RICKETTSIA & CHLAMYDIA

General characteristics

- ➢ Structurally similar to gram (-) bacilli
  - ✓ DNA & RNA
  - ✓ Enzymes for Kreb's cycle
  - ✓ Ribosomes for protein synthesis
  - ✓ Inhibited by antibiotics · Tetracycline & Chloramphenicol

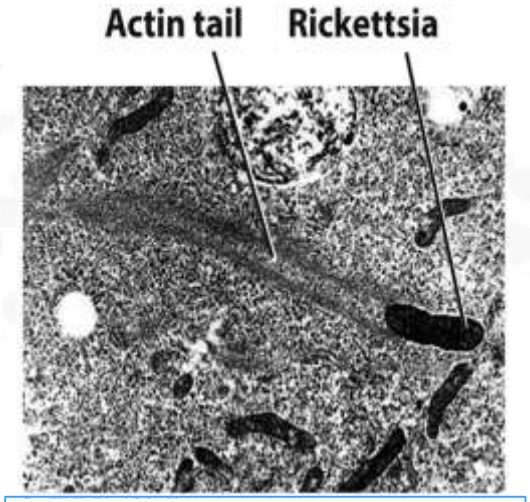
- ➢ Originally thought to be viruses
  - ✓ Small size
  - ✓ Stain poorly with gram stain
  - ✓ Grows only in cytoplasm of Eukaryotic cells
  - ✓ Obligate intracellular parasites EXCEPT Coxiella

- ➢ *Rickettsia* survival depends on entry, growth, and replication within the cytoplasm of eukaryotic host cells. That's why, they cannot live in artificial nutrient environments and is grown either in tissue or embryo cultures.

- ➢ Reservoirs – animals & arthropods

General characteristics

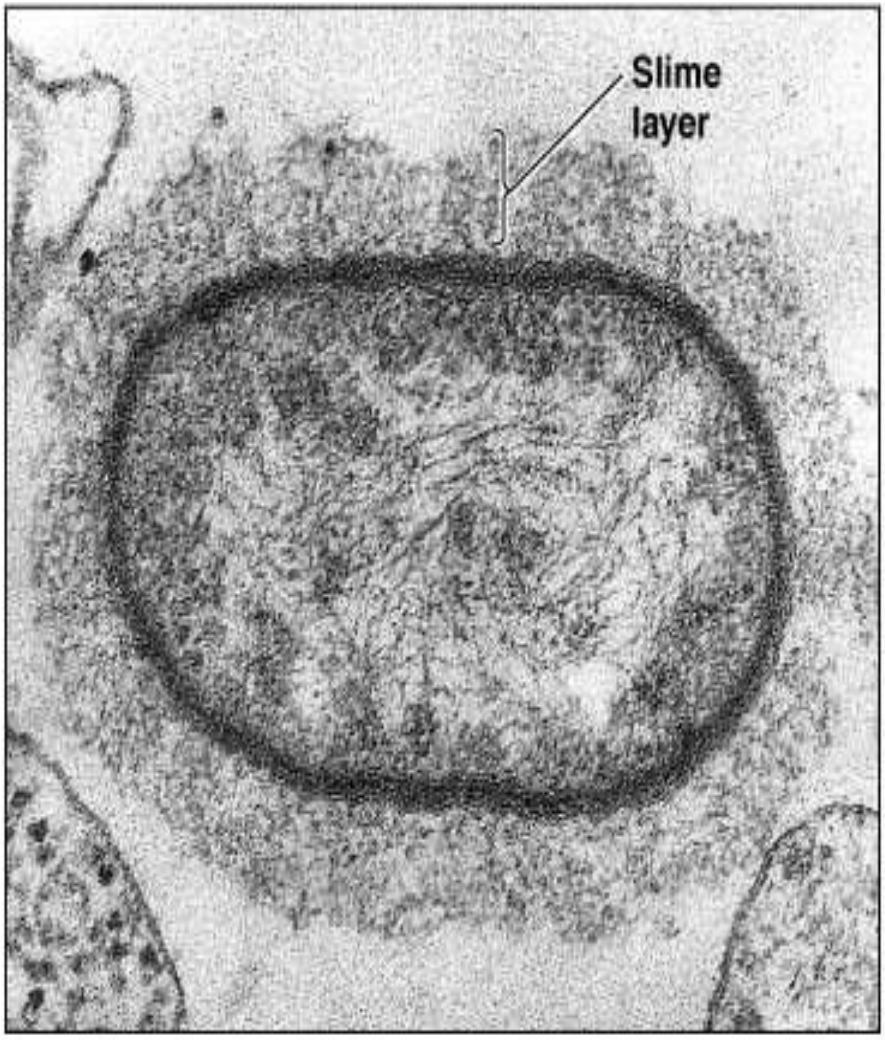
- Humans are accidental hosts
- Cell wall is composed of peptidoglycan & LPS (similar to gram negative bacteria)
- Consists of 3 genera
  - ✓ Rickettsia
  - ✓ Ehrlichia
  - ✓ Coxiella
- Intracellular location
  - ✓ Typhus group – cytoplasm
  - ✓ Spotted fever group – nucleus
  - ✓ Coxiella & Ehrlichia – cytoplasmic vacuoles



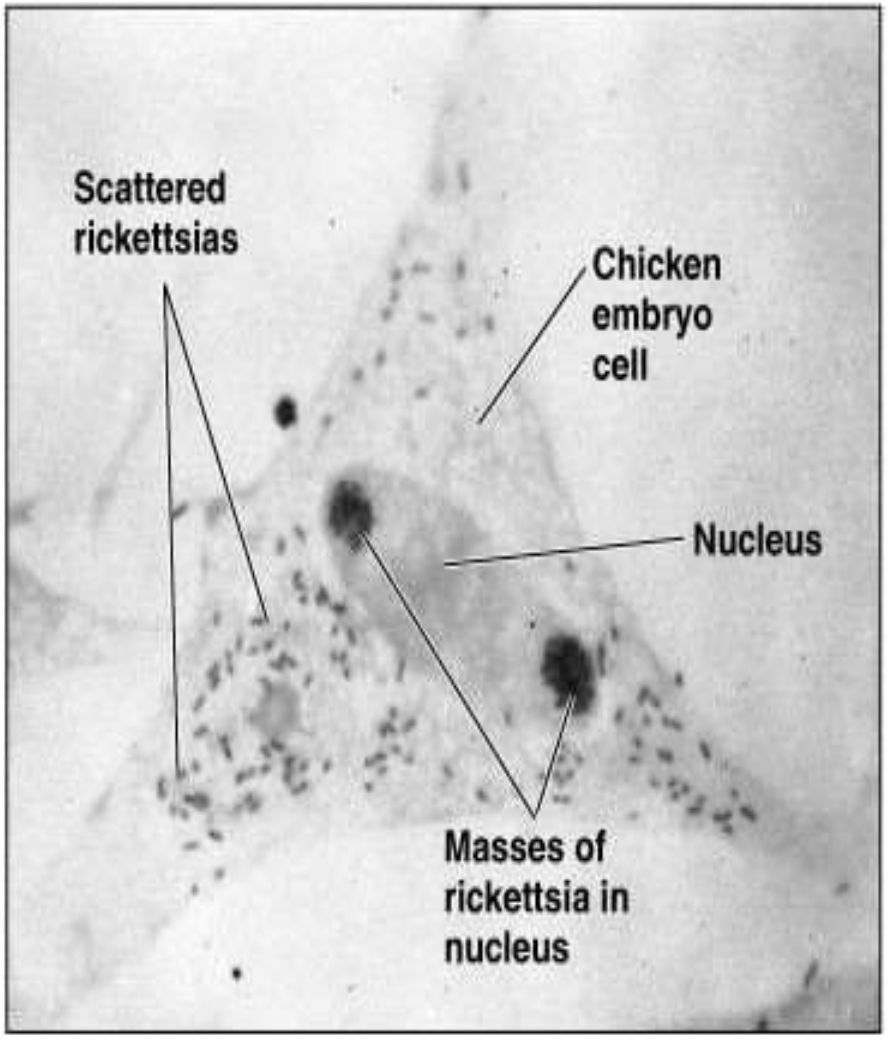
Rickettsia rickettsii

Engorged tick attached to back of toddler's head. Adult thumb shown for scale.

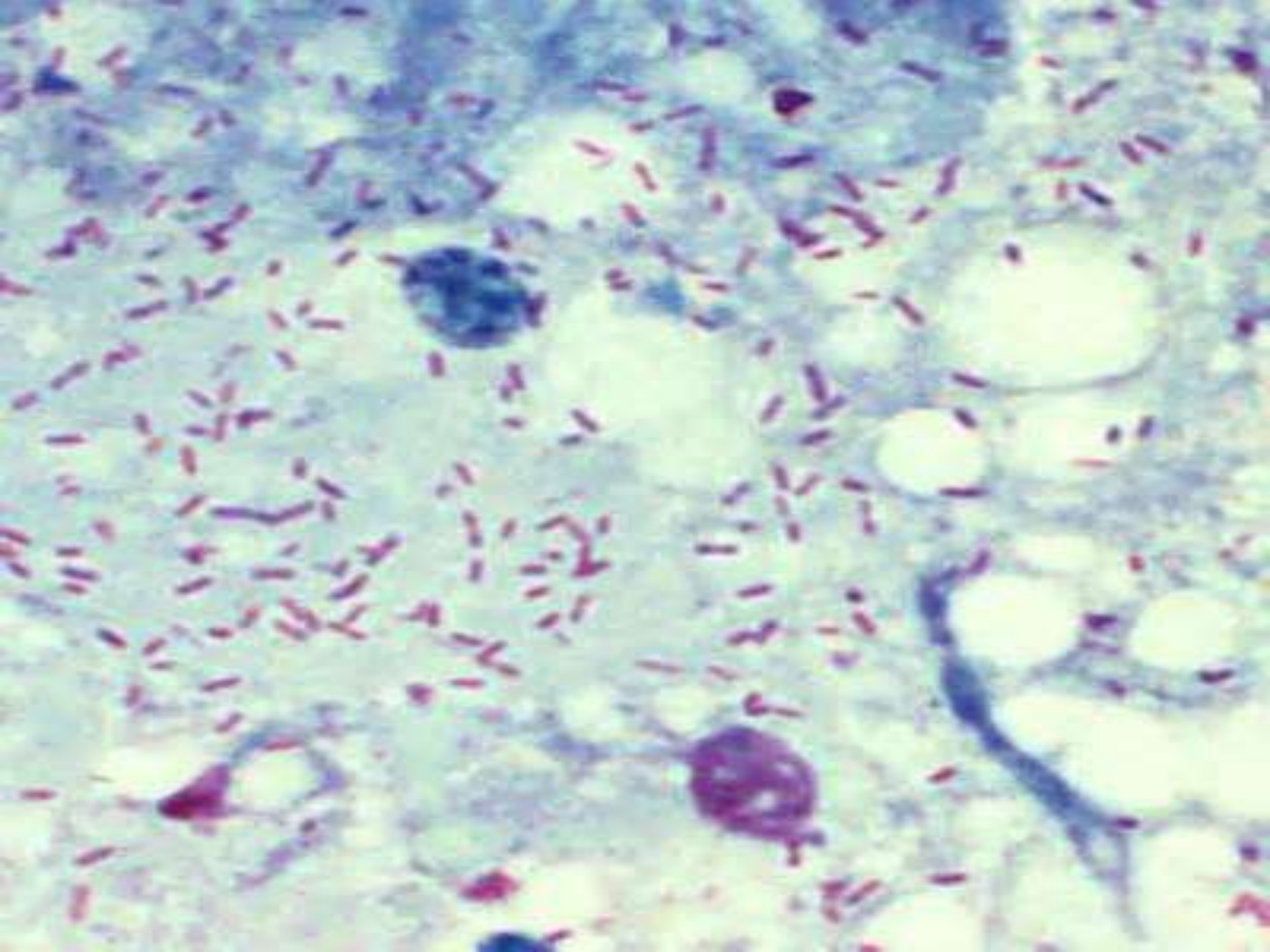
Microscopic figure



(a) Rickettsia



(b) Rickettsias in chicken embryo cell



Rickettsial species and its disease

Species	Disease	Reservoir
R. prowazekii	Epidemic typhus, Brill-Zinsser disease	Human body louse
R. typhi	Endemic typhus	Rat flea
R. rickettsii	Rocky-Mountain spotted fever	Ticks
R. conori	Boutonneuse fever	Ticks
R. australis	Australian tick typhus	Ticks
R. siberica	Siberian tick typhus	Ticks
R. akari	Rickettsial pox	Mites

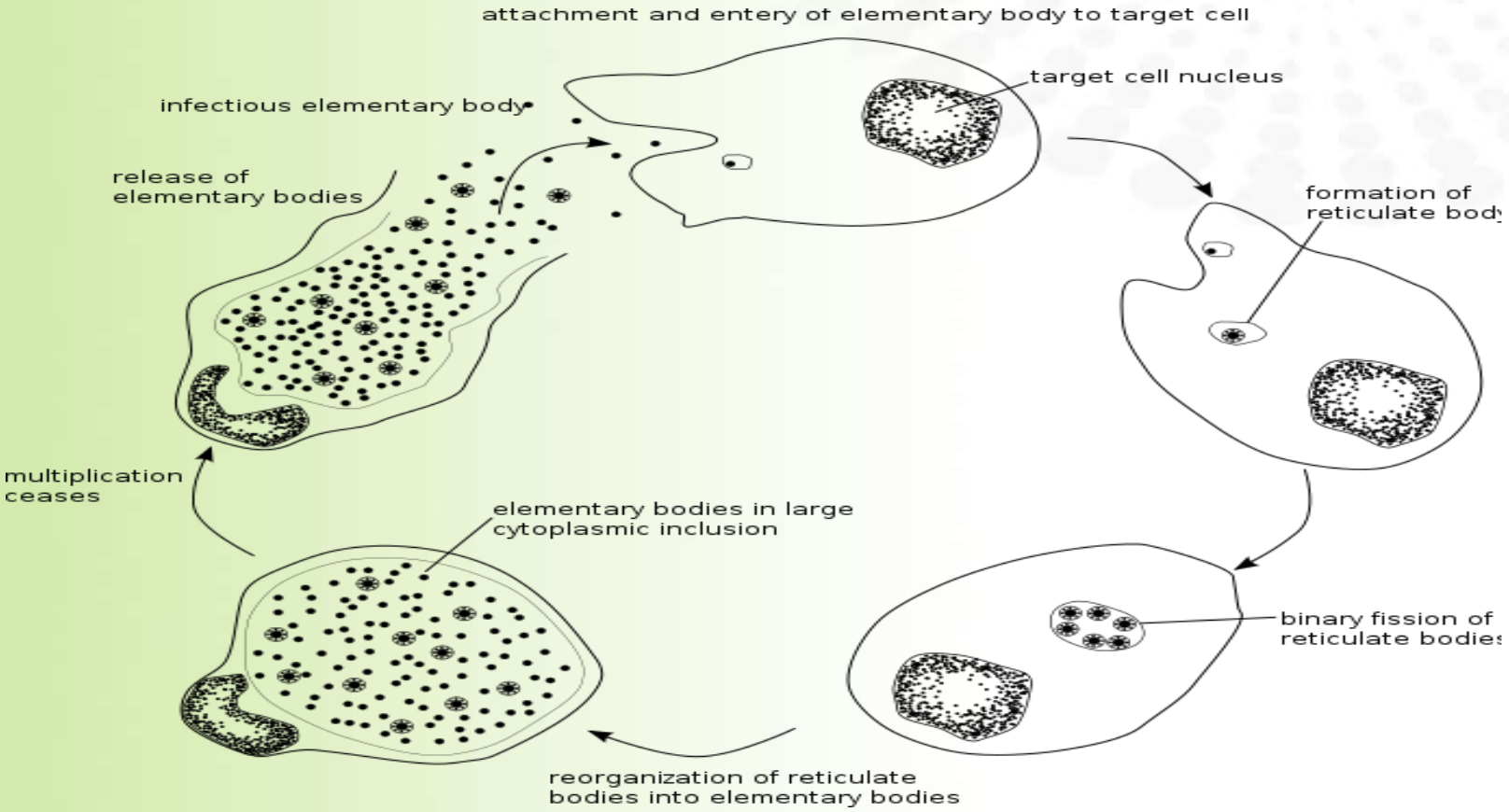
Pathogenesis

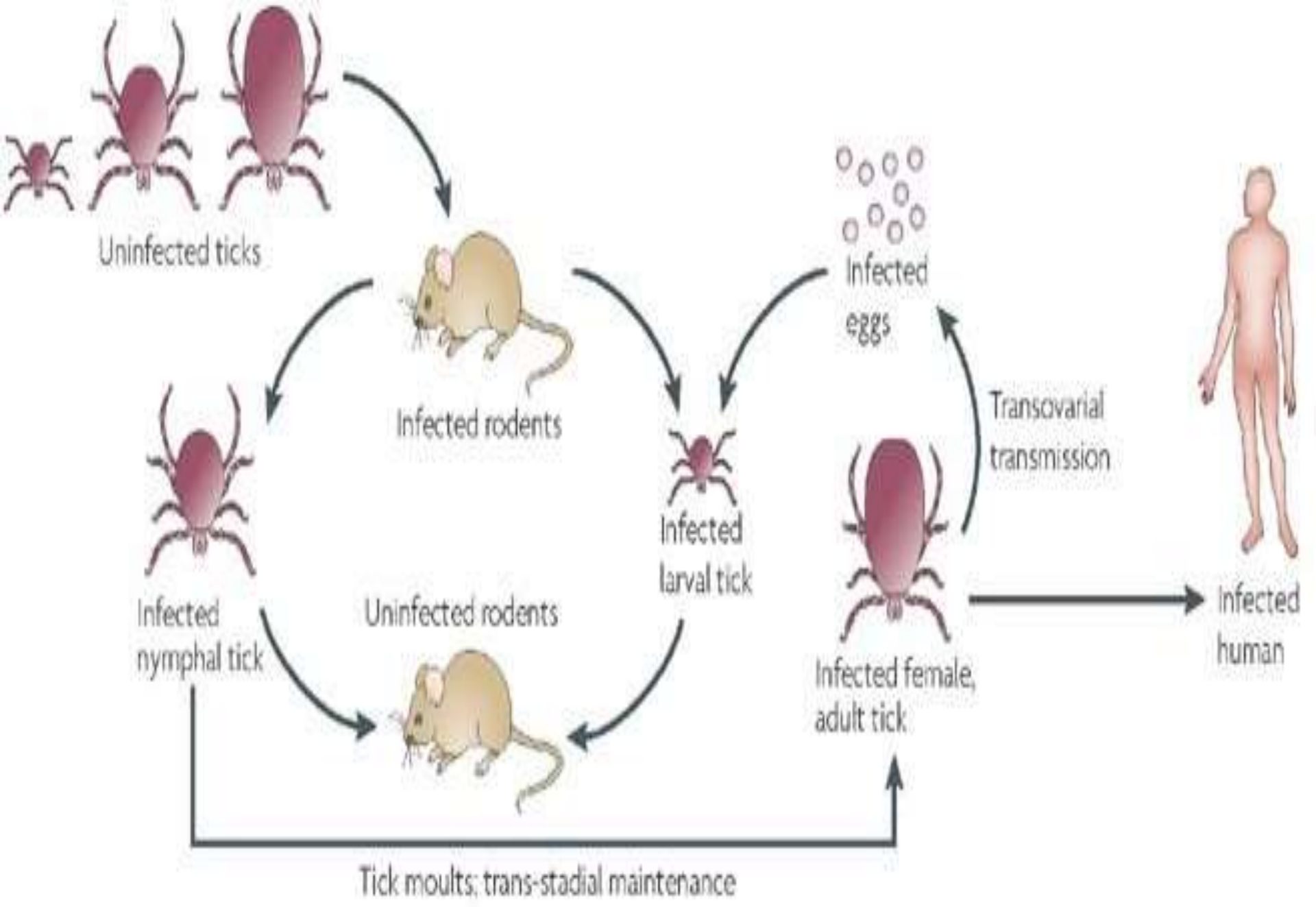
- During the first few days of incubation period
  - local reaction caused by hypersensitivity to tick or vector products
- Bacteria multiply at the site & later disseminate via lymphatic system
- Bacteria is phagocytosed by macrophages (1st barrier to rickettsial multiplication)
- If not, after 7-10 days
  - organisms disseminate
  - replicate in the nucleus or cytoplasm
- Infected cells show intracytoplasmic inclusions & intranuclear inclusions
- Endothelial damage & vasculitis progress causing
  - Development of maculopapular skin rashes
  - Perivascular tissue necrosis
  - Thrombosis & ischemia

Pathogenesis

- 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)





Nature Reviews | Microbiology

Rickettsial infections: Classification

➢ **Typhus fever group**

✓ Epidemic typhus/Brill-Zinsser typhus
✓ Endemic typhus

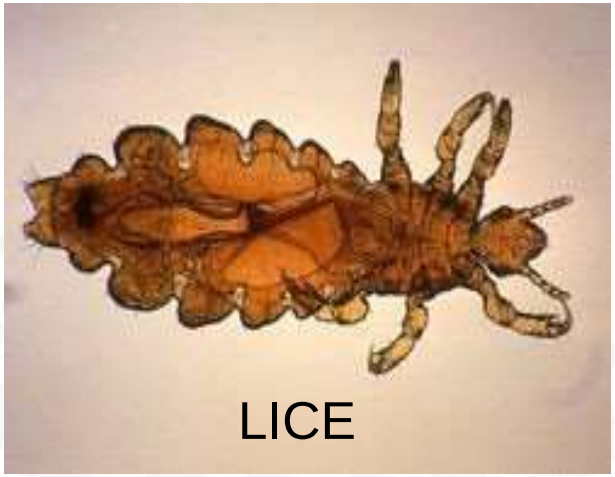
➢ **Spotted fever group**

✓ Rocky mountain spotted fever
✓ Siberian tick typhus
✓ Boutonneuse fever
✓ Australian tick typhus
✓ Rickettsial pox

Epidemic typhus (classical typhus)

- Cause: *Rickettsia prowazekii*

- Vector:
  - ✓ Human body louse
  - ✓ Human head louse


LICE

- Incubation period – 5-21 days

- Mortality rate is 20-30% in untreated cases.

- Symptoms
  - ✓ Severe headache
  - ✓ Chills
  - ✓ Generalised myalgia
  - ✓ High fever (39-41^{0}C)
  - ✓ Vomiting
  - ✓ Macular rash after 4-7 days
  - ✓ Lacks conciousness.

Brill –Zinsser/ Recrudescent typhus

- This occurs after the person is recovered from epidemic typhus and reactivation of the *Rickettsia prowazekii*.
- The rickettsia can remain latent and reactivate months or years later, with symptoms similar to or even identical to the original attack of typhus, including a maculopapular rash.
- This reactivation event can then be transmitted to other individuals through fecal matter of the louse vector, and form the focus for a new epidemic of typhus.
- Mild illness and low mortality rate.

Endemic typhus (Murine typhus)

- Cause: *Rickettsia typhi*
- Vector:
  - ✓ Rat flea
- Infection occurs after rat flea bite
- Murine typhus is an under-recognized entity, as it is often confused with viral illnesses.
- Most people who are infected do not realize that they have been bitten by fleas.

Scanning electron microscope (SEM) depiction of a flea

Endemic typhus (Murine typhus)

➢ Symptoms

✓ Headache
✓ Fever
✓ Muscle pain
✓ Joint pain
✓ Nausea
✓ Vomiting

✓ 40–50% of patients will develop a discrete rash six days after the onset of signs.

✓ Up to 45% will develop neurological signs such as confusion, stupor, seizures or imbalance.

Rocky Mountain spotted fever

- Cause: *R. rickettsii*
- Infection occurs after tick bite
- Incubation period: 1 week
- Most serious form
- More similar to typhus fever but the rash appears earlier and is more prominent.

- Initial symptoms:
  - ✓ Fever
  - ✓ Nausea
  - ✓ Emesis (vomiting)
  - ✓ Severe headache
  - ✓ Muscle pain
  - ✓ Lack of appetite
  - ✓ Parotitis

- Later signs and symptoms:
  - ✓ Maculopapular rash
  - ✓ Petechial rash
  - ✓ Abdominal pain
  - ✓ Joint pain
  - ✓ Forgetfulness

Rickettsial pox

- Cause: *R. akari*
- Vector: Mite
- Benign febrile illness with vesicular rash resembling chickenpox.
- Self-limiting, non-fatal.
- The first symptom is a bump formed by the bite, eventually resulting in a black, crusty scab.
- Many of the symptoms are flu-like including
  - ✓ Fever
  - ✓ Chills
  - ✓ Weakness
  - ✓ Achy muscles
  - ✓ The most distinctive symptom is the rash that breaks out, spanning the infected person's entire body.

Other spotted fever

- The clinical symptoms of other spotted fevers are very similar to Rocky mountain spotted fever

Maculopapular rash

Early (macular) rash on sole of foot

Late petechial rashes on palm and forearm

Complications of rickettsial diseases

- Bronchopneumonia
- Congestive heart failure
- Multi-organ failure
- Deafness
- Disseminated intravascular coagulopathy (DIC)
- Myocarditis (inflammation of heart muscle)
- Endocarditis (inflammation of heart lining)
- Glomerulonephritis (inflammation of kidney)

Diagnosis

Clinical diagnosis

- These diseases present as:
  - febrile illnesses after exposure to arthropods or animal hosts or aerosols (endemic areas).
  - High mortality from Spotted fever (delayed diagnosis).
- The spread of the rash (characteristic):
  - spread from the trunk to the extremities (centrifugal)- **typical for typhus**;
  - spread from the extremities to the trunk (centripetal) **-typical for spotted fever**.

Laboratory Diagnosis

1.Macchiavello stain:

- organisms bright red V blue background.

1.Castaneda stain:

- blue organisms V red background.

1.Giemsa stain:

- bluish purple organisms.

4. Use of immunofluorescent antibodies:

NB: The organism can be inoculated into tissue culture and grown over 4-7 days (very hazardous to personnel).

Culture & isolation

➢**Blood is inoculated in guinea pigs/mice.**

➢**Observed on 3rd – 4th week.**

➢**Animal responds to different rickettsial species can vary.**

➢ **Difficult & dangerous because of the highly infectious nature of rickettsiae.**

➢**Symptoms:**

✓**Rise in temperature – all species.**

✓**Scrotal inflammation,swelling,necrosis – *R.typhi, R.conori, R.akari***

(*except R.prowazekii*)

Serologic test

➢**Weil-Felix test**
✓**Antibody detection**
✓**Based on cross-reactivity between some strains of *Proteus* & *Rickettsia***

➢**Complement fixation**
✓**Not very sensitive & time consuming**

➢**Indirect fluorescence (EIA)**
✓**More sensitive & specific**
✓**Allows discrimination between IgM & IgG antibodies which helps in early diagnosis**

➢**Direct immunofluorescence**
✓**The only serologic test that is useful for clinical diagnosis**
✓**100% specific & 70% sensitive allowing diagnosis in 3-4 days into the illness**

Weil-felix test

- Heterophile agglutination test

- Using non motile *Proteus vulgaris* strains (OX 19, OX 2, OX K) to find rickettsial antibodies in patient's serum.

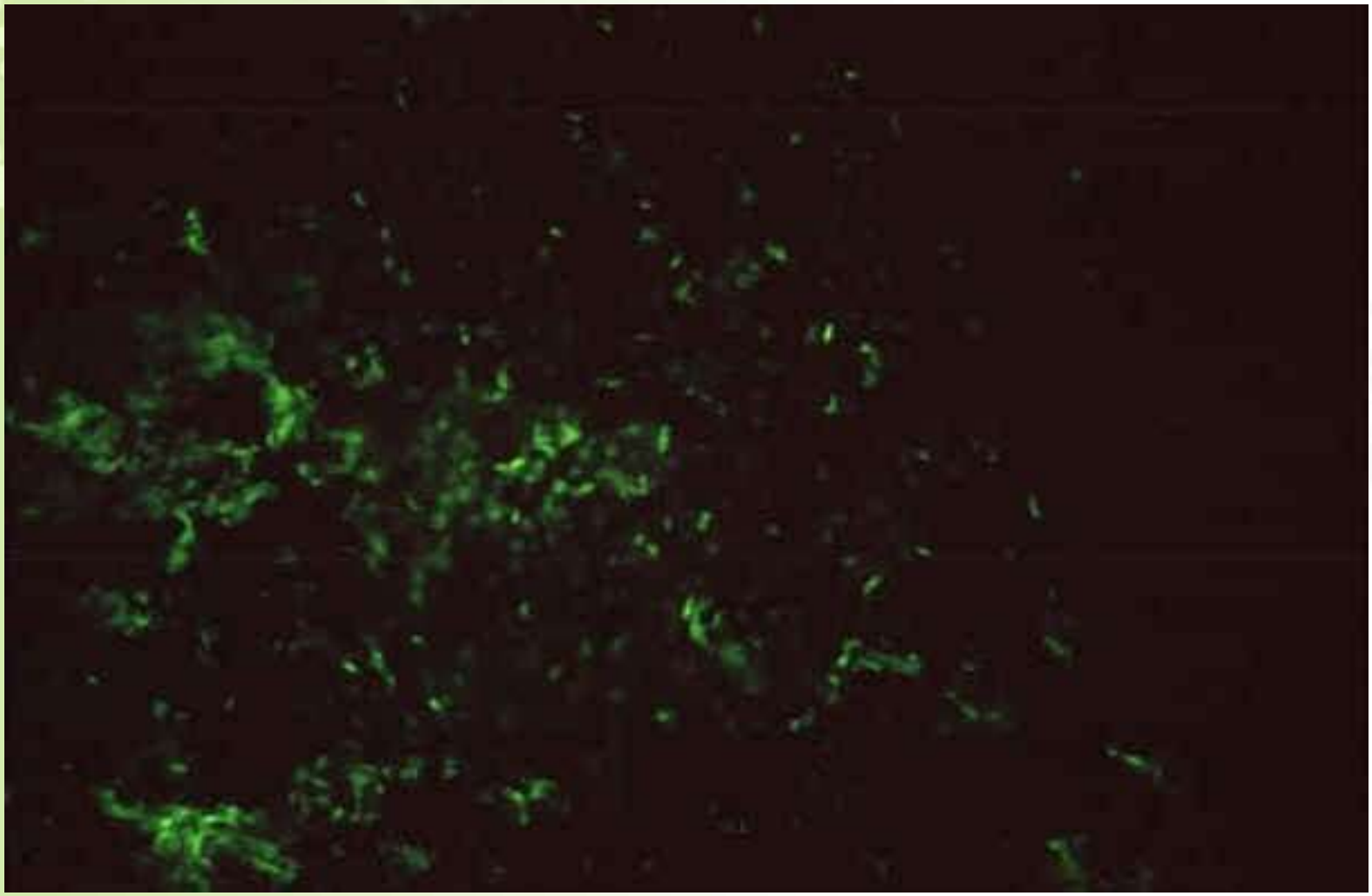
- Procedure:

✓ Serum is diluted in three separate series of tubes followed by the addition of equal amount of OX 19, OX 2, OX K in 3 separate series of tubes.
✓ Incubation at 37^0C for overnight.
✓ Observe for agglutination.

- Interpretation:

✓ Strong Agglutination with OX 19 => epidemic & endemic typhus.
✓ Strong agglutination with OX 19 & OX 2 => Spotted fever
✓ Strong agglutination with OX K => Scrub typhus (Scrub typhus by *Orientia tsutsugamushi*)

Immunofluorescent antibody technique

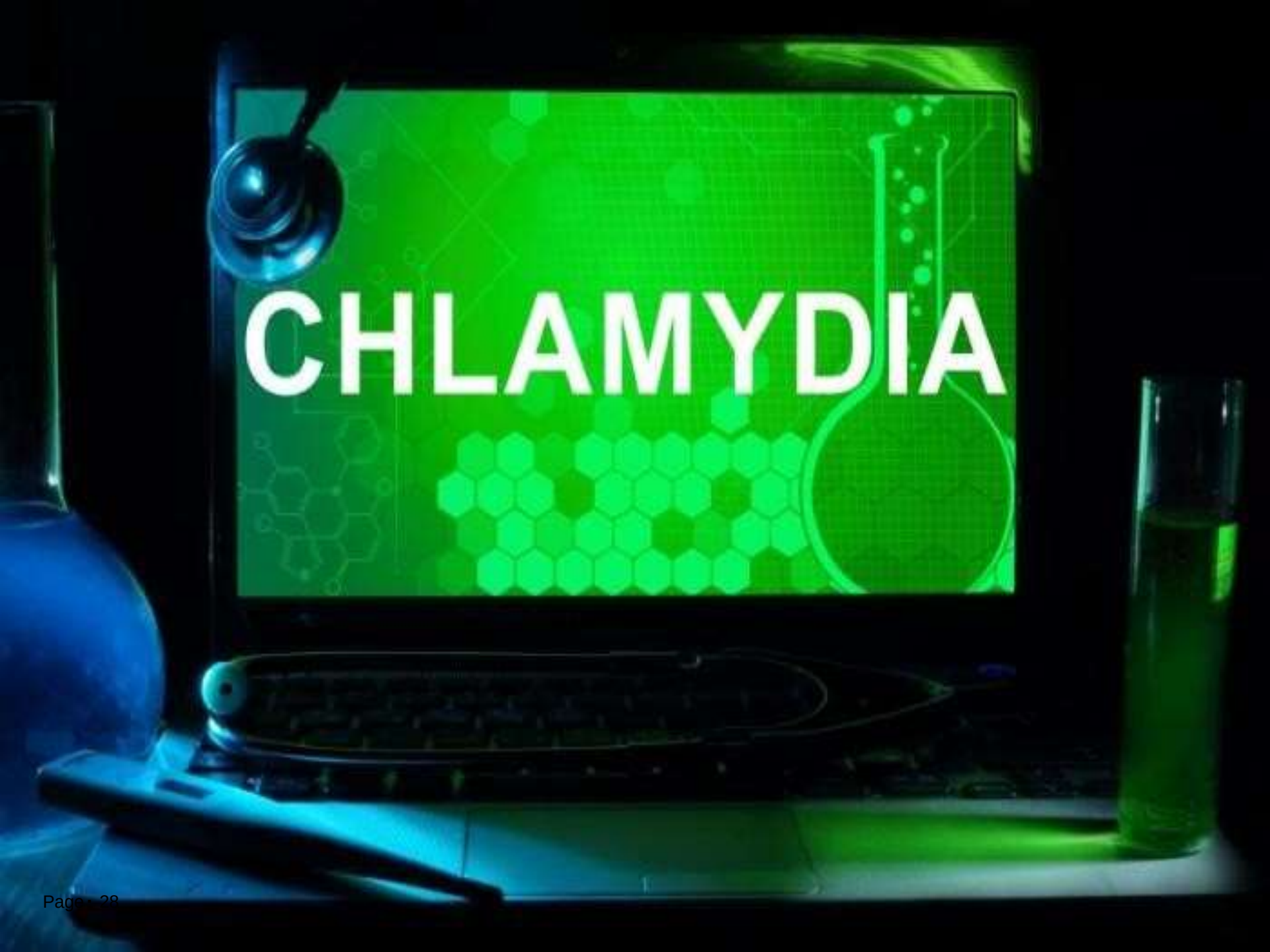


Immunofluorescent Antibody Technique

(utilizes fluorescent antibody to detect rickettsial antigen in infected tissues)

Treatment & Control

- **Chemotherapeutic:**
  - Tetracycline or
  - Chloramphenicol
- **Sanitary:**
  - Arthropod and rodent control are possible but difficult.
- **Immunological:**
  - No vaccines - currently available.


CHLAMYDIA

Fundamental differences between Chlamydiae and Rickettsiae.

Rickettsiae	Chlamydiae
Cytochromes +ve	No cytochromes
Aerobic metabolism	Anaerobic metabolism.
Multiply by binary fision.	Single development cycle.

Similarities

- Small, pleomorphic coccobacillary forms
- Obligate intracellular parasites.
- All contain DNA and RNA.
- Susceptible to various antibiotics.
- Cell walls resemble those of Gram –ve bacteria.
- Require exogenous cofactors from animal cells.
- Most grow readily in the yolk sac of embryonated eggs and in cell cultures.

Risk Factors

- Adolescence
- New or multiple sex partners
- History of STD infection
- Presence of another STD
- Oral contraceptive user
- Lack of barrier contraception

Chlamydiaceae Family (species that cause disease in humans)

Species (genus)	Disease
C. trachomatis 2 biovars, non-LGV LGV	Trachoma, NGU, MPC, PID, conjunctivitis, Infant pneumonia, LGV
C. pneumoniae	Pharyngitis, bronchitis, pneumonia
C. psittaci	Psittacosis

Chlamydia General characteristics

- **Species:** *trachomatis*, *psittaci*
- The *Chlamydia*
  - Obligate intracellular parasites.
- *C. trachomatis*
  - Trachoma,
  - Inclusion conjunctivitis,
  - Lymphogranuloma venereum (LGV)
  - nongonococcal urethritis (NGU). I.e, oculourogenital infections.
- *C. psittaci* produces systemic diseases:
  - psittacosis,
  - ornithosis and
  - pneumonitis.

Distinctive properties.

- Have two distinct forms:-
  - Infectious elementary bodies and
  - Intracellular reticulate bodies.
- Elementary bodies attach and are internalized by susceptible host cells.
- Once inside, they reorganize into a replicative form (the reticulate body)
- Over a 24 hour period:
  - Reticulate bodies divide and begin to reorganize back into elementary bodies.

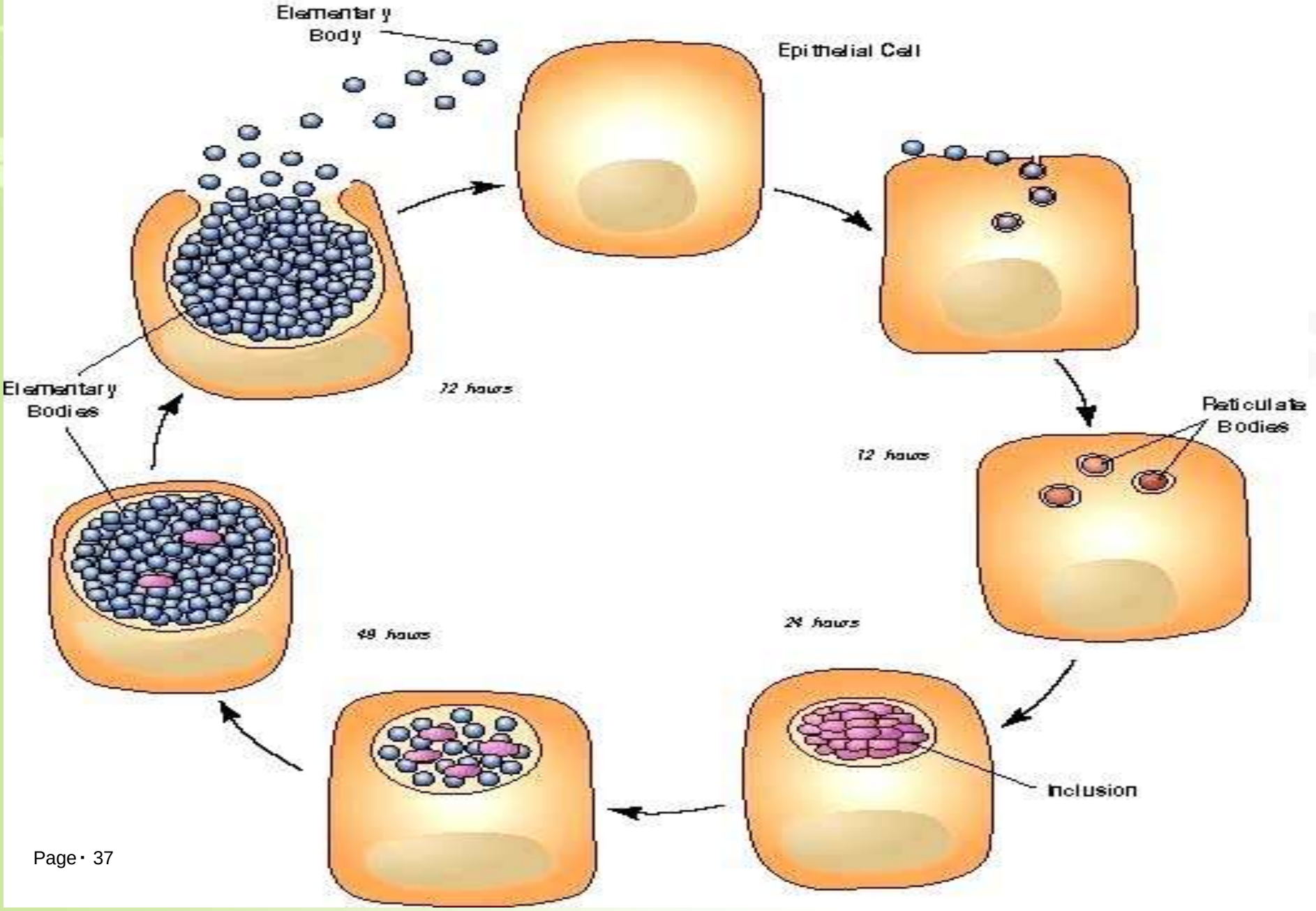
Distinctive properties…

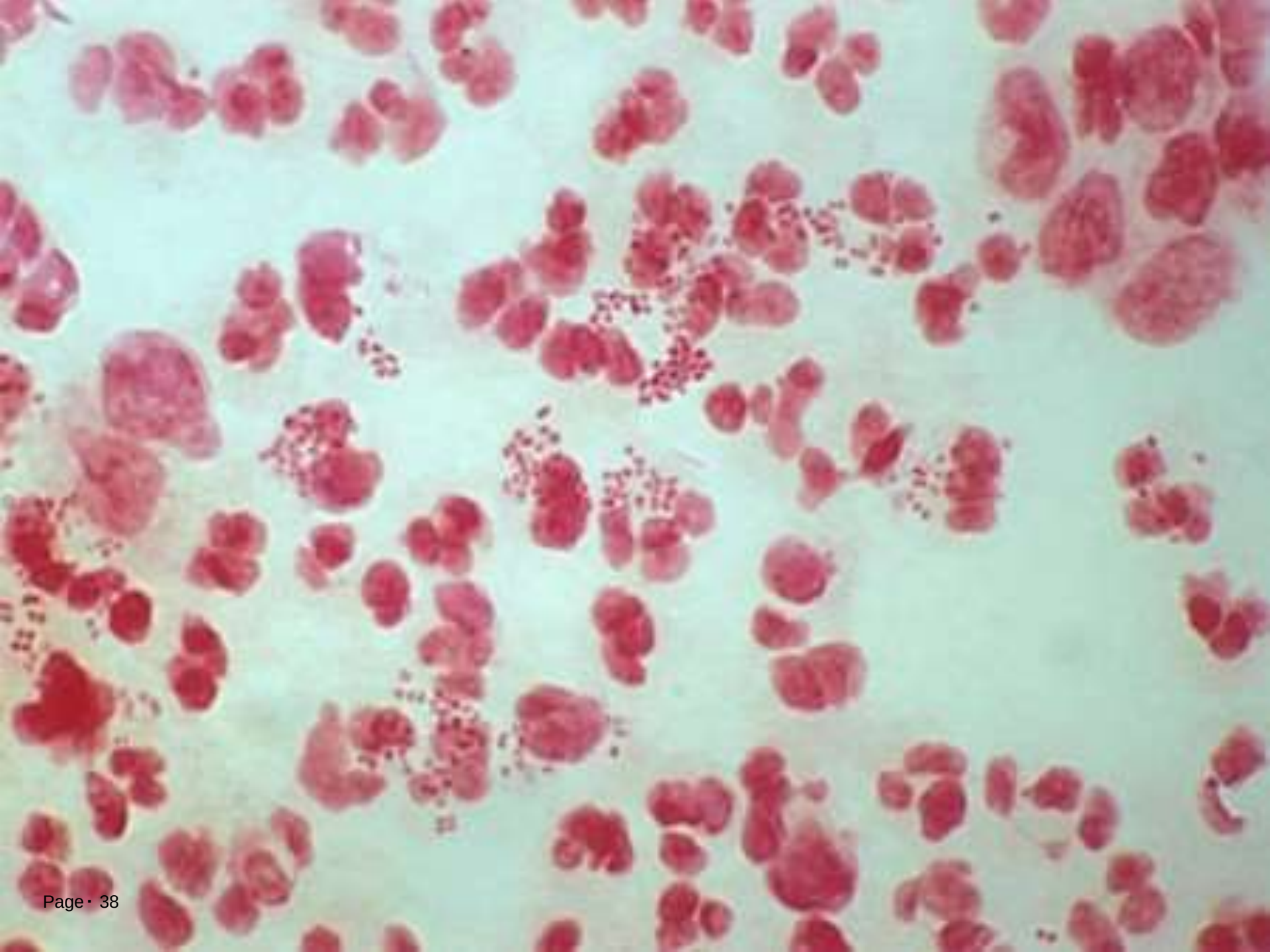
- 48-72 hours after infection:
  - The cell is lysed and
  - numerous infectious elementary bodies released.
- The genome of *Chlamydia* is only 25% the size of *E. coli (*i.e one of the smallest prokaryotes).
- The pathogenic mechanisms employed by *Chlamydia* - not well understood.

Microbiology

- Obligatory intracellular bacteria
- Infect columnar epithelial cells
- Survive by replication that results in the death of the cell
- Takes on two forms in its life cycle:
  - Elementary body (EB)
  - Reticulate body (RB)

Life Cycle of Chlamydia






Chlamydia

Transmission

- Transmission is **sexual** or **vertical**
- Highly transmissible
- Incubation period 7-21 days
- Significant asymptomatic reservoir exists in the population
- Re-infection is common
- Perinatal transmission results in neonatal conjunctivitis in 30%-50% of exposed babies

Clinical Syndromes Caused by *C. trachomatis*

	Local Infection	Complication	Sequelae
Men	Conjunctivitis Urethritis Prostatitis	Reiter's syndrome Epididymitis	Chronic arthritis (rare) Infertility (rare)
Women	Conjunctivitis Urethritis Cervicitis Proctitis	Endometritis Salpingitis Perihepatitis Reiter's syndrome	Infertility Ectopic pregnancy Chronic pelvic pain Chronic arthritis (rare)
Infants	Conjunctivitis Pneumonitis Pharyngitis Rhinitis	Chronic lung disease?	Rare, if any

C. trachomatis Infection in Men

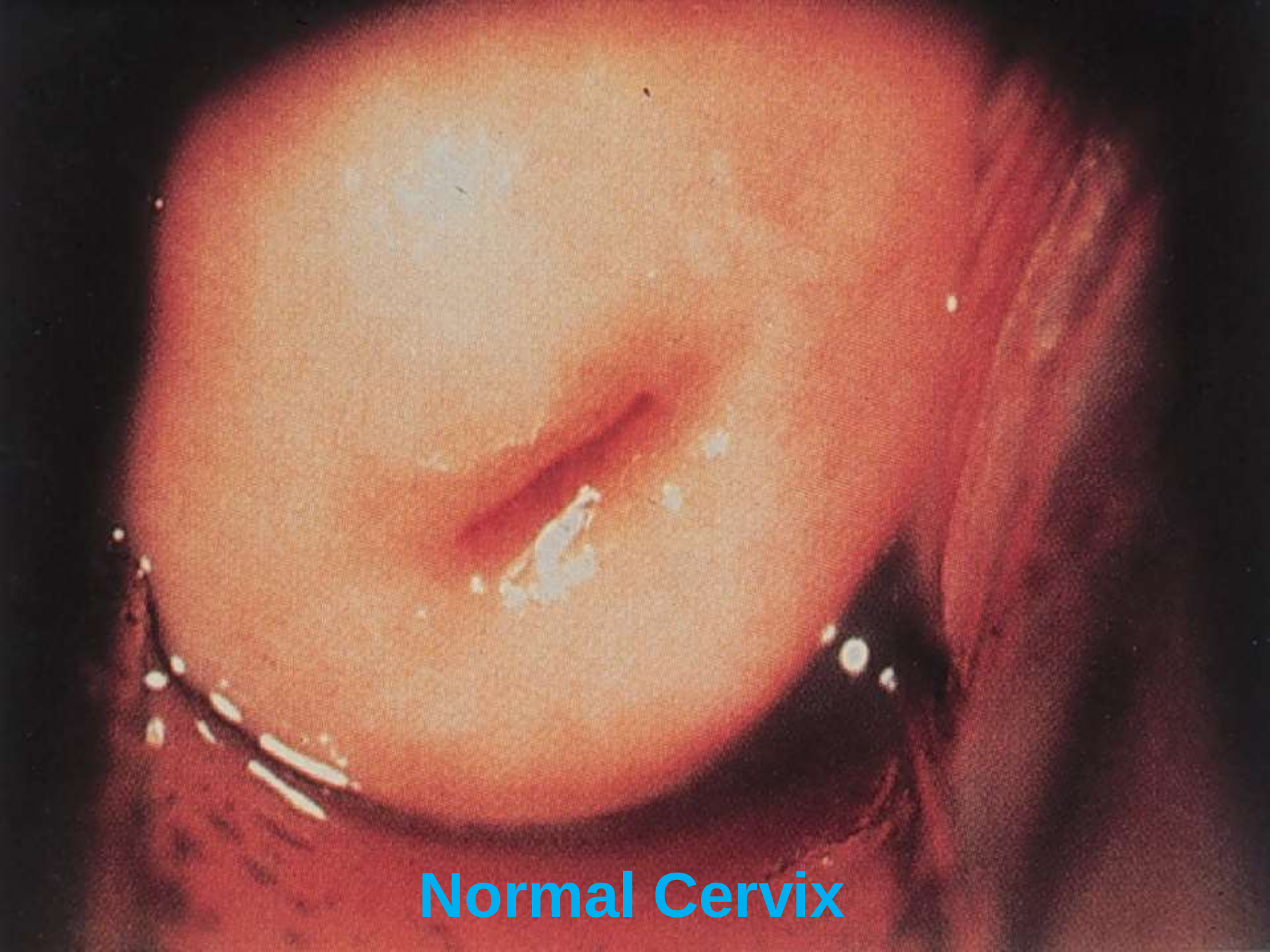
- Urethritis–One cause of non-gonococcal urethritis (NGU)
  - Majority (>50%) asymptomatic
  - Symptoms/signs if present: mucoid or clear urethral discharge, dysuria
  - Incubation period unknown (probably 5-10 days in symptomatic infection)

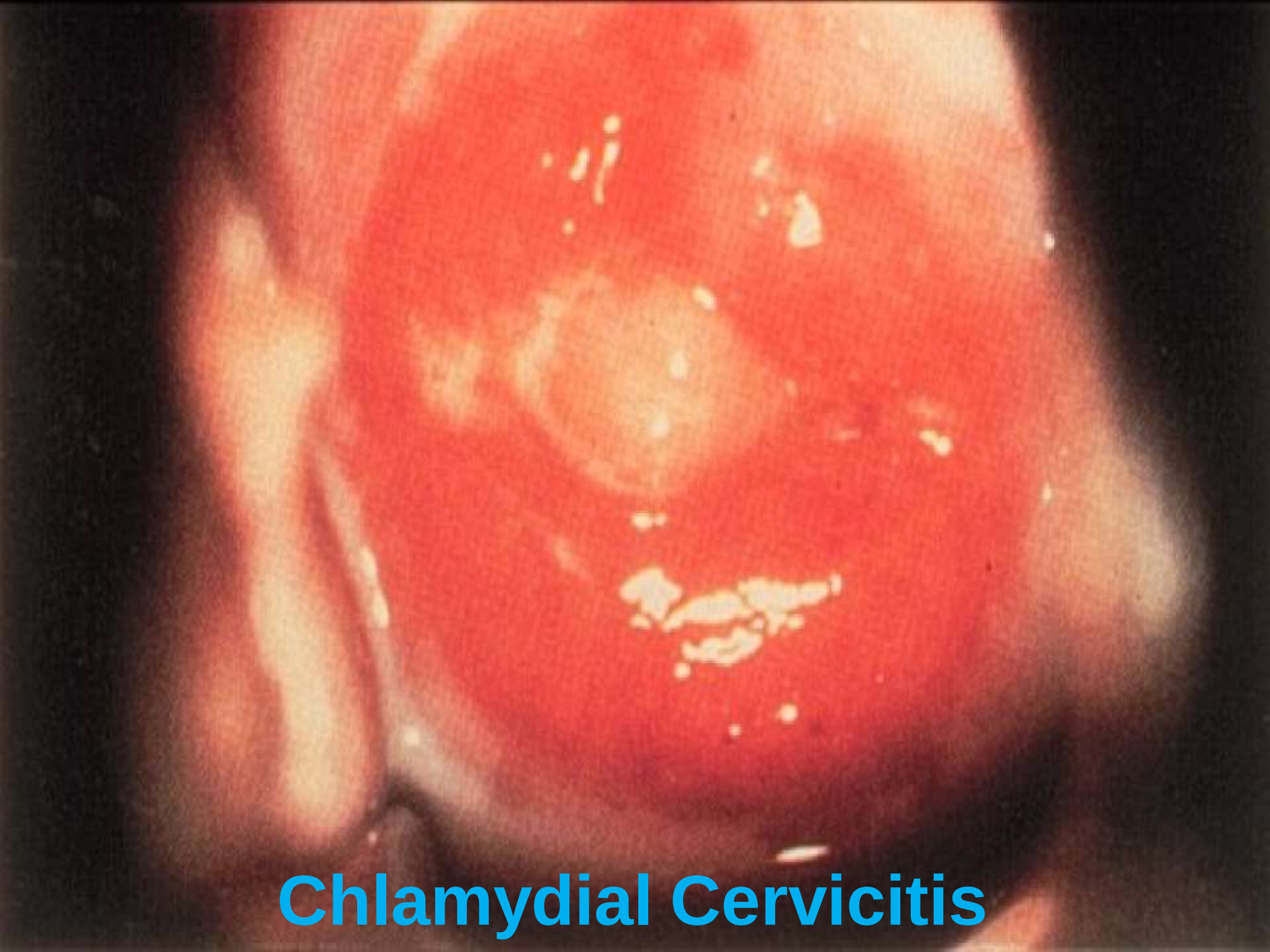
C. trachomatis Complications in Men

- Epididymitis
- Reiter's Syndrome
  - –Rarely occurs in women

C. trachomatis Infections in Women

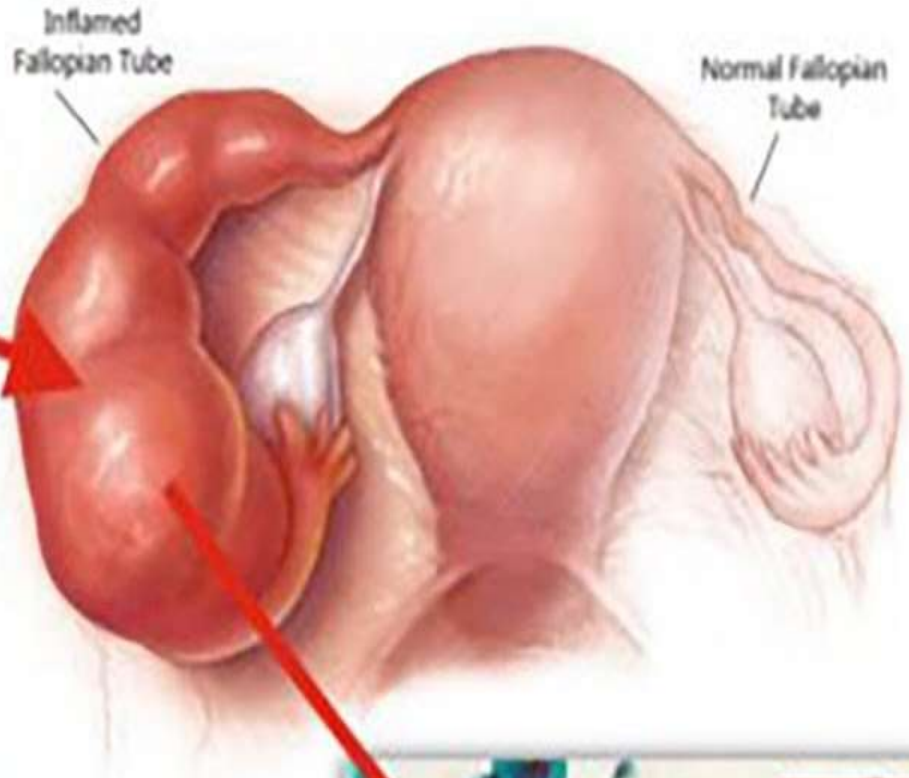
- Cervicitis
  - Majority (70%-80%) are asymptomatic
  - Local signs of infection, when present, include:
    - Mucopurulent endocervical discharge
    - Edematous cervical ectopy with erythema and friability
- Urethritis
  - Usually asymptomatic
  - Signs/symptoms, when present, include dysuria, frequency, pyuria

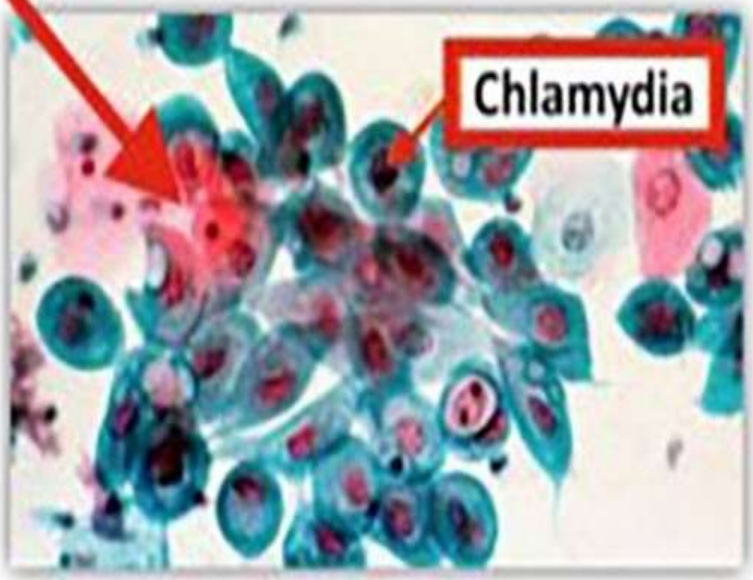

Normal Cervix

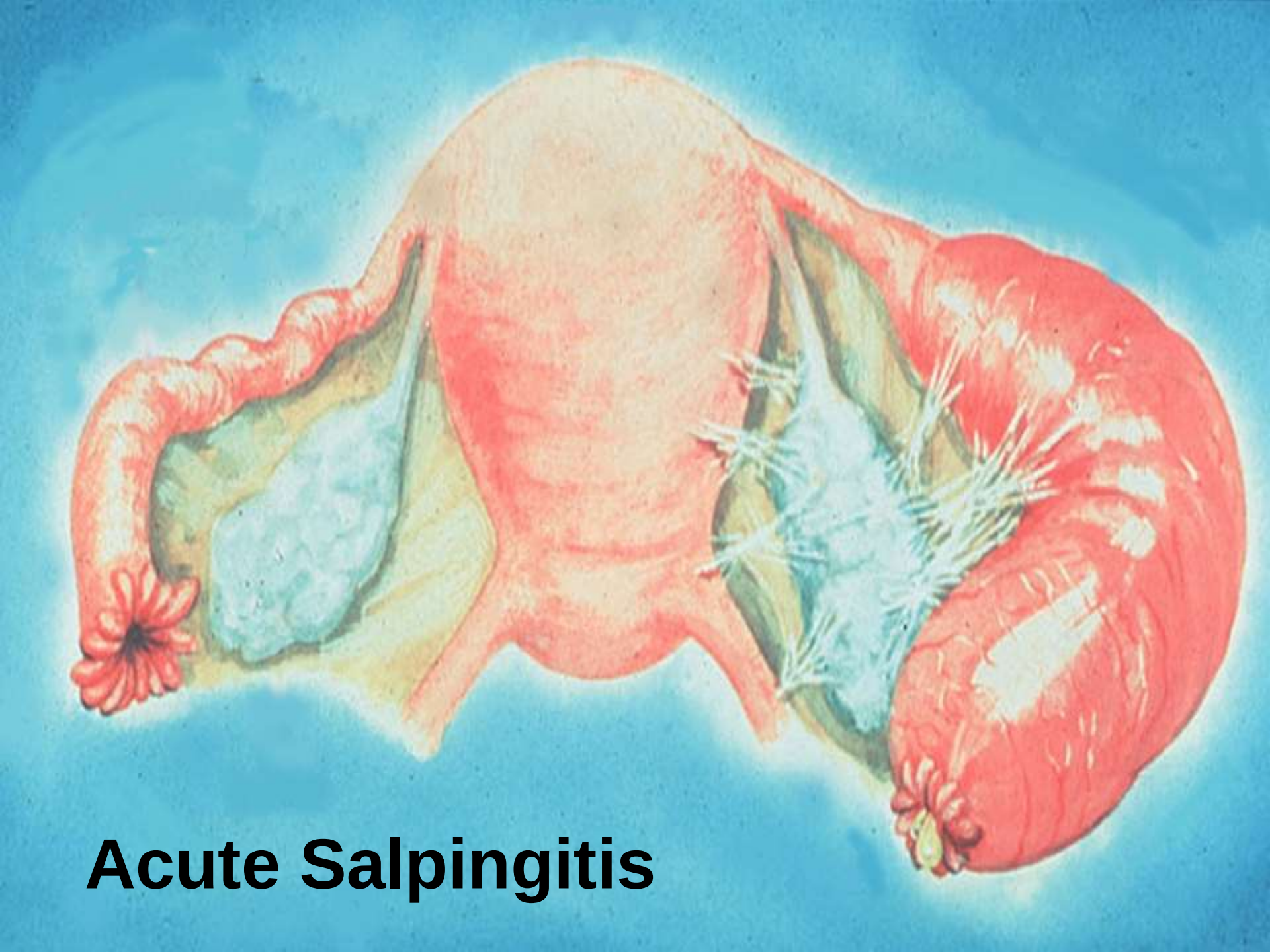

Chlamydial Cervicitis

C. trachomatis Complications in Women

- Pelvic Inflammatory Disease (PID)
  - –Salpingitis
  - –Endometritis
- Perihepatitis (Fitz-Hugh-Curtis Syndrome)
- Reiter’s Syndrome


Inflamed
Fallopian Tube
Normal Fallopian
Tube


Chlamydia



Acute Salpingitis

C. trachomatis Syndromes Seen in Men or Women

- Non-LGV serovars
  - –Conjunctivitis
  - –Proctitis
  - –Reiter's Syndrome
- LGV serovars
  - –Lymphogranuloma venereum


LGV Lymphadenopathy

C. trachomatis Infections in Infants

- Perinatal clinical manifestations:
  - Inclusion conjunctivitis
  - Pneumonia

C. trachomatis Infections in Children

- Pre-adolescent males and females:
  - –Urogenital infections
    - Usually asymptomatic
    - Vertical transmission
    - Sexual abuse

Testing Technologies

- Culture
- Non-culture tests
  - Nucleic Acid Amplification Tests (NAATs)
  - Non-Nucleic Acid Amplification Tests (Non-NAATs)
  - Serology

Culture

- Historically the “gold standard”
- Variable sensitivity (50%-80%)
- High specificity
- Use in legal investigations
- Not suitable for widespread screening

NAATs

- NAATs amplify and detect organism-specific genomic or plasmid DNA or rRNA
- FDA cleared for urethral swabs from men/women, cervical swabs from women, and urine from both
- Commercially available NAATs include:
  - Becton Dickinson *BDProbeTec*
  - Gen-Probe *AmpCT, Aptima*
  - Roche *Amplicor®*
- Significantly more sensitive than other tests

Non-NAATs

- Direct fluorescent antibody (DFA)
  - Detects intact bacteria with a fluorescent antibody
  - Variety of specimen sites
  - Can be used to determine quality of endocervical specimens
- Enzyme immunoassay (EIA)
  - Detects bacterial antigens with an enzyme-labeled antibody
- Nucleic acid hybridization (NA probe)
  - Detects specific DNA or RNA sequences of *C. trachomatis* and *N. gonorrhoeae*

Serology

- Rarely used for uncomplicated infections (results difficult to interpret)
- Criteria used in LGV diagnosis
  - Complement fixation titers >1:64 suggestive
  - Complement fixation titers > 1:256 diagnostic
  - Complement fixation titers < 1:32 rule out

Treatment and Control.

- Chlamydia exhibit low pathogenicity in a compromised host.
- Chemotherapeutic:
  - Tetracycline or erythromycin are drugs of choice.
- Sanitary:
  - Good hygiene,
  - Treatment of sexual partners and
  - Quarantine of birds all reduce the incidence.

Treatment and control.

- **Immunological:**
  - No vaccine – available since specific antibodies fail to neutralize elementary bodies *in vivo*.

NB:

- Chlamydial d'ses –relatively easy to treat, but:

1. Latency of infection
2. Susceptibility to reinfection.