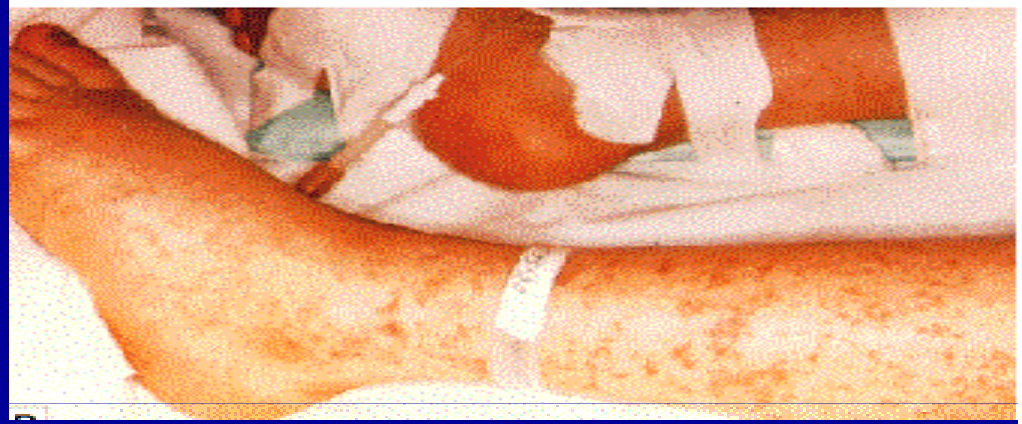
Rocky Mountain spotted fever

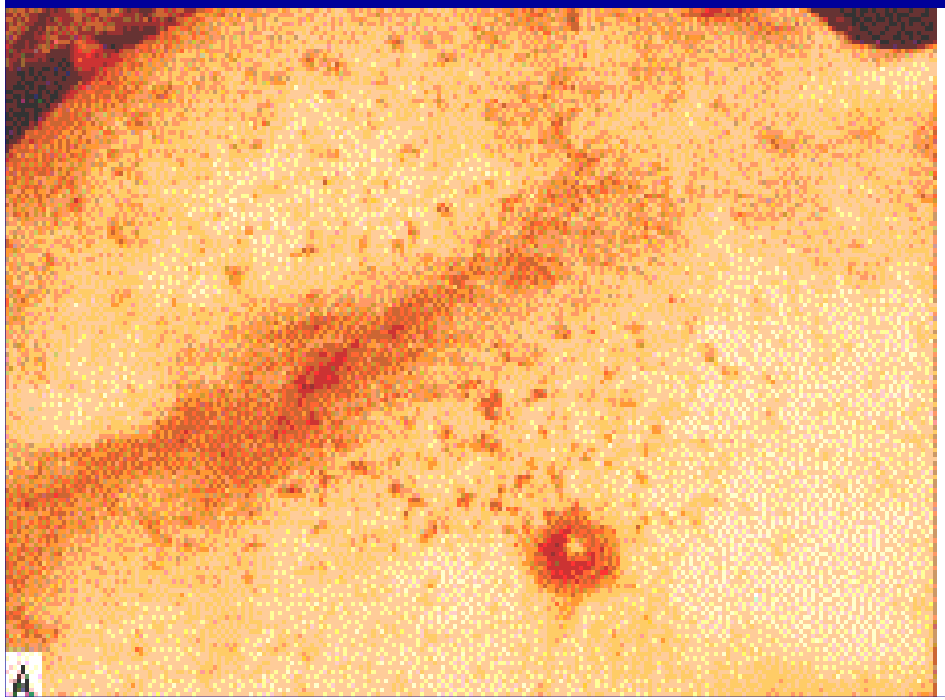
Clinical Manifestations

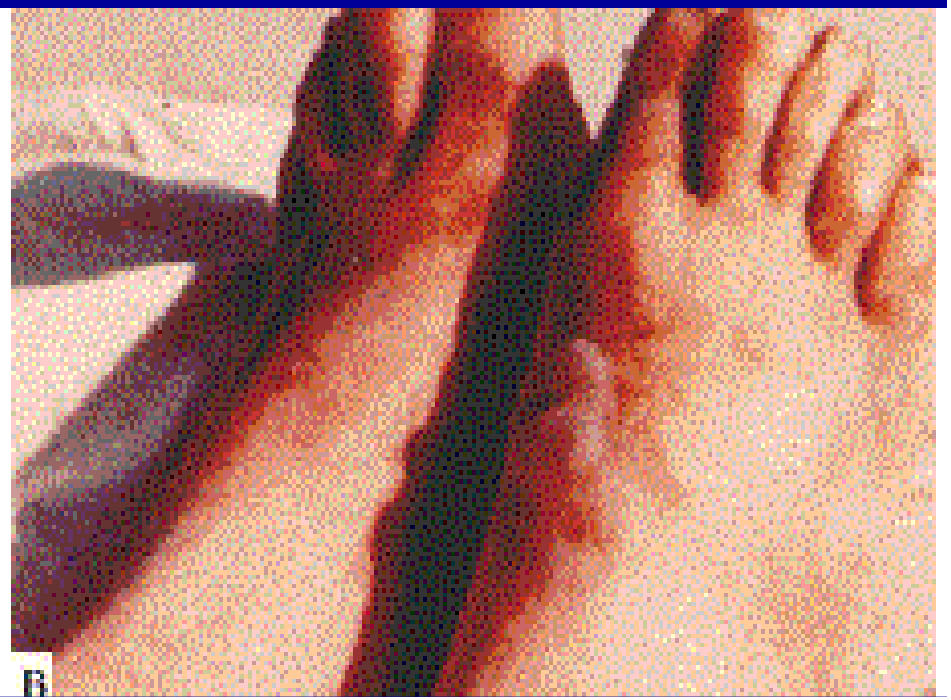
- **Fever, myalgia/severe headache**
- **Skin rash erythematous and macular and later macolopapular and often petechial= wrists and ankles to trunk/palms of hands, soles of feet**
- **Thrombocytopenia, leucopenia, anemia and hyponatremia**

Rash









Rocky Mountain spotted fever
Complications

Last as long as three weeks and can be severe with involvement of:

- Central nervous system
- Cardiac
- Pulmonary
- Gastrointestinal
- Renal tract

And develop DIC and shock leading to death

Other Rickettsial Spotted Fever Infections

- *Rickettsia africae* -(African tick bite fever)
- *Rickettsia conorii* -(Mediterranean spotted fever, Indian tick typhus, Marseilles fever,Isrealy tick typhus,and Astrachan spotted fever)
- *Rickettsia sibirica* -(Siberian tick typhus)
- *Rickettsia australis*- (North Queensland tick typhus)
- *Rickettsia japonica*-(Japanese spotted fever)
- *Rickettsia honey* -(Thai tick typhus)
- *Rickettsia slovaca* -(Tick born lymphadenopathy)
- *Rickettsia felis* -(Cat flea rickettsiosis)

Other Rickettsial Spotted Fever Infections

- Each of these diseases has some clinical and pathologic features similar to those of Rocky Mountain spotted fever
- These diseases are of importance among people traveling to or returning from areas these agents are endemic

Typhus Group
Epidemic typhus

- **Etiology**

–*Rickettsia prowazekii*

-body louse = bite/feces

(*Pediculus humanus sp corporis*)

Epidemic typhus (*Rickettsia prowazekii*)

Epidemiology

- Humans are the usual reservoir
- Transmission from person to person by the human **body louse**
- Infected louse feces are rubbed into broken skin or mucous membranes or inhaled
- Direct person-to-person spread of the disease does not occur in the absence of louse vector
- **Poverty, crowing, poor sanitary conditions, and poor personal hygiene contribute of spread of lice and hence the disease**
- Occurred throughout the world most common during winter

Epidemic typhus
Clinical manifestations

- **Abrupt onset of fever, chills, myalgias/severe headache and malaise**
- **Skin rash: macopurulent becomes petechial or hemorrhagic = trunk to extremities (face,palms and soles usually are not affected)**
- Changes in mental status are common and delirium or coma can occur

Typhus Group
Endemic typhus
(Fleaborne Typhus or Murine Typhus)

- **Etiology**

Rickettsia typhi

(R.mooseri)

Endemic typhus
(*Rickettsia typhi*)
Epidemiology

- Rats are the natural reservoirs
- Opossums and domestic cats and dogs can also be infected and served as hosts
- Vector for transmission a rat flea (usually *Xenopsylla cheopis*)
- Infected flea feces are rubbed into broken skin or mucous membranes or inhaled
- In Mediterranean and Developing Countries occur more frequently in summer and autumn

Endemic typhus
Clinical manifestations

- Resembles epidemic typhus but usually has less abrupt onset with less severe symptoms:
  - fever
  - persistent headache
  - myalgias
  - rash (macular or maculopapular and no hemorrhagic)
- Ilness seldom lasts longer than 2 weeks; visceral involment is uncommon, but untreated severe diseases can be fatal

Scrub Typhus Group

Scrub typhus

– ***Rickettsia tsutsugamushi***

- **mite bite**
- **fever/severe headache**
- **skin rash = covers body/eschar**

R.tsutsugamushi has been reclassified into a new genus, ***Orientia***

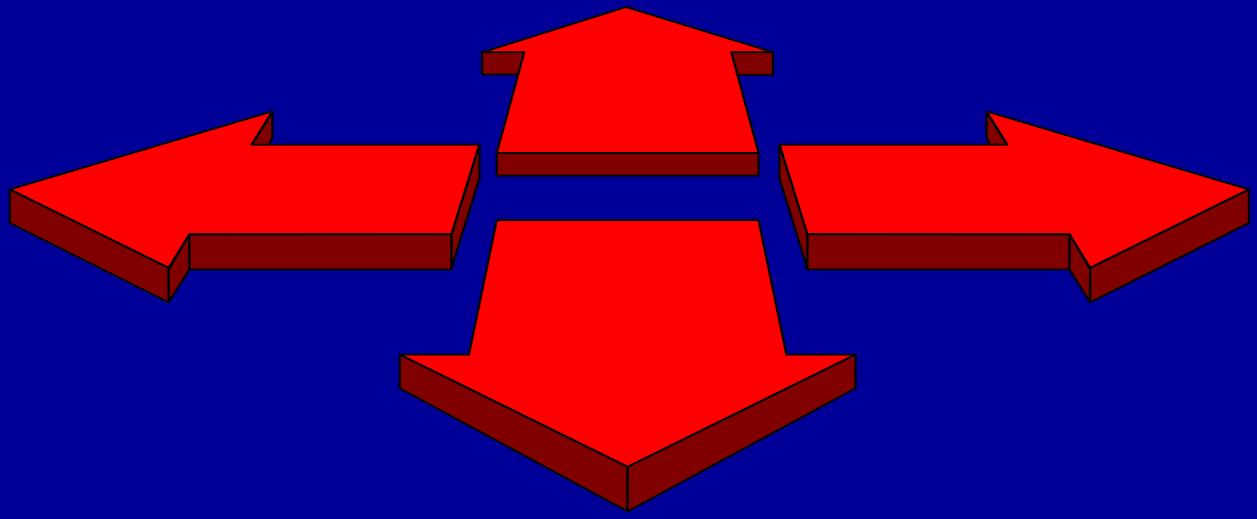
Scrub Typhus Group

Scrub typhus

- Out of mites, fleas, ticks and lice, mites are the only arthropods that burrow beneath the skin, laying eggs that hatch to form more “baby” mites.
- Note skin rash that covers the body; an eschar or black spot is found at the mite bite site.

Diagnosis of Rickettsial Diseases

- **Rise in serum antibody/often do not develop in early stages**



Spoted Fever Group (SFG) Laboratory Diagnosis

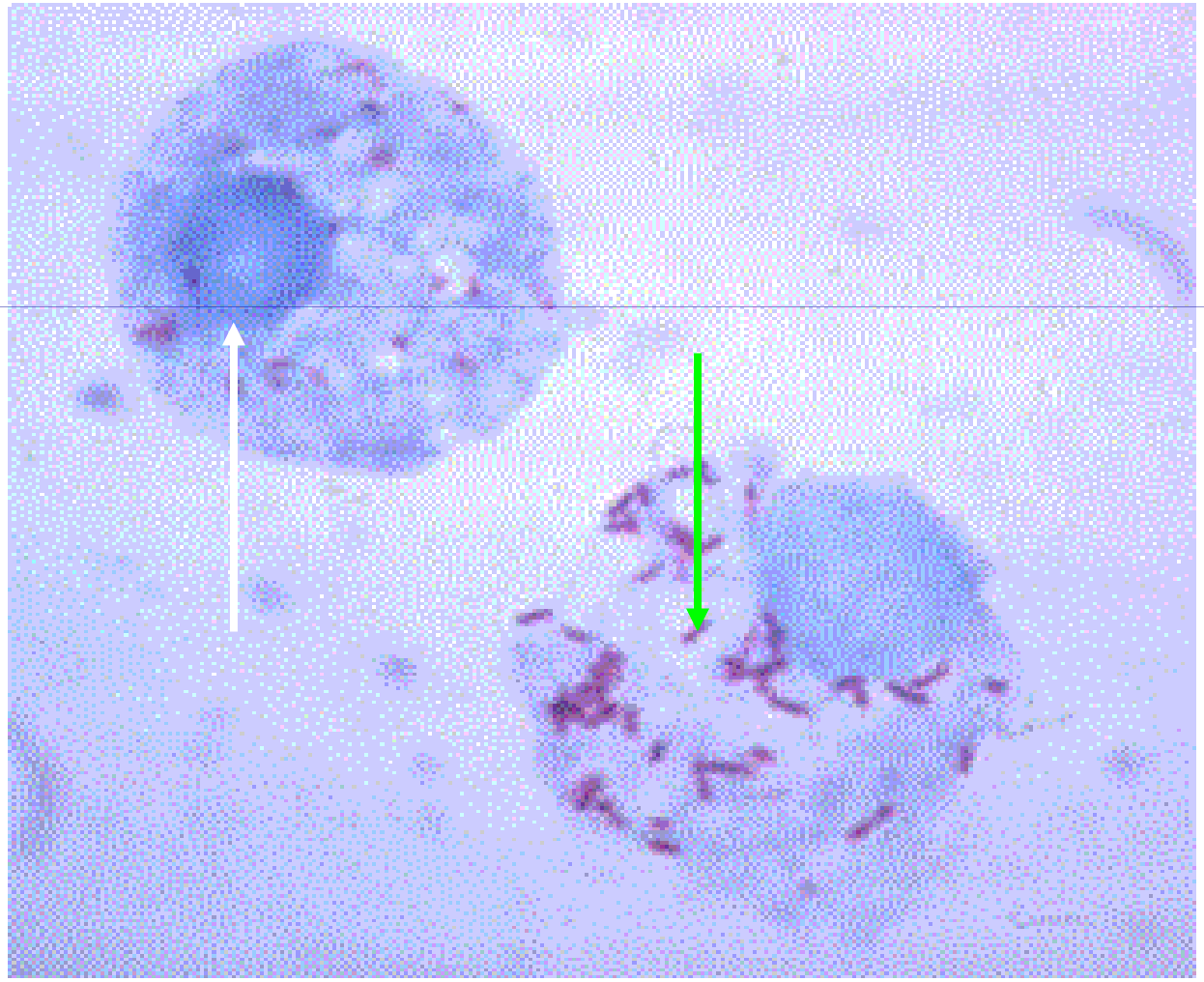
- Isolation
- Immunohistochemistry
- PCR
- Serology

Isolation of *Rickettsia*

- Requires specialized techniques
- Requires BSL-3 Lab
- Limited to research labs, not used for routine diagnosis
- Organism may not be detected for a week or more after inoculation

Compliments of the CDC

Figure 5. Gimenez stain of tick hemolymph cells infected with *R. rickettsii*



Immunohistochemistry

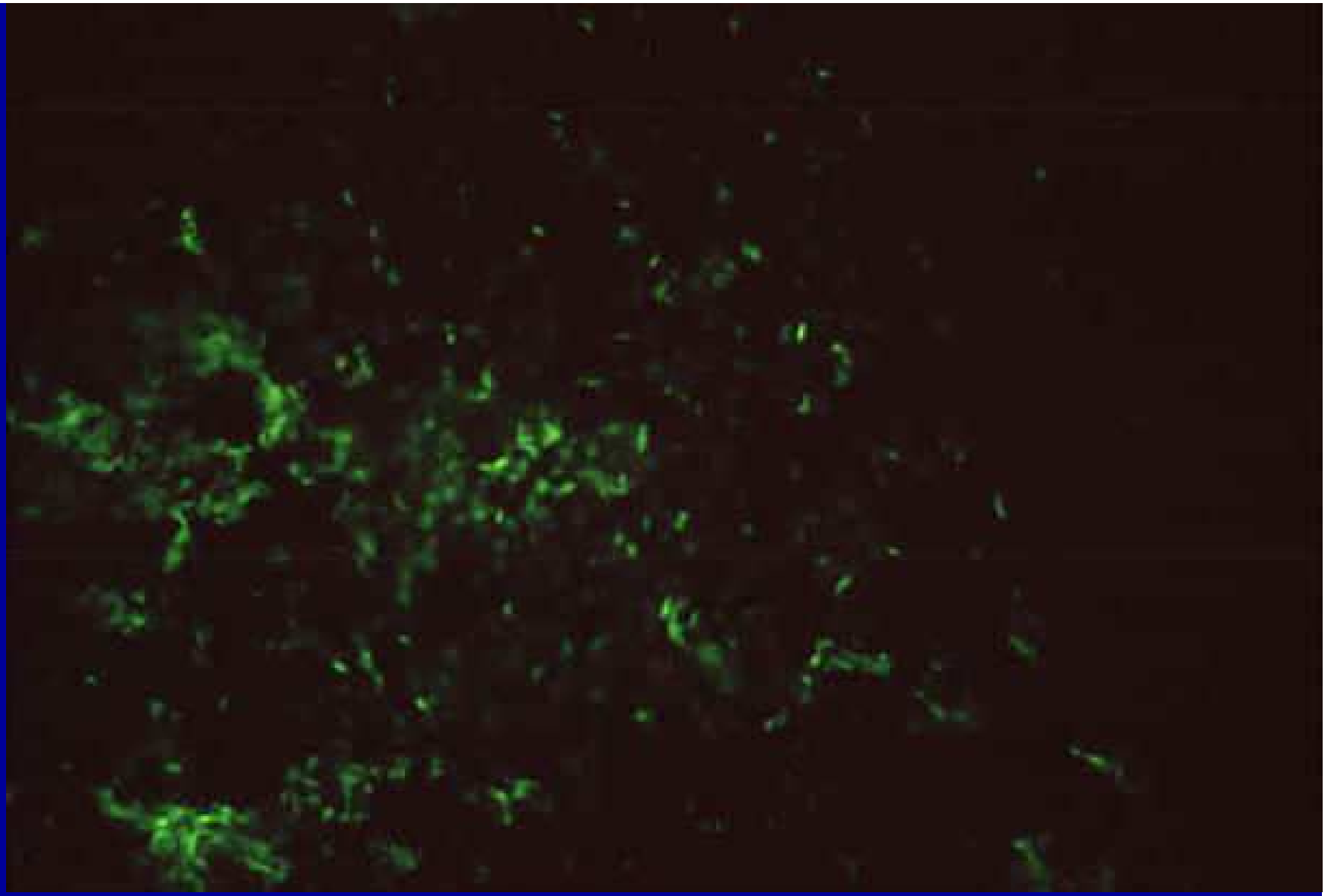
- This test has been used to diagnose SFG in the acute stage especially if eschar was formed
- Limited to research and reference labs
- Sensitivity probably about 70%
- This test could be applied to autopsy specimen and has been used to confirm unexplained cause of death
- Very useful in *R.akari* caused eschar which could be misdiagnosed as cutaneous anthrax

PCR

- PCR can be used to detect rickettsial DNA in whole blood and tissue specimen (preferred)
- Positive PCR in acute case could be considered confirmatory from diagnostic point of view
- PCR methods can be rickettsia-specific, but are usually confirmed by DNA sequencing of the amplified gene fragments and referencing with existing sequence databases
- Varying degrees of sensitivity depending on timing of sample collection, treatment with *Doxycycline*
- Not widely available

SFG Serology

- *Weil-Felix* test is a classic test, widely available, but unacceptable for accurate diagnosis
- IFA is the “gold standard”
- ELISA, latex agglutination, hemagglutination, Western blot could be used as well for specific diagnostic purposes
- Patients presenting early into the illness may have antibody level below detection threshold of any test, including Western blot



IFA reaction of a positive human serum on *R.rickettsii* grown in chicken yolk sacs, CDC, 2005

SFG Serology (cont'd)

- **IgM and IgG antibodies appear 3-10 days after disease onset, and peak after 3-4 weeks.**
  Initial negative test should not be used to exclude diagnosis. The 2-d specimen should be tested 1-3 weeks later to establish diagnosis
- **IgM and IgG could be still detectable after 1 and 4 years, respectively**
- **Treatment within 2 days of disease onset may abrogate antibody production**
  Following prompt treatment, IgG titers decline below detectable levels within 8-11 months

SFG Serology (cont'd)

False-positive

Cross-reaction with *Ehrlichia(little), Bartonella, Legionella, Proteus*, other rickettsias, also rheumatoid factor,*CMV, EBV*

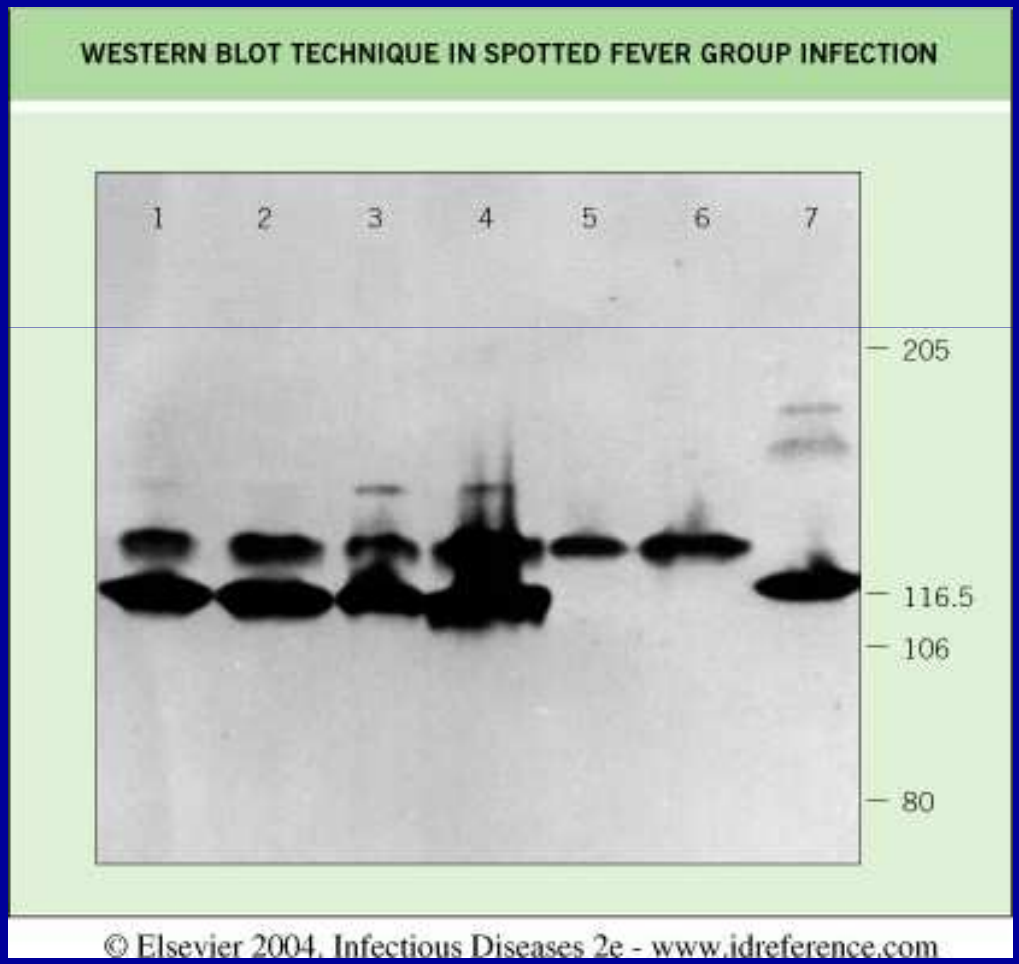
IgG antibodies are more specific and reliable than IgM antibody titers

Western blot assay is especially useful in differentiating true-positive from false-positive tests

False-negative

if tested early into the illness

Western blot of pooled mouse antisera to *R.africae*
– human isolate (lane 1),
R.africae – tick isolate (lanes 2–4),
R.conorii – Kenyan strain (lane 5),
R.conorii – Moroccan strain (lane 6)
and Israeli SFG rickettsia (lane 7).



Molecular masses (in thousands) are shown

Routine Laboratory Tests

- Neutropenia in the acute phase, leukocytosis in the later stages(not always); thrombocytopenia
- Hypoproteinemia, hypoalbuminemia, and decreased sodium, potassium, chloride during the first 10 days
- Elevated ALT, AST, and alkaline phosphatase
- CPK and LDH often elevated in acute infection

Typhus Group Rickettsias

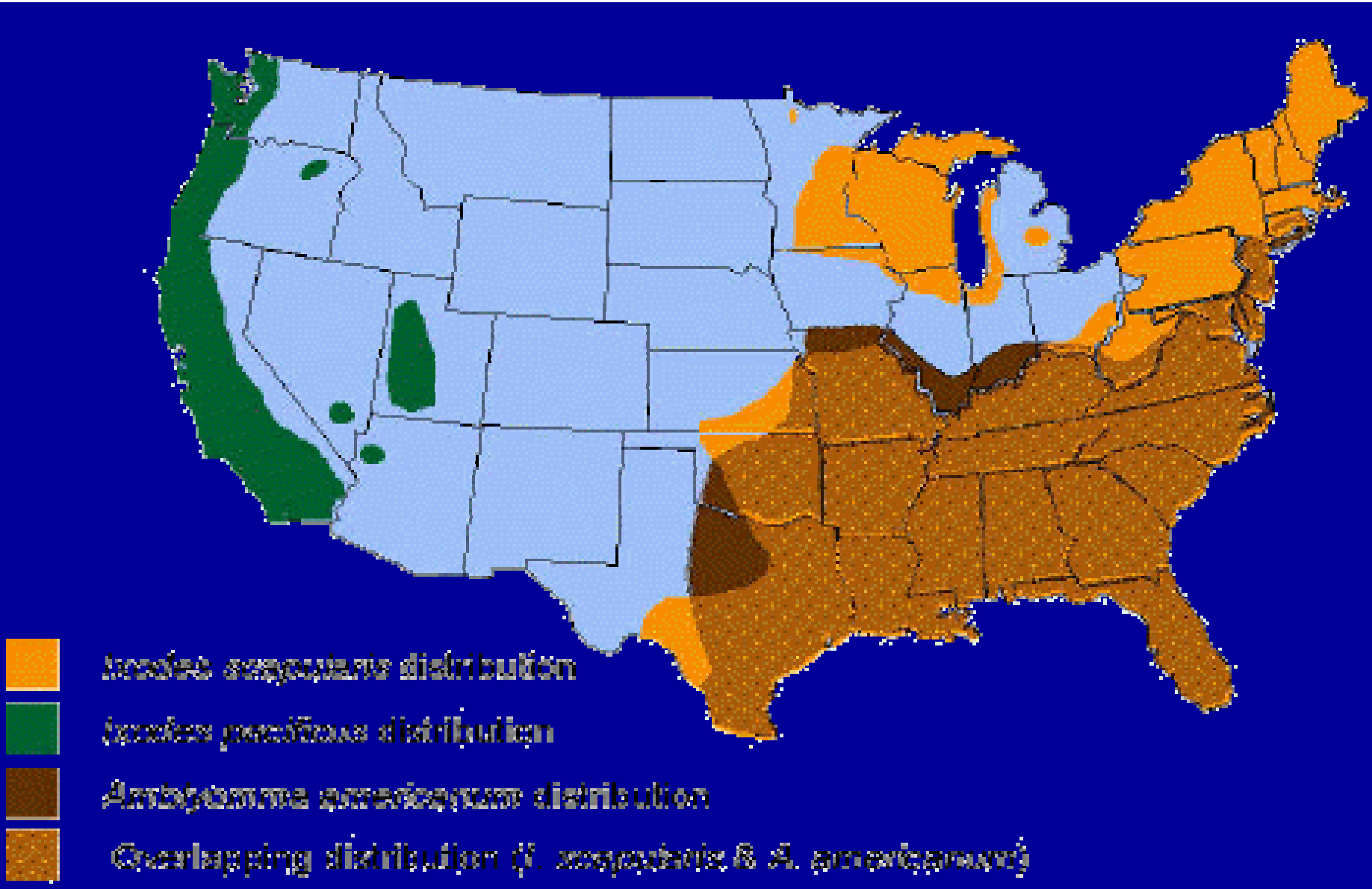
- ***R. typhi*** – agent of endemic or murine typhus (rat flea vector)
  R. prowazekii – agent of epidemic typhus (body lice vector)
- Considerable cross-reactivity between two; may also cross-react with SFG antigens
- Interpretation of serology similar to SFG
- Combination of IFA and Western blot can help differentiate epidemic and endemic typhus

Summary of Rickettsial Tests

- No widely available sensitive tests exist to make early diagnosis of rickettsiosis
- Most existing assays generally provide only retrospective confirmation
- Most Labs report IFA reactivity to the group-specific rickettsia
- Do not compare group-specific antibodies with agent-specific antibodies when available

Ehrlichiosis

- **Human *Ehrlichiae* encountered in USA**:
  1. ***E.chaffeensis*** – human monocytic ehrlichiosis (HME)
  2. ***E.phagocytophilia (Anaplasma)*** – human granulocytic ehrlichiosis (HGE)
  3.E.ewingii – HME-like illness in MO, OK, and TN
- Most cases of HME in SE and South-central USA
- Most cases of HGE in Upper Midwest and "Lyme-areas"
- Incidence of Ehrlichiosis likely 5-6 times that of RMSF
- **Significant numbers of human cases of Ehrlichiosis could be asymptomatic**



Areas where human ehrlichiosis may occur based on approximate distribution of vector tick species.
CDC, 2000

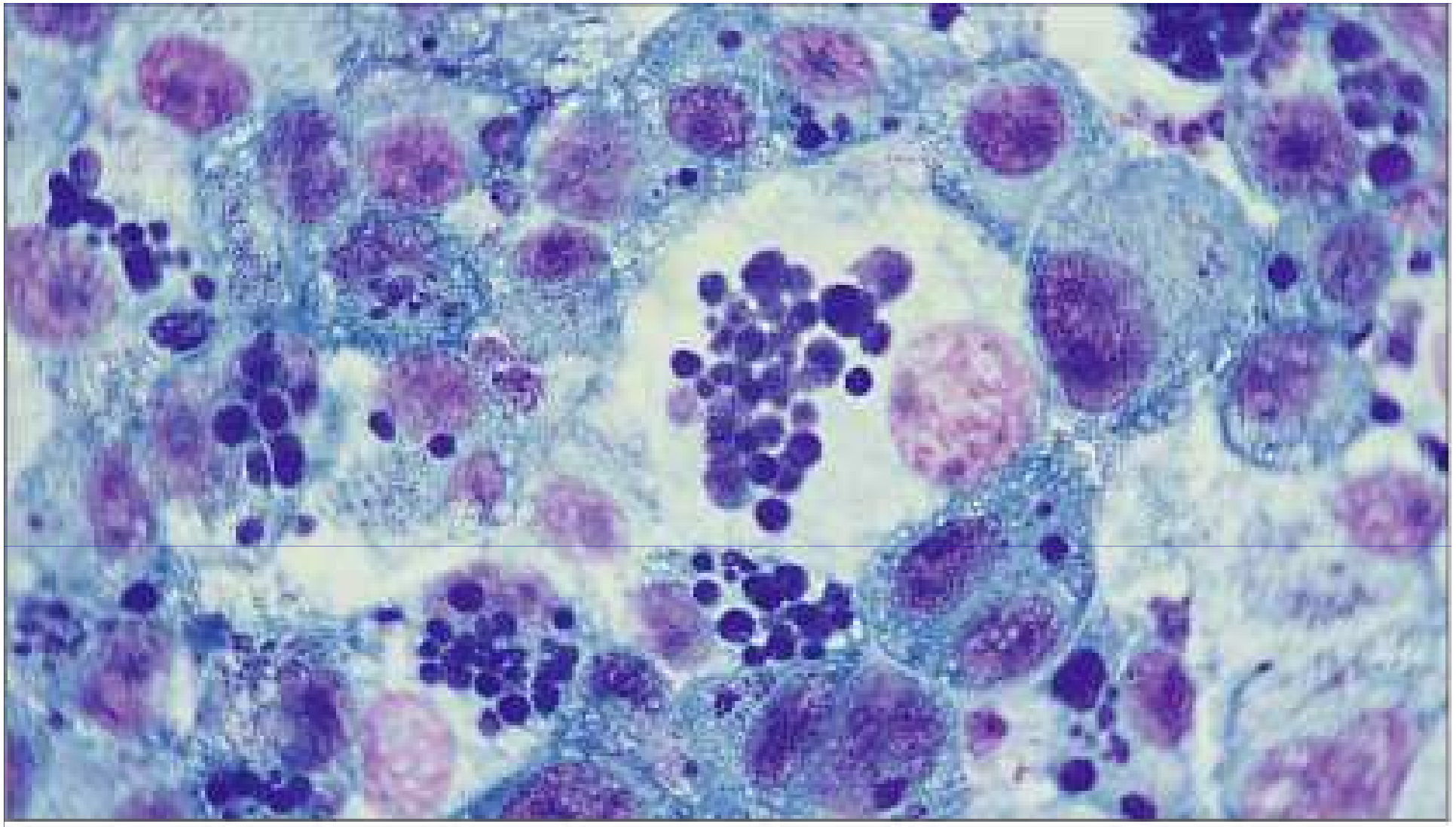
Ehrlichia Pathogenesis:

- Tick bite (deer or dog)
- Invasion of white cells: lymphocytes, neutrophils, monocytes
- 20% of patients show rash
- Lymph nodes up, high fever, headache, malaise, myalgia
- Treat with doxycycline

Ehrlichiosis
Laboratory Tests

- Culture-based
- Molecular (PCR)
- Serologic (IFA)

Routine tests indicative of possible ehrlichiosis include leukopenia, lymphopenia, thrombocytopenia, elevated liver enzymes. The organisms can be occasionally seen in blood smears by staining



Multiple morulas of *Ehrlichia canis* in culture DH82 cells

Ehrlichiosis
Polymerase Chain Reaction (PCR)

- PCR tests remain unstandardized; diagnostic sensitivity (50-80% acute phase) and specificity vary
- Positive results indicate presence of specific DNA from *E.chaffeensis*, *E.phagocytophilia, or E.ewingii;* species-specific PCR tests are available; further testing,e.g. sequencing, needed for final identification
- A negative result doesn't indicate absence of disease
- PCR detected organisms 3-5 weeks into the illness in HGE, and up to 7 weeks in HME

Ehrlichiosis
Serology

- Most commonly used; IFA is a principal tool
- There is no agreement on what constitutes a positive test among different laboratories; consult individual lab for their threshold levels
- In some PCR-confirmed cases IFA has been negative possibly due to insufficient sensitivity
- Seroprevalence of HGE ehrlichiosis in endemic areas is about 11-15%

Ehrlichiosis

Serology (cont'd)

- Most patients have increased IgM and IgG by the 2-d or 3-d week;
- Antibody levels decline significantly in 1 year, but in some cases may persist up to 2.5 years
- Antibodies can be cross-reactive (10-30% sera) among different *Ehrlichia* species. For example, *E.ewingii* antibodies cross-react with E.chaffeensis
- *E.ewingii* accounted for 7% of all ehrlichia-positive specimens at Washington University Lab

Trench fever (Bartonellosis)

- **Trench Fever Group –*Bartonella* transferred to *Rochalimaea quintana* -**
  - **body louse bite/feces**
  - **fever/headache/mild symptoms**
  - **skin rash - global**

Bartonellosis

- *B.quintana* and *B.henselae* are the most important pathogens in this group of Gram-negative rods
- *B.quintana-* agent of trench fever and endocarditis, *B.henselae-* agent of cat-scratch disease (CSD), peliosis hepatitis, and endocarditis; Both can cause bacillary angiomatosis;
- Both can be important pathogens in AIDS and transplant patients

Bartonellosis
Laboratory Diagnosis

- Primary isolation from blood is complicated and may require a month or more
- PCR used for the tissue specimen or blood; may differentiate *B.quintana* and *B.henselae.* Negative test indicates absence of detectable DNA, but does not rule out recent disease
- Serology using IFA and ELISA
  Little cross-reactivity with other rickettsias, but may cross-react with *Coxiella* and *Chlamydia spp*.

Q-Fever Group

- ***Coxiella burnetii*** **- Q fever**
  - **inhale contaminated aerosol; resist dessication = up to 3 years outside host**
  - **intermittent fever/pneumonia**
  - **<u>NO</u> skin rash**

Q Fever
Epidemiology

- Infections occur worldwide, except for New Zealand
- The causative agent, *Coxiella burnetii,* is a Gram-negative intracellular coccobacillus
- *C.burnetii* survives extreme environmental conditions for years; very low infectious dose; transmitted by inhalation of infectious aerosol
- Up to 60% of all cases are asymptomatic.
- Infection may also occur by ingestion of infected milk or meat. Most such infections result in seroconversion without disease symptoms.

Q-Fever
Acute Disease

Clinical Manifestations

- Abrupt onset of fever, chills, weakness, headache, anorexia and other non specific systematic symptoms.
- Weight loss weakness can be pronounce
- Cough and chest pain can accompany pneumonia (20%-50%)
- Hepatitis (40%-60%)
- The illness typically lasts 1-4 weeks
- Rare complications meningoencephalitis and myocarditis

Q-Fever
Chronic disease

- Occurs in approximately 1% of acutely ill

Clinical manifestations

Endocarditis

Hepatitis

Mortality

- Fatal if be untreated
- Endocarditis despite treatment :10%

Q Fever
Laboratory Diagnosis

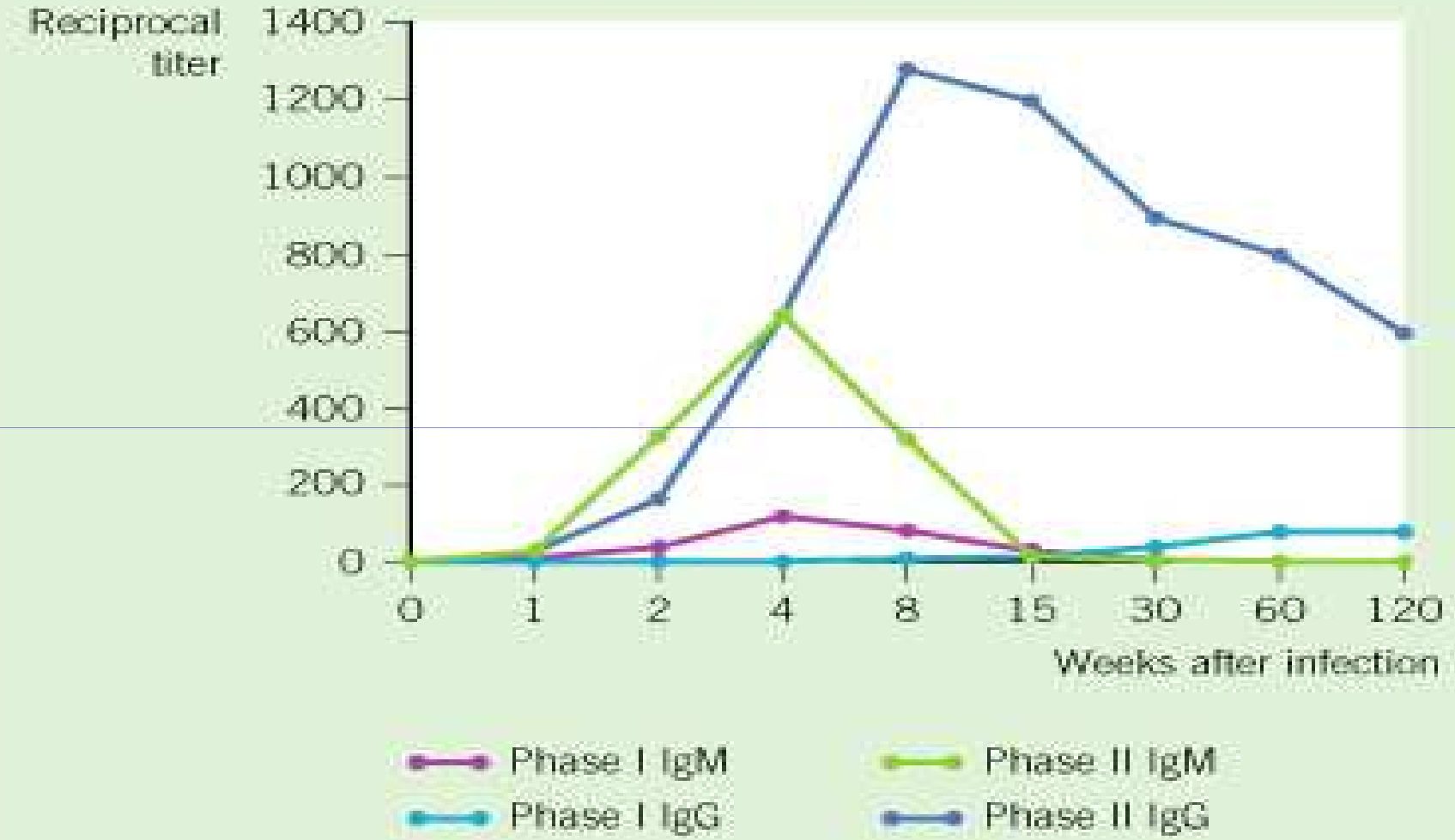
- Isolation from body fluids in acute disease; only specially equipped laboratory should do that
- PCR assay for different human specimens
- Serology using IFA, ELISA, complement fixation. During the course of infection, the outer membrane of the agent undergoes changes called phase variation. Differences in Phase I and Phase II antigen presentation help in distinguishing acute and chronic infection
- All specimens of suspected Q fever cases should be handled with extreme care!

Q Fever

Serology (cont'd)

- **IgM** antibody appear early, reaching maximal phase II titers by week 3 and declining by week 14; phase I IgM titers follow same pattern at much lower level
- In some cases IgM may persist for nearly 2 years
- **IgG** antibody is seen early, peak by week 8, and persist for longer than 1 year
- It is possible to see IgG titers to both phases simultaneously
- **IgA** antibody to phase I –» chronic infection likely

KINETICS OF ANTIBODY RESPONSES TO PHASE VARIANTS OF *COXIELLA BURNETII*



Conclusion

- Serological tests are most commonly used for the diagnosis of rickettsial diseases
- Serology may not allow early diagnosis
- Follow-up testing required for reliable diagnosis
- Good **epidemiologic history** is helpful for laboratory data interpretation
- **When in doubt, call testing Lab !**

Rickettsiosis

Treatment, Prevention, and Control

- **tetracycline/chloramphenicol or a fluoroquinolone**
- **vaccines under study/one=**

  Q fever
- **Control = insecticides/cleanliness**

Questions?