MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

DEPARTMENT OF VETERINARY PATHOLOGY

Chlamydia & Rickettsia

General Characteristics

- Obligate intracellular parasites
  - Dependent on a host for survival
- Can not be cultured on agar plates

HISTORY

- *'Chlamys'* is Greek for **"cloak draped around the shoulder"**
- **It was once thought to be a virus**
- **Isolation from**
  - **Embryonated eggs in 1957**
  - **Cell culture in 1963**
- **Energy parasite**

CLASSIFICATION

- Order - *Chlamydiales*
  - Family – *Chlamydiaceae* + 3 other families
    - Genus – *Chlamydia* and *Chlamydophila*
      - *Species – 9*
        - *C. trachomatis* (human)
        - *C. suis* (swine)
        - *C. muridarum* (mouse, hamster).
        - *C. psittaci* (avian)
        - *C. fells* (cats)
        - *C. abortus* (sheep, goats, cattle)
        - *C. caviae* (guinea pigs)
        - *C. pecorum* (sheep, cattle)
        - *C. pneumonia* (human)

CHARACTERISTICS

- **Obligate intracellular parasites**
- Tropism for squamous epithelial cells and macrophages
- Cell walls - similar to **G-B**, but lack muramic acid

- Deficient in independent energy metabolism
  - **energy parasites**

- Habitat
  - Intestine of healthy animals
  - Organisms are excreted in feces

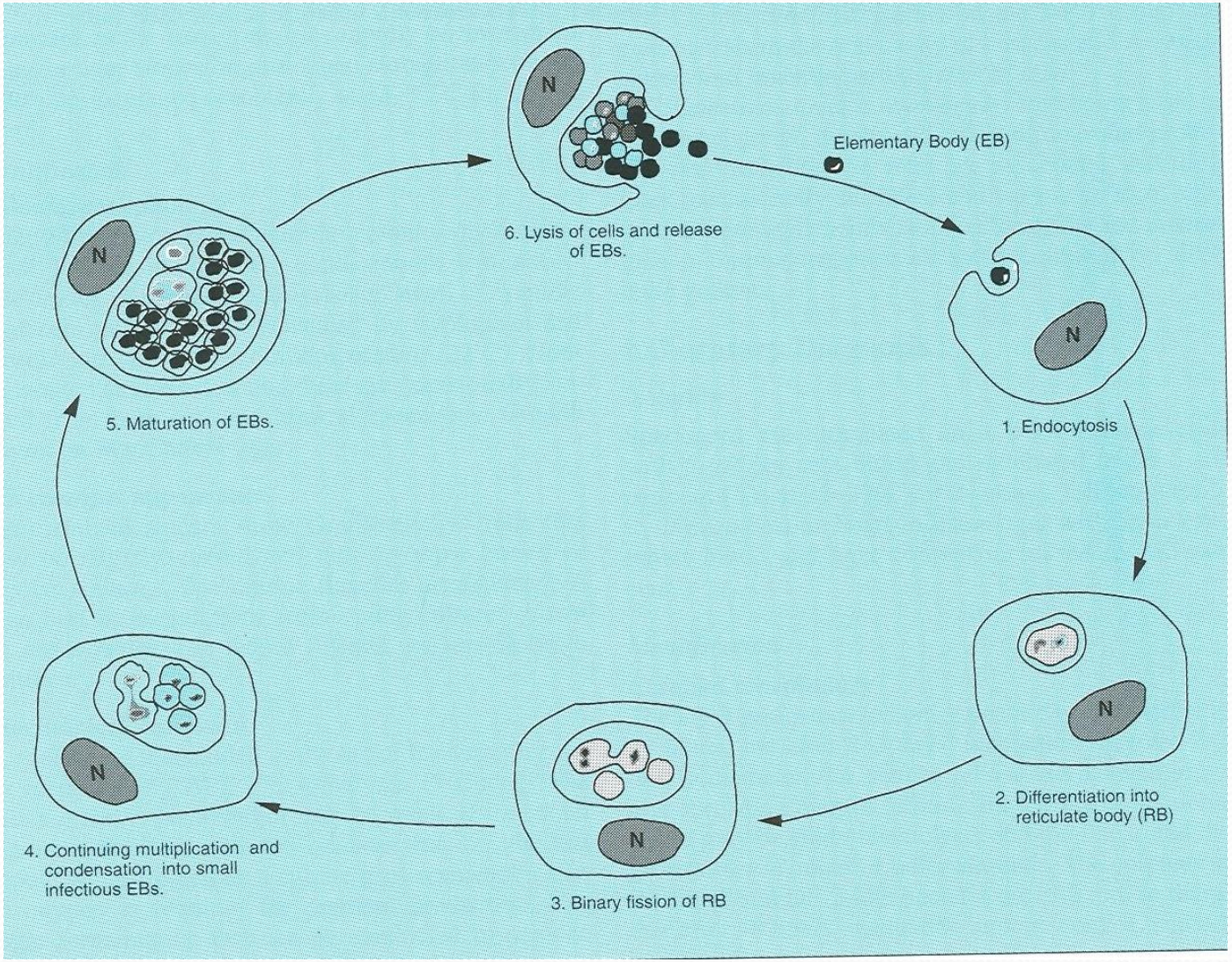
CHLAMYDIA – DEVELOPMENTAL CYCLE

- Unique/Unusual developmental cycle
  - Two forms
    - **Elementary body (EB)**
      - Small (200-300nm), Infectious, extracellular, metabolically inert, osmotically stable
    - **Reticulate body (RB)**
      - Large (1 µm dia.), intracellular, metabolically active, osmotically fragile,
      - **Reproductive form** - Replicates by binary fission

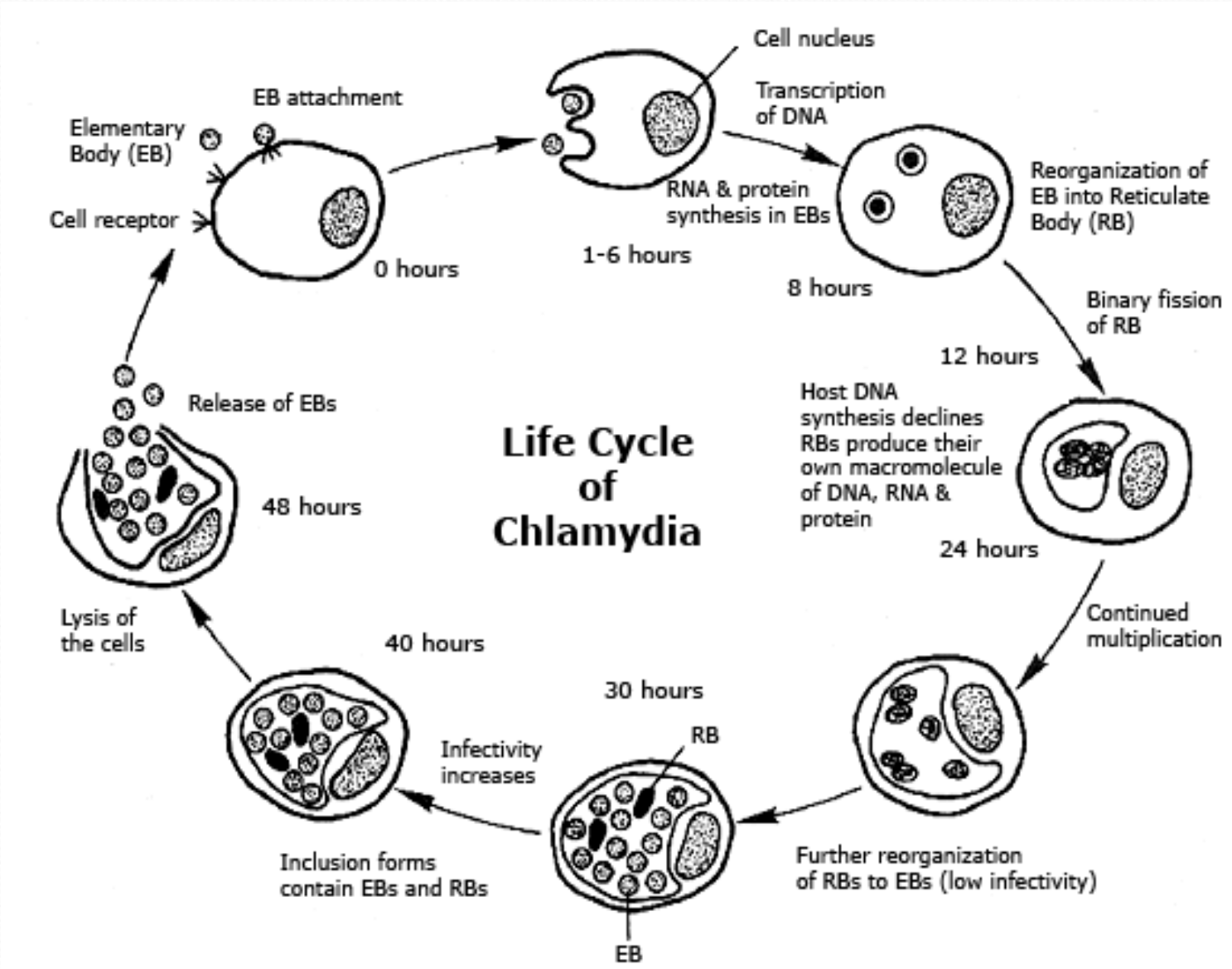
CHLAMYDIA – DEVELOPMENTAL CYCLE

Characteristic	Elementary Body	Reticulate Body
Size	0.2 - 0.3 microns	1 micron
Morphology	Electron dense core ,rigid	Fragile, pleomorphic
Infectivity to host	Infectious	Non-infectious
RNA : DNA ratio	1 : 1 (condensed DNA core)	3 : 1 (increased ribosome's)
Metabolic activity	Relatively inactive	Active, replicating stage
Trypsin digestion	Resists	Sensitive
Projections and rosettes	Few	More

CHLAMYDIA – DEVELOPMENTAL CYCLE



CHLAMYDIA – DEVELOPMENTAL CYCLE

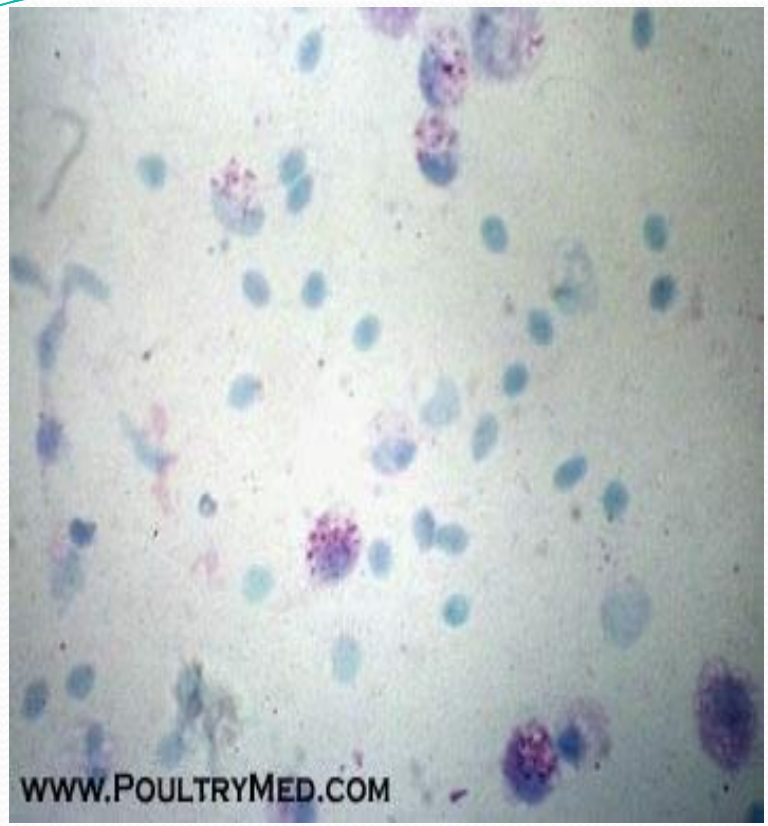


Laboratory Diagnosis

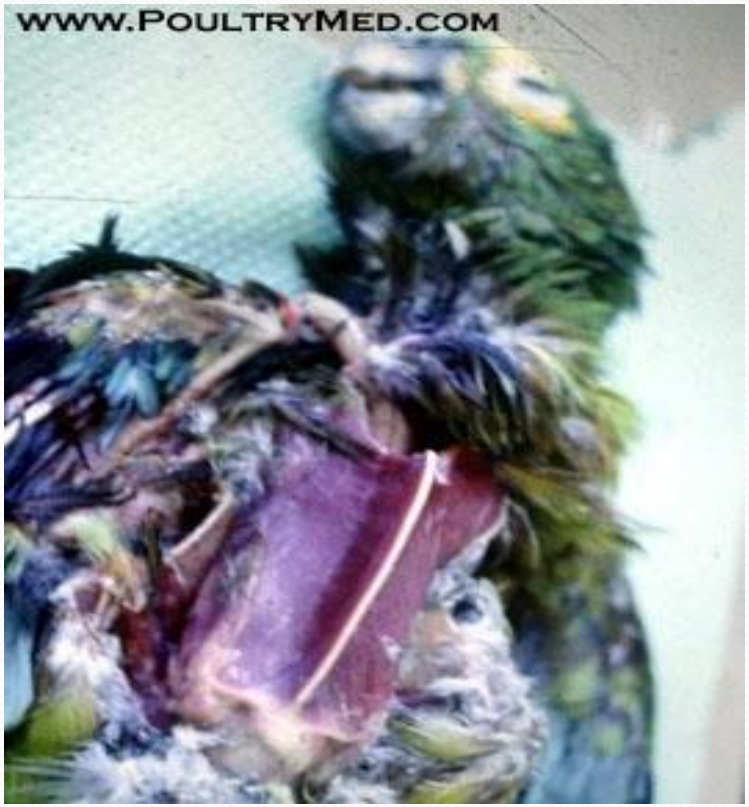
- History, Clinical signs & Pathological changes
- Specimen
  - TM – SPG (Sucrose-Phosphate-Glutamate) medium + FCS + AB +AF
- Thermolabile
  - Inactivated at 60^0C for 10 minutes
  - – 4^0C or Frozen at -70^0C
- Susceptible to penicillin, tetracycline & chloramphenicol
- Resistant to streptomycin
  - So streptomycin is added in suspected samples before cultivation of Chlamydia to destroy contaminating organism

Laboratory Diagnosis

- **Direct microscopic** examination to find EB's
  - Special staining – Modified ZN, Giemsa stain, Modified **Macchiavello's** and **Castaneda**
  - Immunofluorescent staining - fluorescein-conjugated Ab
- **Isolation** - cell
  - E. eggs – Yolk sac
  - CC – McCoy, L929, BHK & Vero
  - Lab Animals – Mice & G. Pigs
- **Serologic tests** are method of choice
  - Exceptionally high or Four-fold rise in titer
  - ELISA, CFT, Indirect or micro-immunofluorescent
- **Molecular test**
  - PCR – rRNA operon or *ompA* gene



Giemsa stain: intracellular chlamydial inclusion (reddish-purple).



Chronic chlamydia - (cachexia)

Chlamydia in tissues

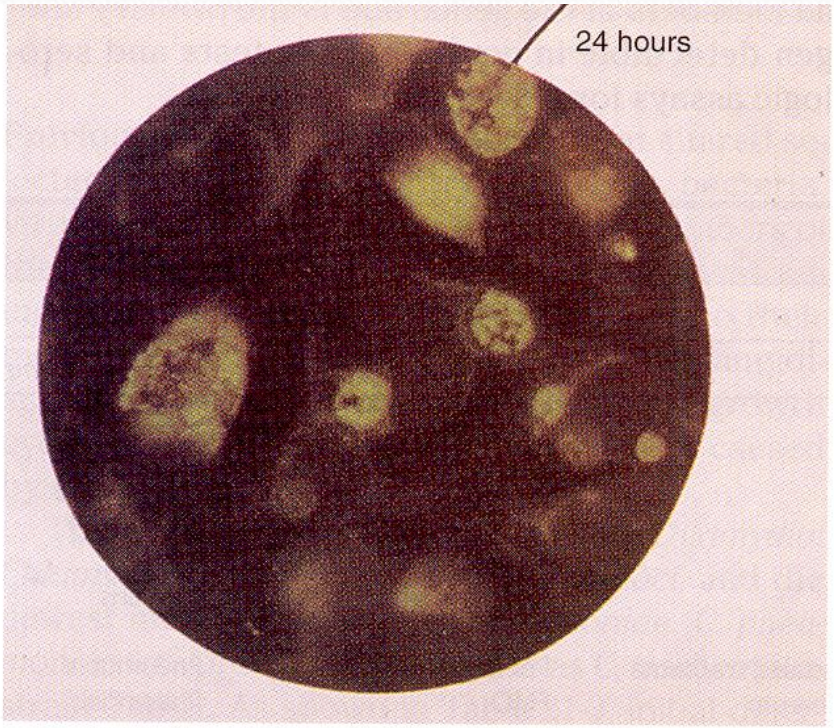


Figure 21-2

Chlamydia spp. growth cycle highlighting reticulate bodies (RBs), sometimes referred to as *initial bodies.* (Courtesy Syva-Microtrak, Palo Alto, Calif.)

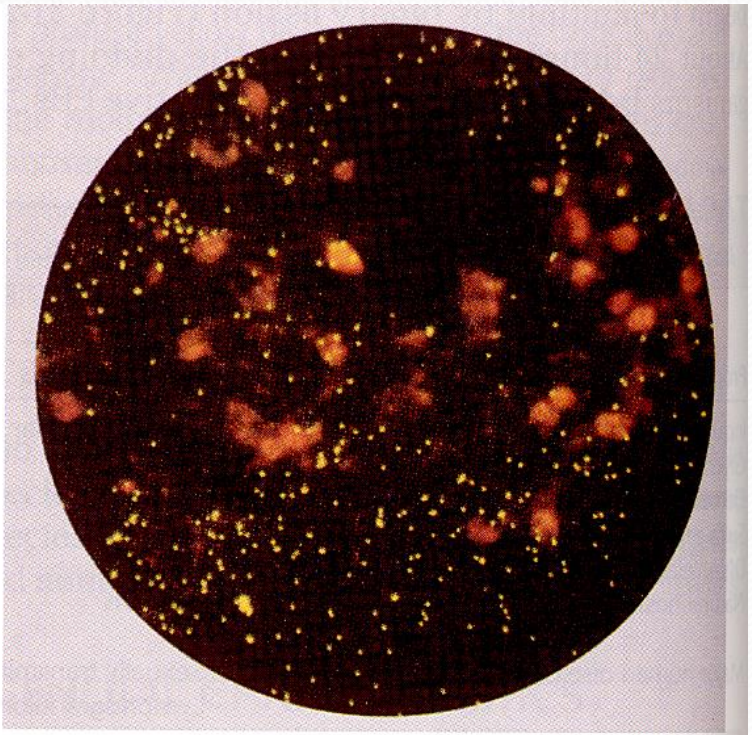
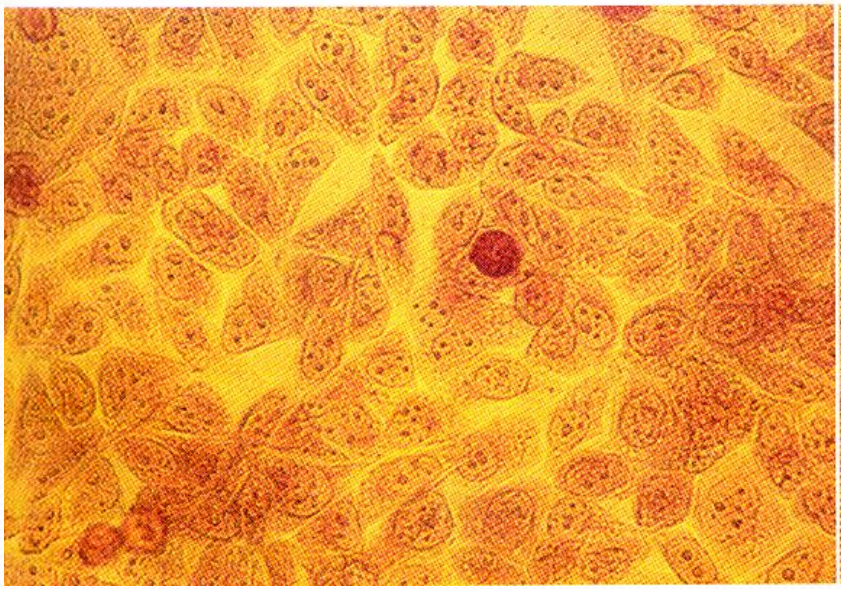
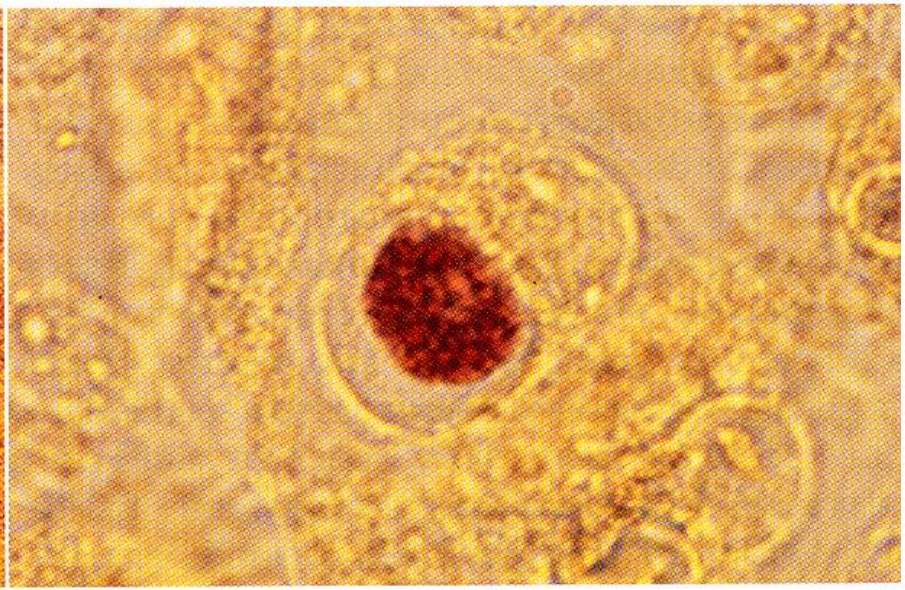


Figure 21-3

Elementary bodies (EBs) and cells in *Chlamydia trachomatis*–positive direct specimen. (Courtesy Syva Microtrak, Palo Alto, Calif.)

Chlamydia inclusion bodies





Diseases caused by *Chlamydia*

Species	Diseases caused	
C.psittaci	• **Ornithiosis/ Psittacosis /Parrot fever** • Pneumonia • Arthritis • Encephalomyelitis • Abortion, Conjuctivitis & enteritis	Birds Cattle, s/g, pigs, horses Calves & lambs Calves Cattle, sheep, goats
C. trachomatis	Endemic trachoma/ Lymphogranuloma venerium/ Inclusion conjunctivitis/ Sexually transmitted disease (STD)	Human
C. abortus	Enzootic abortion	Ewes
C. pneumoniae	Pneumonia	Human

Chlamydia psittaci

Avian Chlamydiosis/ Parrot fever/ Ornithosis/Psittacosis

- Psittacosis – Human, mammals and psittacine birds [e.g. parrots(parrot fever), parakeets, etc.]
- Ornithosis - affects birds other than psittacines
- is acquired by contact with an infected animal
- Infection can range from subclinical to fatal pneumonia
- Most commonly causes an atypical pneumonia with fever, chills, dry cough, headache, sore throat, nausea, and vomiting
- Identification based on history of close contact with birds and serologic evaluation

OVINE CHLAMYDIOSIS

Enzootic abortion of ewes

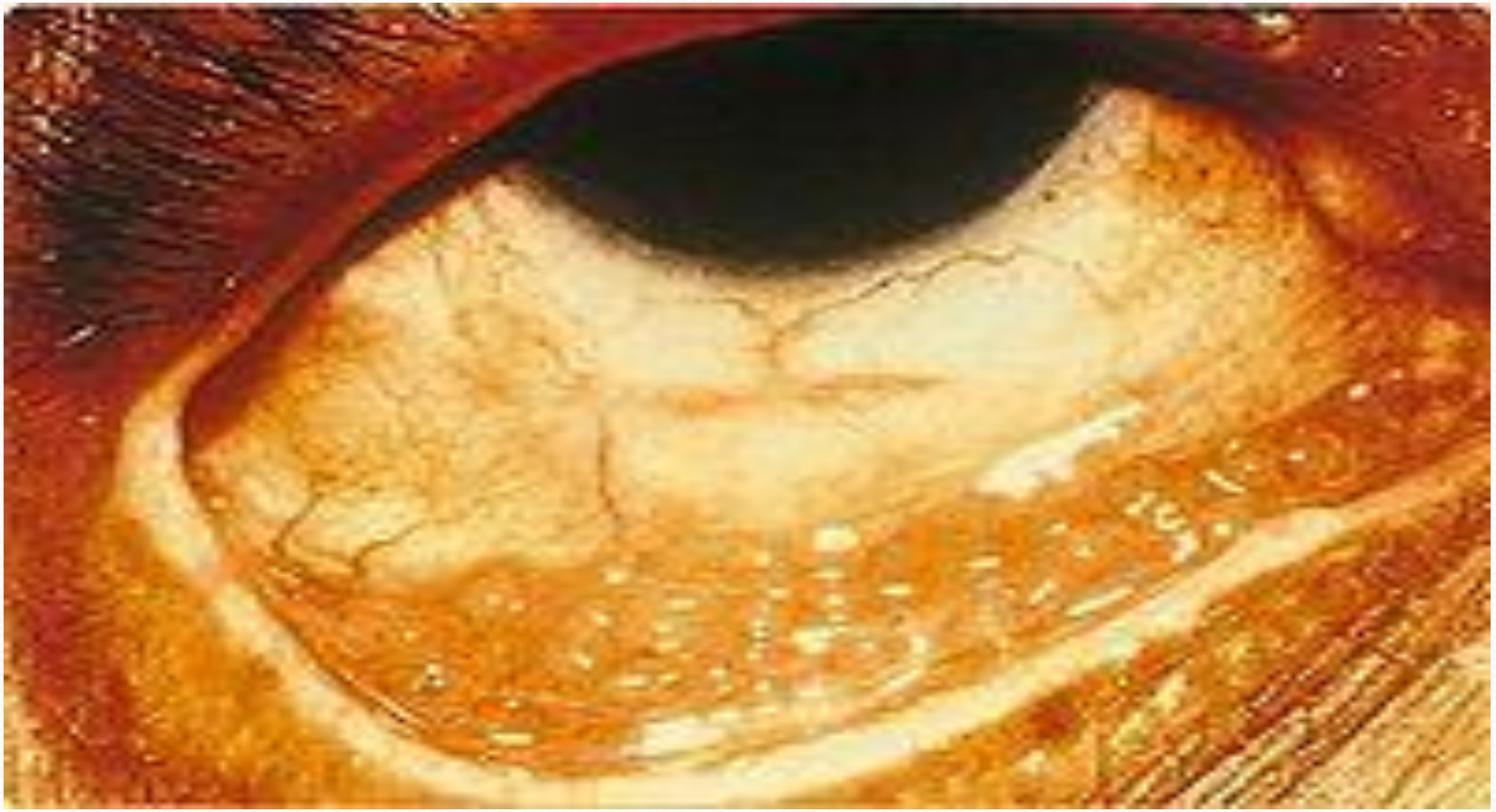
- **Chlamydial abortion of ewes**
- In sheep, abortion in late pregnancy with expulsion of necrotic fetal membranes are the important symptoms
- Invasion of placentomes produces a progressively diffuse inflammatory response, thrombotic vasculitis and tissue necrosis

Chlamydia trachomatis

- Most commonly sexually transmitted bacterial pathogen
  - Major cause of sterility
  - May be transmitted to newborns during delivery
    - Results in conjunctivitis
  - Trachoma – infection of the conjunctiva, resulting in scarring and blindness (Mostly in India and Egypt)
  - Lymphogranuloma venereum
    - Infects lymph nodes
    - STD found in immigrants from the tropics

Chlamydial cervicitis in a female patient characterized by mucopurulent cervical discharge erythema and inflammation

Male patients may develop a white, cloudy or watery discharge (shown) from the tip of the penis



Conjunctivitis due to chlamydia

Chlamydia pneumoniae

- Important respiratory pathogen (acute respiratory disease, pneumonia, and pharyngitis)
- Common (50% of adults have antibodies)
- Implicated in asthma
- Reinfection common
- Biphasic clinical picture
  - Prolonged sore throat and hoarseness, followed by flu-like lower respiratory symptoms
  - Pneumonia and bronchitis

Chlamydial vaccines

- There are no commercial chlamydial vaccines that produce long-lasting protective immunity against chlamydia.
- Live and killed vaccines (bacterin) have been found to be protective.
  - But live vaccines may result in carriers (birds carrying infection without showing symptoms)
  - whereas several injections of the killed vaccines are necessary to induce protection.
- There are no commercially available vaccines for the protection of poultry against chlamydiosis in India.

Rickettsia

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Assistant Professor
Department of Veterinary Microbiology

Classification

- Order - *Rickettsiales*
  1. ***Coxiella*** (1 species) – obligate intracellular parasite which grows preferentially in vacuoles of host cells
  2. ***Bartonella*** (3 species) – intracellular parasite which attacks theRBC
- Family - *Rickettsiaceae*
  1. ***Rickettsia*** (11 species)--obligate intracellular parasites which do not multiply within vacuoles and do not parasitize white blood cells.
- Family – *Anaplasmataceae*
  1. ***Ehrlichia*** (2 species) – obligate intracellular parasites which do not multiply within vacuoles but do parasitize white blood cells.
  2. ***Anaplasma***
  3. Aegyptianella
  4. Neorickettsia

Characteristics

- Extremely small/minute, non-motile and variable shape
- Posses a cell wall - gram negative organism
- Host cell dependence - Obligate intracellular parasites
  - multiply via binary fission only inside host cells
    - cultivated in living tissues
      - embryonated chicken eggs (yolk sac) or
      - vertebrate cell cultures
- Arthropod vector – replicate, survive and spread
- inhibited by antibiotics
- Poor affinity to basic dye
  - Romanowsky methods (Giemsa or Leishman)
- Endothelial cells

Rickettsia and Related Organisms

Disease	Agent	Arthropod Vector
Rocky Mountain spotted fever	*Rickettsia rickettsii*	Wood tick
Rickettsial pox	*Rickettsia akari*	House mouse mites
Murine typhus	*Rickettsia typhii*	Rat flea
Epidemic typhus	*Rickettsia prowazekii*	Human body louse
Scrub typhus	*Orientia tsutsugamushi*	Chigger mites
Ehrlichiosis	*Ehrlichia chaffeenis*	Lone star tick
Q fever	*Coxiella burnetti*	Not- spread by inhalation

Pathogenicity

- In **arthropod vectors** multiply in
  - epithelium of the intestinal tract - excreted in the feces
  - occasionally in salivary glands - transmitted through a bite
- In **mammalian host** (Incubation period: ~1 week)
  - During the first few days of incubation period
    - local reaction caused by hypersensitivity to tick or vector products
  - Bacteria multiply at the site & later disseminate via lymphatic system
  - Bacteria is phagocytosed by macrophages (1st barrier to rickettsial multiplication)
  - After 7-10 days
    - organisms disseminate
    - replicate in nucleus/cytoplasm of endothelial cells → vasculitis

- Infected cells show intracytoplasmic inclusions & intranuclear inclusions
- Endothelial damage & vasculitis progress causing
  - development of maculopapular skin rashes
  - perivascular tissue necrosis
  - thrombosis & ischemia
- Disseminated endothelial lesion lead to increased capillary permeability, edema, hemorrhage & hypotensive shock
- Endothelial damage can lead to activation of clotting system ---> Disseminated intravascular coagulation (DIC)

Rickettsia: cell-to-cell spread

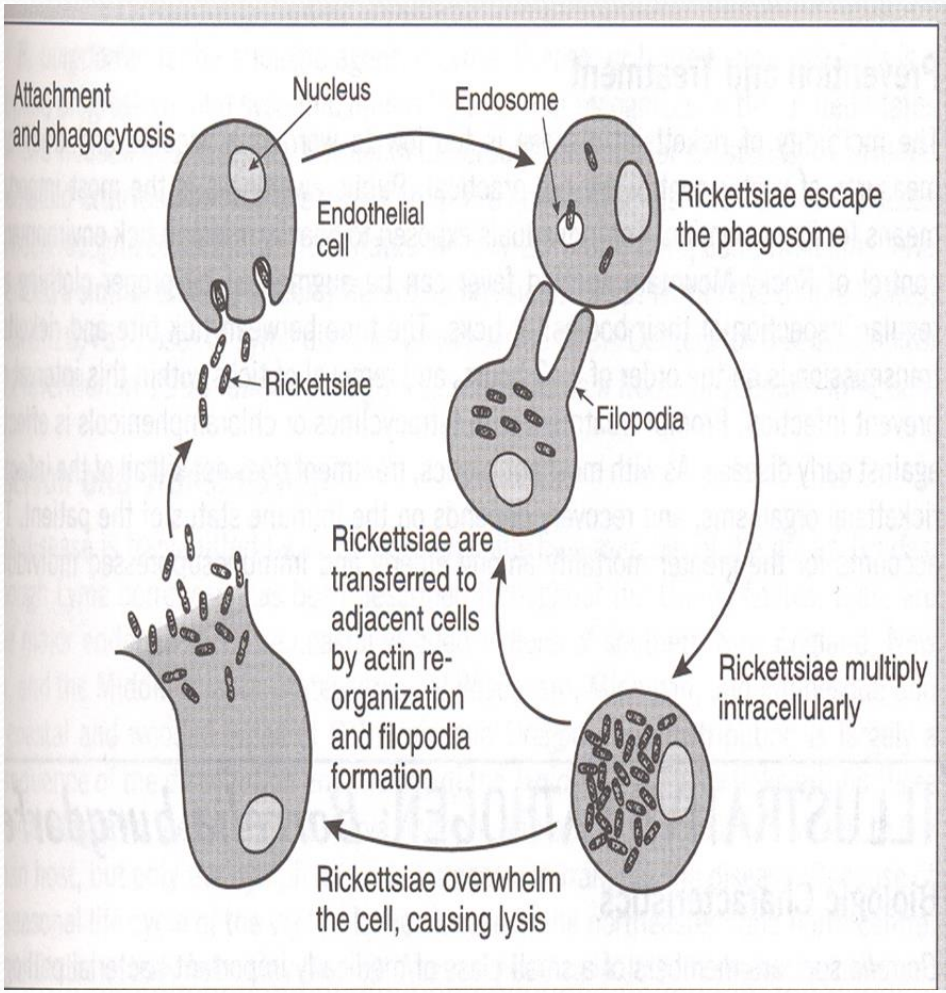
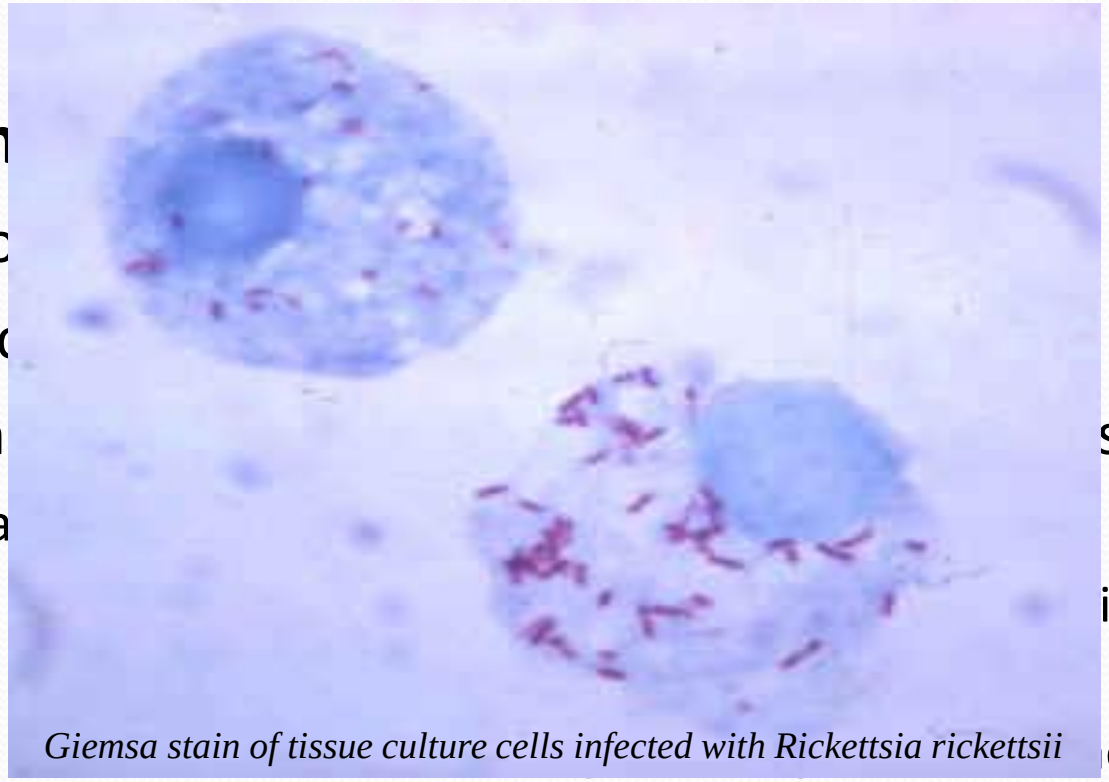


FIGURE 8-3
Cell-to-Cell Spread by Rickettsia rickettsii

Diagnosis

- **Presun**
  - Histo
  - Clini
  - Dem sue or blood
    - gra
      1. inst the blue
      2. ckground
      3. Giemsa stain--bluish purple organisms
    - Immunohistochemical detection



Giemsa stain of tissue culture cells infected with Rickettsia rickettsii

- **Confirmative diagnosis**
  - **Serological tests**
    - Weil-Felix reaction
      - Ab of *Rickettsial strain* react with Ag of OX strain of *Proteus* *(R. prowazekii X P vulgaris OX19)*
    - complement fixation test
    - Indirectfluorescent antibody test (IgM & IgG)
      - diagnostic test of choice for ehrlichiosis
  - **Culture & Isolation**
    - Will not grow on artificial media but can be grown in embryonated eggs or tissue culture and require growth co-factors
    - Cultivation is costly and hazardous (aerosol transmission)
  - **Molecular tests**
    - PCR
    - Real time PCR etc

Treatment

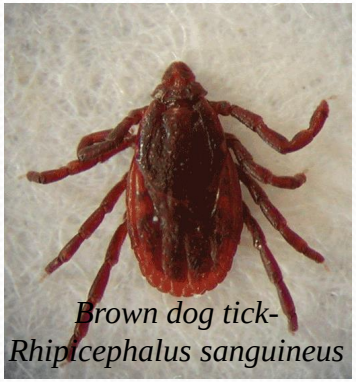
- The drugs of choice for the treatment of rickettsial diseases are
  - chloramphenicol and
  - Tetracycline
- Sulfonamides - contraindicated → stimulate rickettsial growth

Diseases

1. **Louse-borne:**
   - **European epidemic typhus (*Rickettsia prowazekii*)**
   - Brill's disease (*Rickettsia prowazekii*)
   - Trench fever (*Bartonella quintana*)
2. **Flea-borne**
   - **Endemic murine typhus (*Rickettsia typhi*)**
   - Cat scratch fever /Bacilliary angiomatosis/ (*Bartonella henselae*)
3. **Mite-borne**
   - Scrub typhus (Orientia /*Rickettsia tsutsugamushi*)
   - Rickettsialpox (*Rickettsia akari*)

4. **Tick-borne**
   - Rocky Mountain Spotted Fever (*Rickettsia rickettsii*),
   - North Asian tick typhus (*Rickettsia siberica*),
   - Fievre boutonneuse (*Rickettsia conorii*),
   - Queensland tick typhus (*Rickettsia australis*),
   - **Q-fever (*Coxiella burnetii*),**
   - Spotted fever (*Rickettsia rhipicephali*),
   - **Ehrlichiosis** (***Ehrlichia canis***, *Ehrlichia chaffeensis*)
5. **Fly-borne**
   - Oroyo fever / Verruga peruana (Bartonella bacilliformis)


Castor bean tick
Ixodes ricinus


Brown dog tick-
Rhipicephalus sanguineus

Rocky Mountain Spotted Fever

- Etiologic agent: *Rickettsia rickettsiae*

- Most common rickettsial disease
- It is common during summer months

- Individuals younger than 19 years old are usually at risk
- Males affected twice as often as females

- Transmission
  - By ticks - must remain attached for hours
- Incubation of 2-6 days
  - After 2-6 days, a maculopapular rash develops, first on the extremities, including palms, foot soles and spreading to the chest and abdomen
  - If left untreated, the rash will become petechial with hemorrhages in the skin and mucous membranes due to vascular damage as the organism invades the blood vessels
- Death may occur during the end of the second week due to kidney or heart failure
- Serious disease with 35% mortality rate

FIGURE 23.17 The rash caused by Rocky Mountain spotted fever. This rash is often mistaken for measles. People with dark skin have a higher mortality rate because the rash is often not recognized early enough for effective treatment.

Life cycle of the tick vector of Rocky Mountain Spotted Fever

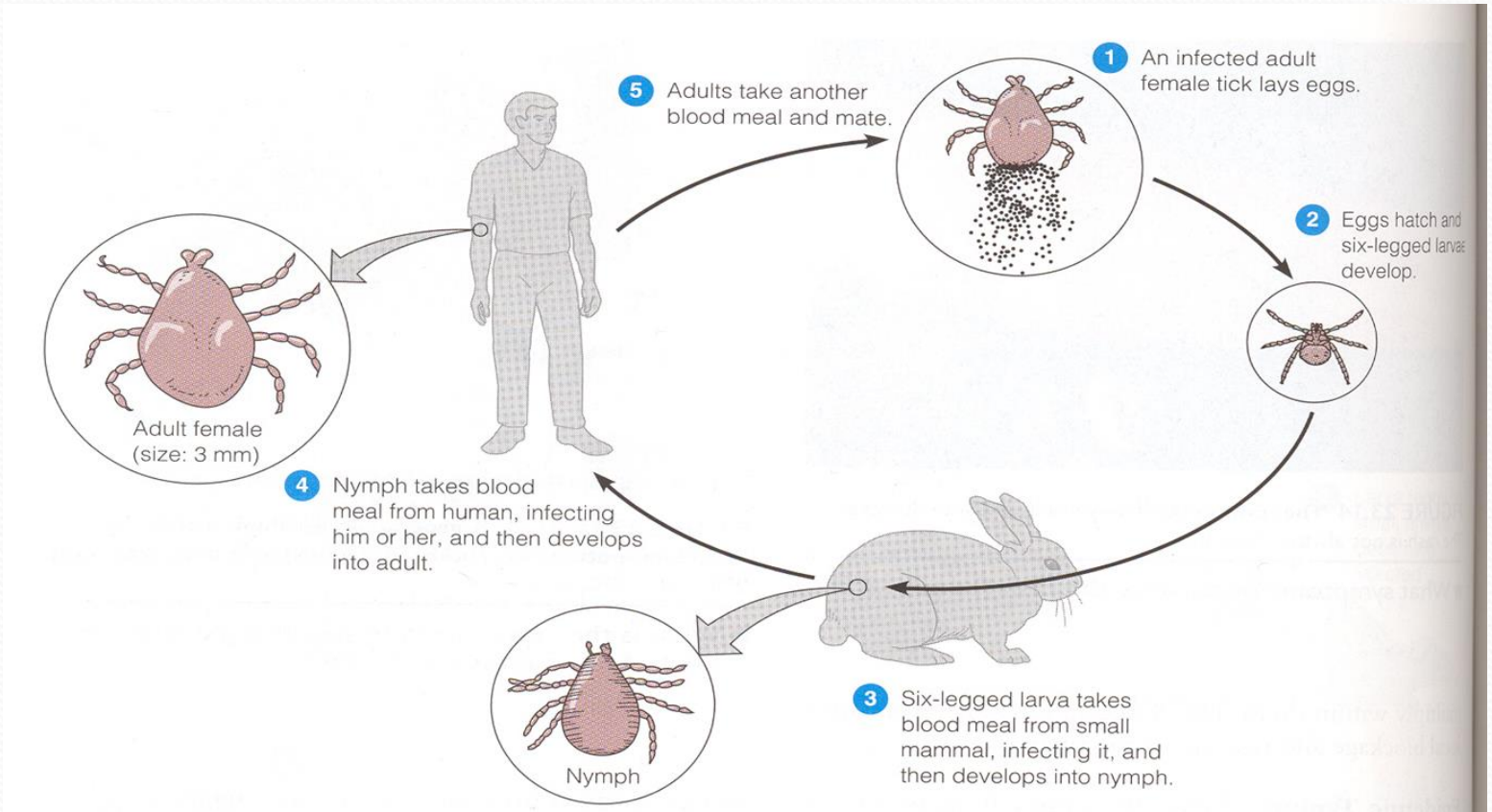


FIGURE 23.16 The life cycle of the tick vector (*Dermacentor* spp.) of Rocky Mountain spotted fever. Mammals are not essential to survival of the pathogen, *Rickettsia rickettsii*, in the tick population; the bacteria may be passed by transovarian passage, so new ticks are infected upon hatching. A blood meal is required for ticks to advance to the next stage in the life cycle.

Endemic Typhus

- Etiologic agent: *Rickettsia typhi*
- Incubation period: 5-18 days
- Transmitted to man by rat fleas
  - cat fleas and mouse fleas are less common modes of transmission
- The disease occurs sporadically

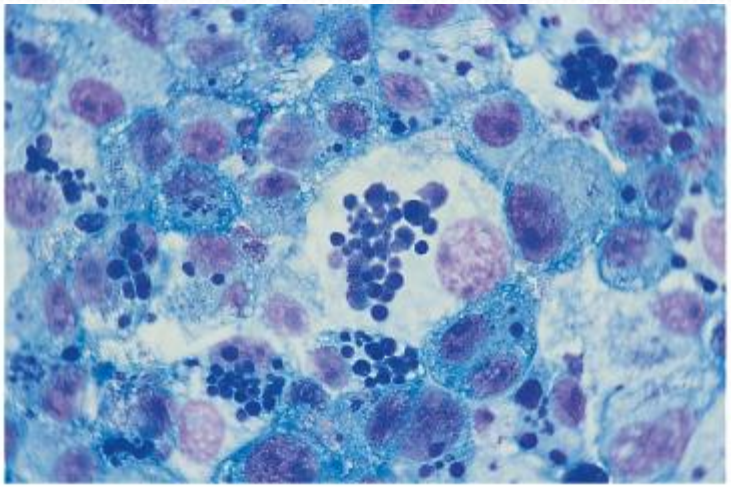
- Symptoms: severe headache, chills, fever, and after a fourth day, a maculopapular rash caused by subcutaneous hemorrhaging as Rickettsia invade the blood vessels

- The rash begins on the upper trunk and spread to involve the whole body except the face, palms of the hands and the soles of the feet

- The disease lasts about 2 weeks and the patient may have a prolonged convalescence

Ehrlichiosis

- Etiologic agent: *Ehrlichia canis, Ehrlichia chaffeensis*
- Transmitted via tick vectors
- Invade leukocytes and grow in cytoplasmic vacuoles making characteristic inclusions known as morulae



Multiple morulae of Ehrlichia canis in DH82 tissue culture cells

- Clinically manifests as acute fever with
  - leucopenia
  - thrombocytopenia
  - elevations of aminotransferase levels
- Symptoms resemble Rocky Mountain spotted fever

- Rash is infrequent
- Vasculitis is rare

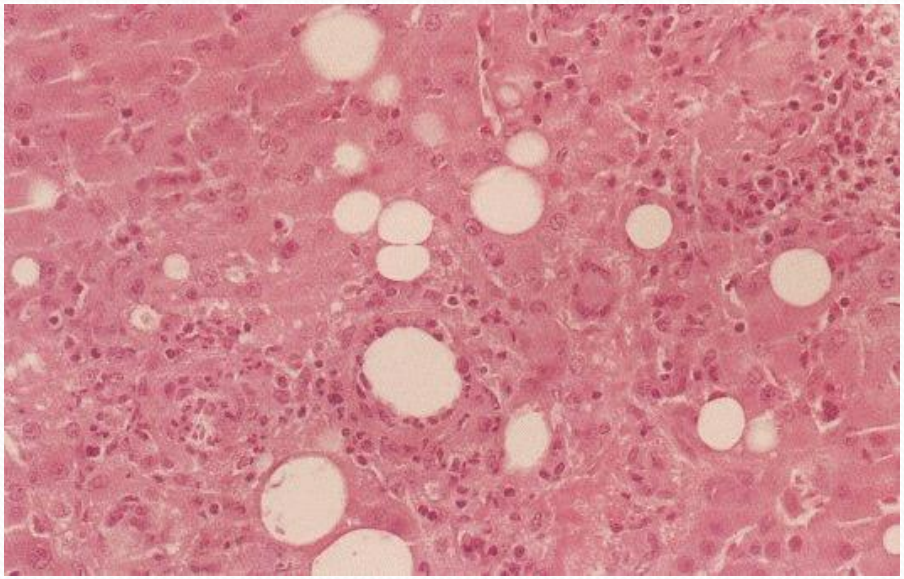
- Salmon poisoning disease
  - *Neorickettsia helminthoeca*
- Elokomin fluke fever
  - *Neorickettsia elokominica*
- Potomac Horse Fever
  - aka Equine monocytic Ehrlichiosis/ Equine Ehrlichial colitis
  - *Neorickettsia risticii*
- Bovine Petechial Fever/ Ondiri disease
  - *Ehrlichia ondiri*
- Heart water disease
  - *Ehrlichia ruminantium*
- Equine Ehrlichiosis
  - *Anaplasma phagocytophilum*
- Tick-borne fever
  - *Anaplasma phagocytophilum*

Q-fever

- **Causal agent**
  - The only species of Coxiella genus - *Coxiella burnetii*
  - Q for “query” or mysterious febrile illness
  - Obligate i/c parasites
  - small, pleomorphic coccobacillus (0.2 to 0.7 µm) → endospore-like form that resists heat and dryness allowing it to survive in extracellular environment
- **Transmission**
  - No arthropod vector for *C. burnetii* – for human
  - Localize & replicates in female genital tract & mammary gland
    - Intermittent or continuous shedding
  - Contact with the infected placenta, milk, urine, feces of infected animals
  - Inhalation of airborne organisms
    - infected dusts in farm and slaughterhouses
  - High risk - veterinarians, ranchers and animal researchers

- **Pathogenesis**
  - Incubation period: 10-28 days
  - *Coxiella* proliferate in the respiratory tract and then disseminate to other organs
  - Acute diseases:
    - characterized by fever, influenza-like syndromes; but no skin rash
    - Some patients present with bronchopneumonia with patchy interstitial infiltrates
  - Chronic disease:
    - hepatitis, endocarditis and meningoencephalitis

- **Diagnosis**
  - Direct microscopy – Smear → Placental or uterine discharge - MZN
  - Isolation & culture (not commonly performed – Lab infection)
    - Yolk sac route – 5-7days old EE
  - Serological tests (most commonly use)
  - Molecular tests – PCR
- Tetracycline or combination of rifampin and either doxycycline or trimethoprim-sulfamethoxazole



Doughnut shaped *non-caseating granuloma of Q fever*


Thank You.

Need to remember

- RB and EB
- Staining methods