AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 5.

Pathogenic Spirochetes, Rickettsiae, Chlamydiae and Mycoplasma

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

•1. **Pathogenic spirochetes**. General characteristics, classification.

•- ***Treponemas***. The causative agent of syphilis, morpho-biological characteristics, antigen structure, virulence factors, pathogenesis. The causative agents of syphilis-like diseases (frambezia, pinta). Principles of microbiological diagnostics. Principles of treatment of syphilis.

•- ***Borrelia***. The causative agents, morpho-biological characteristics, virulence factors, pathogenesis. Microbiological diagnostics. The causative agent of Lyme disease, the pathogenesis of the disease. Microbiological diagnostics.

•- ***Leptospirosis***, morpho-biological characteristics, classification. Source of infection, mode of transmission, pathogenesis, microbiological diagnosis, principles of specific treatment and prevention.

•2. **Pathogenic rickettsiae**, morpho-biological characteristics. Classification of rickettsioses.Causes of typhus group (Rickettsia prowazekii, Rickettsia typhi), virulence factors, pathogenesis and microbiological diagnosis. Principles of specific treatment and prevention.Pathogenesis and microbiological diagnosis of diseases caused by spotted fever group rickettsiae (rocky mountain spotted fever - *R.rickettsii*, Marseille fever - *R.conorii*, flower-like rickettsiosis - *R.akari*, North Asian tick-borne rickettsiosis - *R.sibirica*).

•- *Orientia tsutsugamushi* – Scrub typhus or Bush typhus, morpho-biological characteristics, pathogenesis and microbiological diagnosis.

•- Genus Ehrlichia (monocytic ehrlichiosis – *E.sennetsu, E.chaffeesis*, granulocytic ehrlichiosis – *E.ewingii*, Anaplasma phagocytophilum), morpho-biological characteristics, pathogenesis and microbiological diagnosis of the diseases they cause

•- The causative agent of Q-fever (*Coxiella burnetii*), morpho-biological characteristics, pathogenesis and microbiological diagnosis.

•3. **Pathogenic chlamydia**, classification, morpho-biological characteristics. *Chlamydia trachomatis*, serotypes, characteristics of diseases caused by individual serotypes, pathogenesis. Microbiological diagnostics. *Chlamydia psittaci* – the causative agent of ornithosis. Pathogenesis of the disease in man. Microbiological diagnostics. *Chlamydia pneumonia*, its role in human pathology. Pathogenesis and microbiological diagnosis of the disease caused by it.

•4. **Pathogenic mycoplasmas, morpho-biological characteristics.**

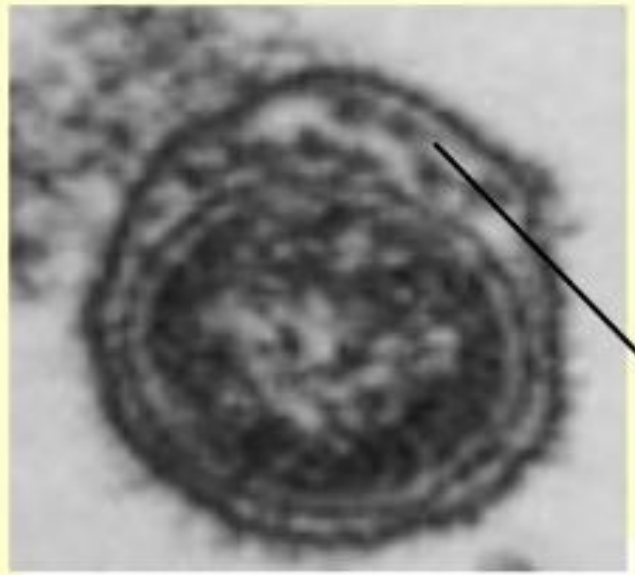
•- *Mycoplasma* genus, morpho-biological characteristics, classification. Pathogenicity factors. Human diseases. Microbiological diagnostics.

•- *Ureaplasmas*, morpho-biological characteristics. Role in urogenital infections and pregnancy pathology. Microbiological diagnostics.

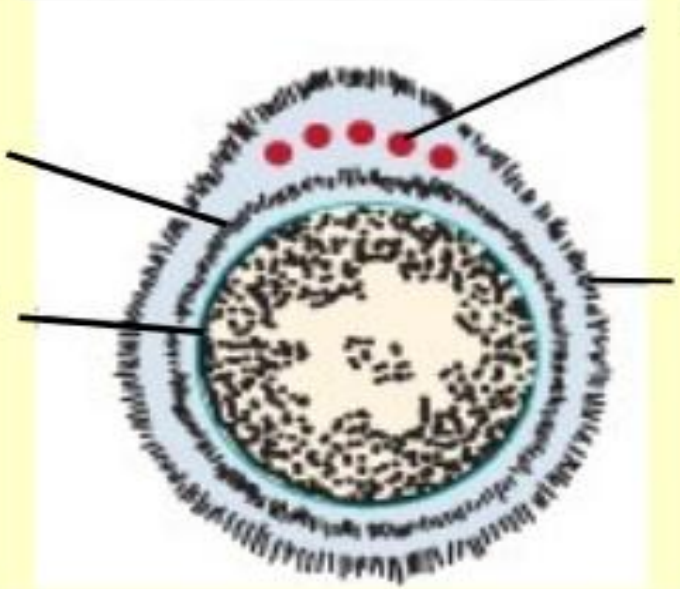
Spirochetes

Spirochetes -are elongated motile, flexible bacteria twisted spirally along the long axis.

spirochetes contain – **endoflegalla** which are polar flagella along the helical protoplasmic cylinder and situated between the outer membrane and cell wall



Endoflagella



Cell wall

Cytoplasmic menbrane

Outer envelope

Human pathogens

Genera Treponema

Borreilia

Leptospira

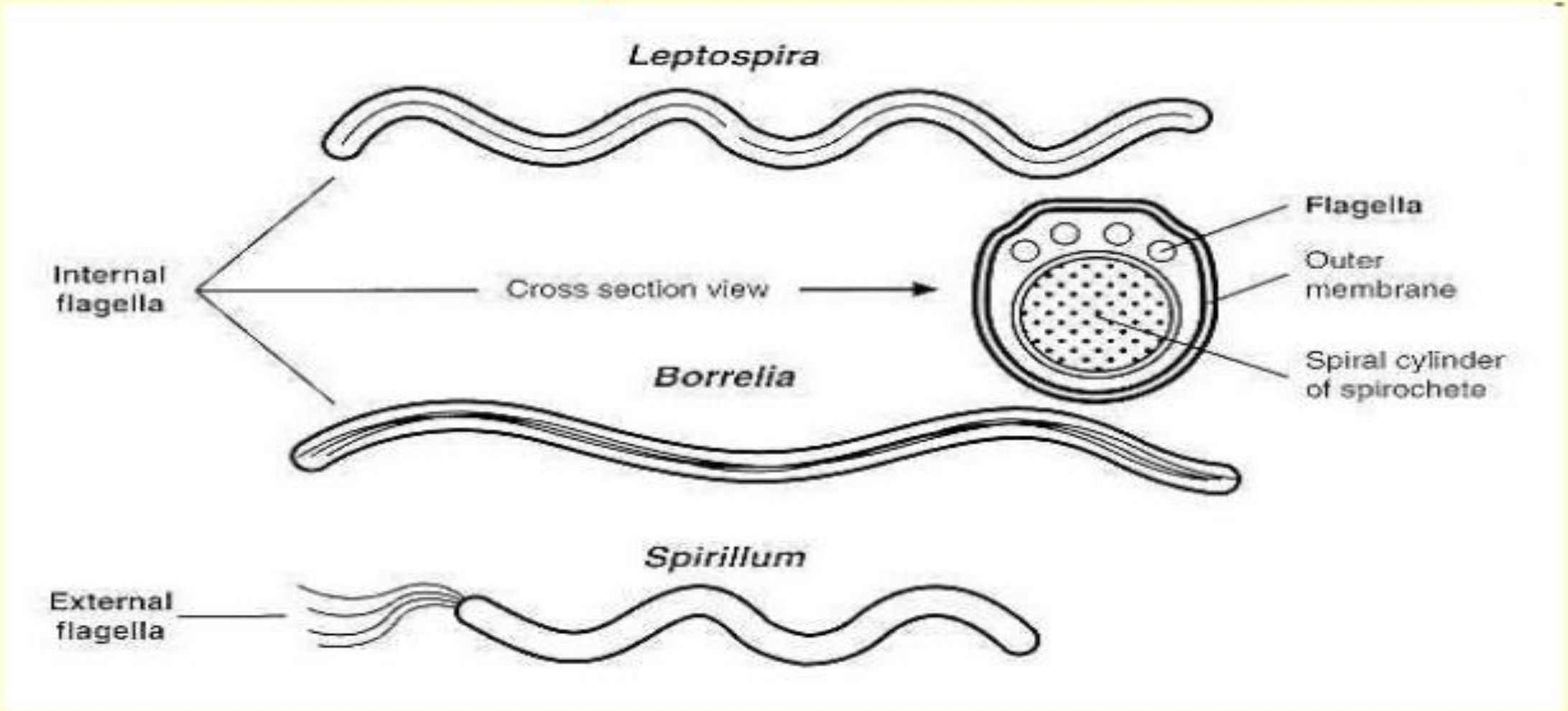
Spirochaetales *Associated Human Diseases*

Genus	Species	Disease
Treponema	*pallidum ssp. pallidum*	*Syphilis*
	pallidum ssp. endemicum	*Bejel*
	pallidum ssp. pertenue	*Yaws*
	carateum	*Pinta*
Borrelia	*burgdorferi*	*Lyme disease (borreliosis)*
	recurrentis	*Epidemic relapsing fever*
	Many species	*Endemic relapsing fever*
Leptospira	*interrogans*	*Leptospirosis (Weil's Disease)*

Medically Important Genera in the Order Spirochaetales

Spirochaetales	Human Disease	Etiologic Agent
FAMILY SPIROCHAETACEAE		
Genus *Borrelia*	Epidemic relapsing fever	*B. recurrentis*
	Endemic relapsing fever	Many *Borrelia* species
	Lyme borreliosis	*B. burgdorferi*, *B. garinii*, *B. afzelii*
Genus *Treponema*	Venereal syphilis	*T. pallidum* subsp. *pallidum*
	Endemic syphilis (bejel)	*T. pallidum* subsp. *endemicum*
	Yaws	*T. pallidum* subsp. *pertenue*
FAMILY LEPTOSPIRACEAE		
Genus *Leptospira*	Leptospirosis	*Leptospira* spp.

Comparative Morphology of Spirochetes



TREPONEMA PALLIDUM

Thin spirochete, sexually transmitted disease, congenital infections, painless ulcer (chancre)

Biology and Virulence

- Coiled spirochete (0.1 to 0.2 × 6 to 20 μm) too thin to be seen with Gram or Giemsa stains; observed by darkfield microscopy
- Outer membrane proteins promote adherence to host cells
- Hyaluronidase facilitates perivascular infiltration
- Coating of fibronectin protects against phagocytosis
- Tissue destruction primarily results from host's immune response to infection

Trepanoma pallidum

Biological Characteristics

Motile, sluggish in viscous['vɪskəs]environments

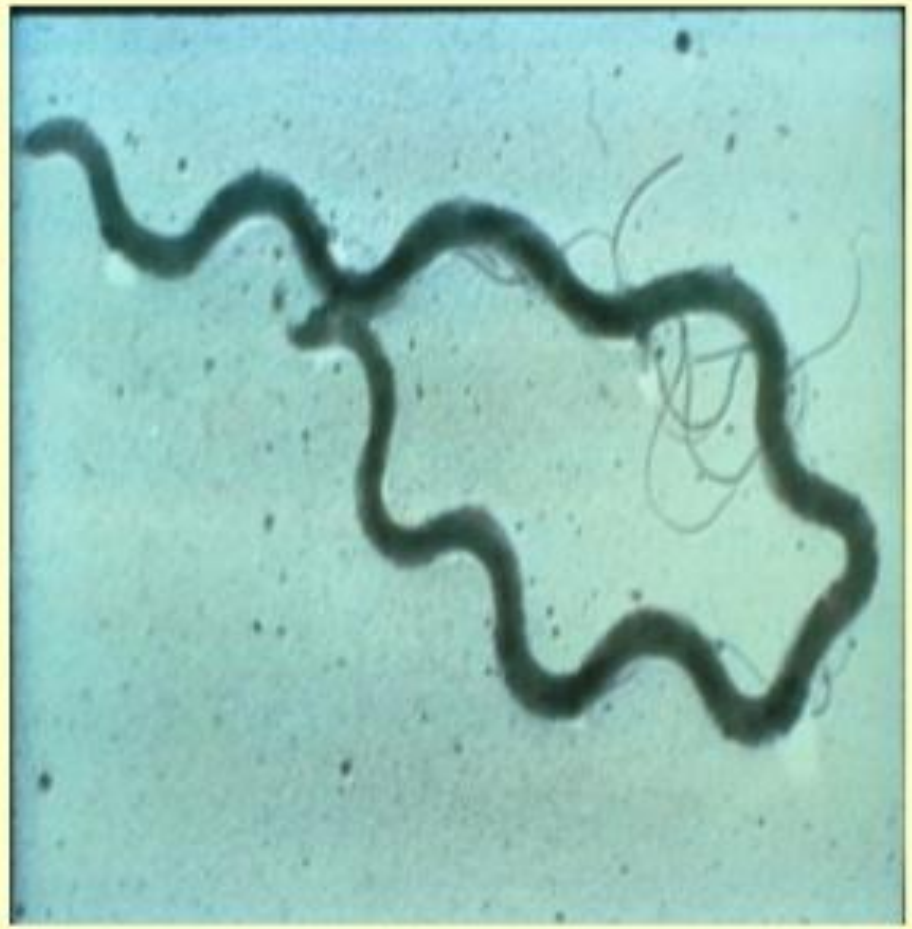
Size: 0.2 μm in width and 5-l5 μm in length

Structure

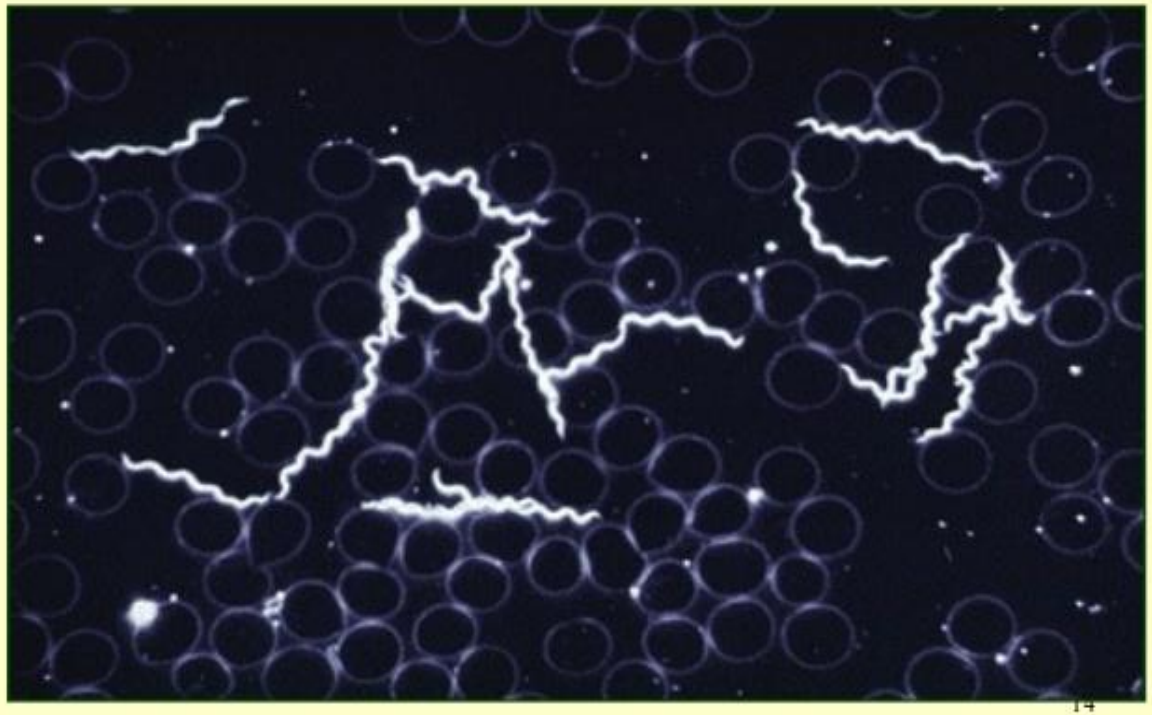
- Multilayer cytoplasmic membrane
- Flagella-like fibrils
- Cell wall
- Outer sheath (outer cell envelope)
- Capsule-like outer coat

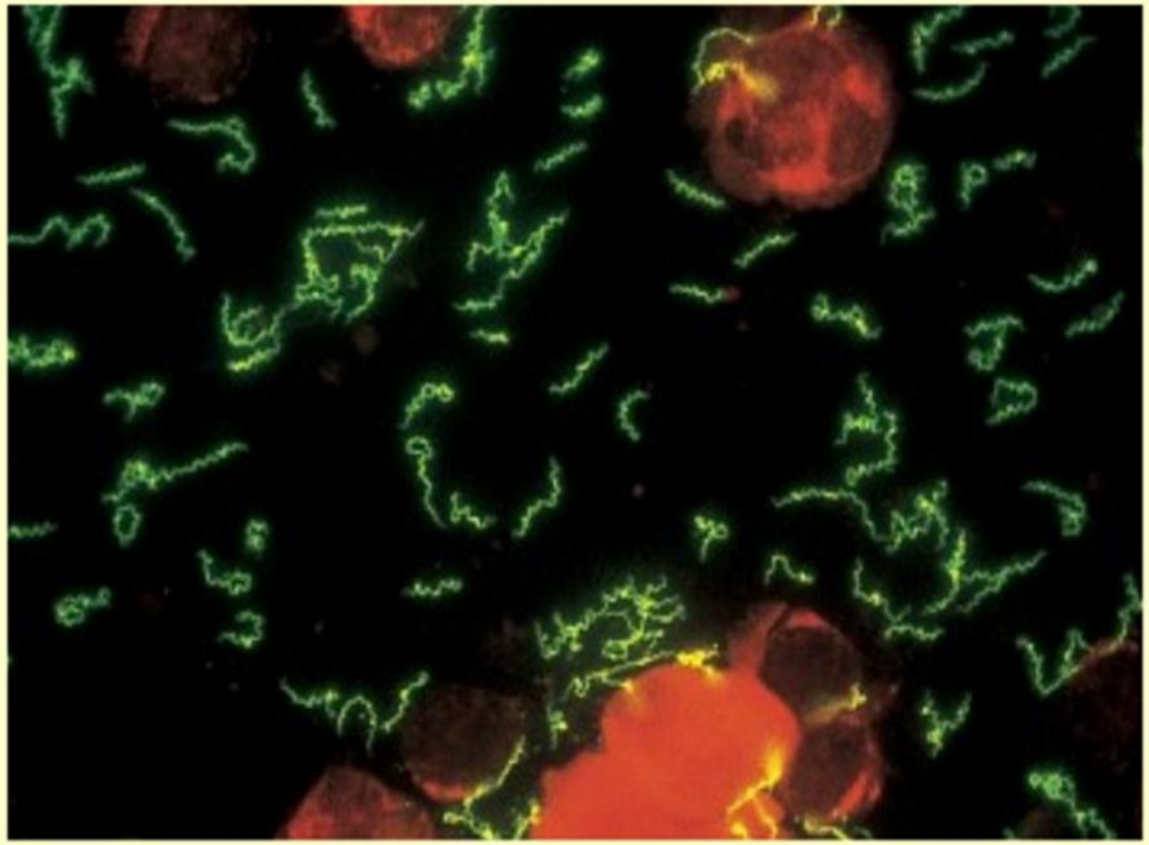
Treponema pallidum

Spiral spirochete can be seen on fresh primary or secondary lesions by **dark field microscopy or fluorescent antibody techniques**



Dark field Microscopy





***T. pallidum* in the direct fluorescent antibody test**

Epidemiology

- Humans are the only natural host
- Syphilis transmitted by sexual contact or congenitally
- Syphilis occurs worldwide, with no seasonal incidenc

Diseases

- Syphilis presents as primary disease (painless ulcer [chancre] at site of infection, with regional lymphadenopathy and bacteremia), secondary syphilis (flulike syndrome with generalized mucocutaneous rash and bacteremia), and late-stage disease (diffuse chronic inflammation and destruction of any organ or tissue); congenital (latent multiorgan malformations, fetal death)

CLASSIFICATION

Syphilis is majorly classified in to two types namely:

- Veneral syphilis.
- Non-veneral syphilis.

VENERAL SYPHILIS:

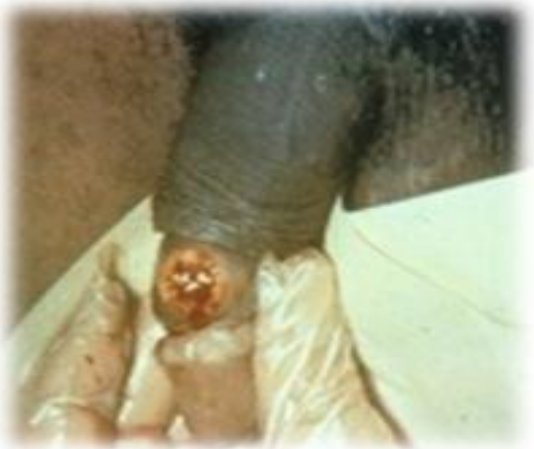
The disease falls in to 3 stages namely:

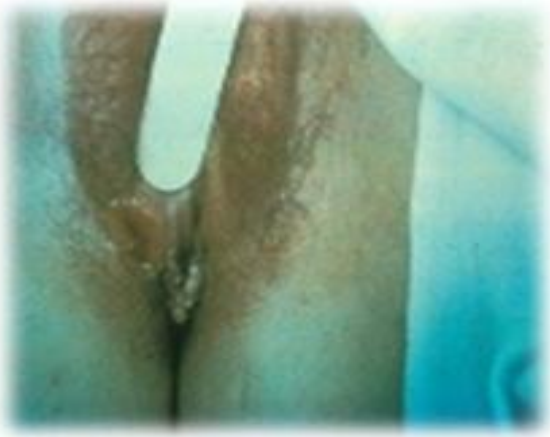
- Primary stage.
- Secondary stage.
- Tertiary stage.

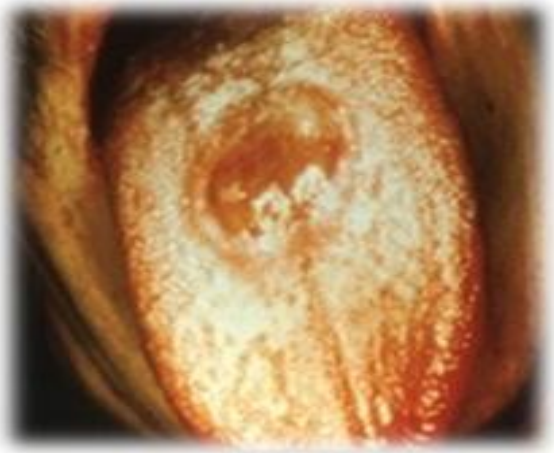
PRIMARY SYPHILIS:

- Primary lesion or "chancre" develops at the site of inoculation.
- **Chancre:**
  - Progresses from macule to papule & then to ulcer.
  - Typically painless, indurated, and has a clean base.
  - Highly infectious.
  - Heals spontaneously within 1 to 6 weeks.
- Regional lymphadenopathy: classically rubbery, painless, bilateral.

Primary syphilis- chancre, labial chancre,tongue



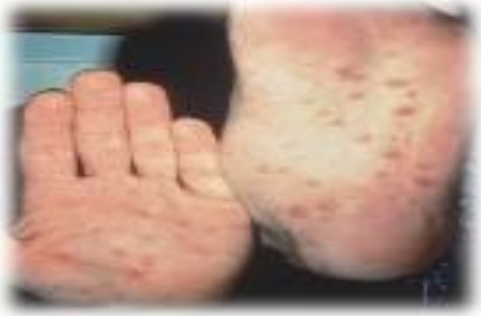


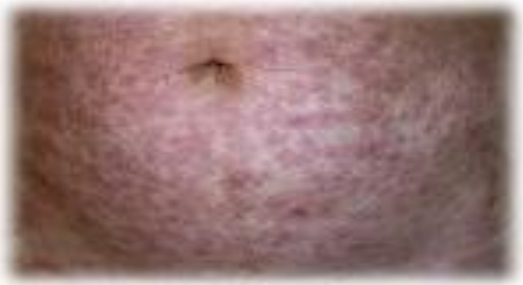


SECONDARY SYPHILIS:

- Secondary lesions occur 3 to 6 weeks after the primary chancre appears; may persist for weeks to months
- Mucocutaneous lesions are most common
- Manifestations:
  - Rash (75%-100%)
  - Lymphadenopathy (50%-86%)
  - Mucous patches (6%-30%)
  - Alopecia (5%)
- Serologic tests are usually highest in titer during this stage

Secondary Syphilis:Palmar/Plantar,generalised body rash





Secondary Syphilis alopecia,Nickel/Dime Lesions

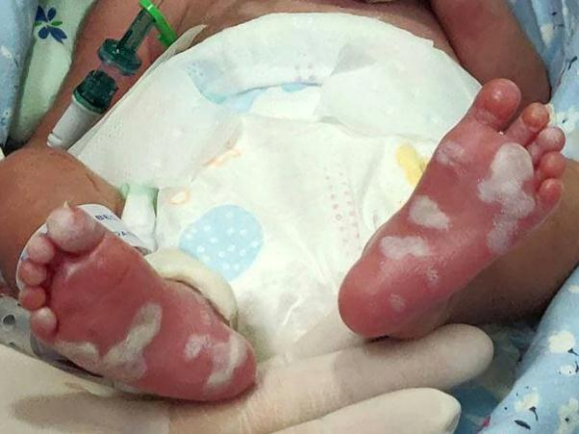

Tertiary (Late) Syphilis

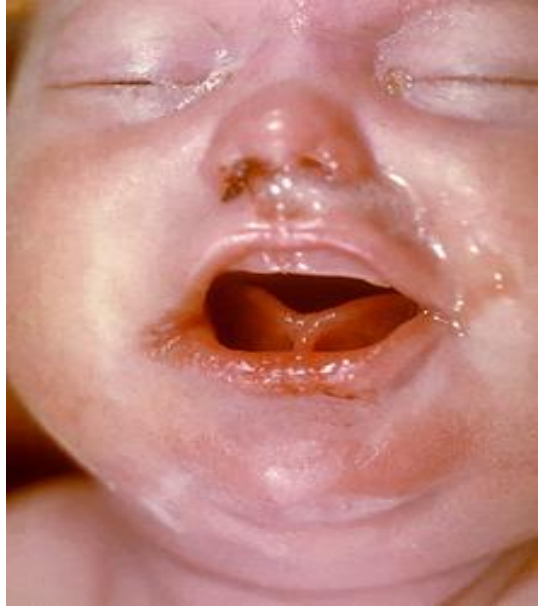
- Approximately 30% of untreated patients progress to the tertiary stage within 1 to 20 years
- Rare because of the widespread and use of antibiotics
- Manifestations
  - Gummatous syphilis (15%)
  - Cardiovascular syphilis (10%)
  - Late neurosyphilis (6.5%)

Ulcerating gumma, cardiovascular

Latent Syphilis

- Host suppresses the infection enough so that no lesions are clinically apparent
- Only evidence is positive serologic test for syphilis
- May occur between primary and secondary stages, between secondary relapses, and after secondary stage
- Categories:
  - Early latent: <1 year duration
  - Late latent: ≥1 year duration





- Occurs when *T. pallidum* is transmitted from a pregnant woman with syphilis to her foetus
- May lead to stillbirth, neonatal death, and infant disorders such as deafness, neurologic impairment, and bone deformities
- The risk is much higher during primary and secondary syphilis

Congenital Syphilis

Hydrops fetalis

Nasal discharge

Rash

Hepatomegaly

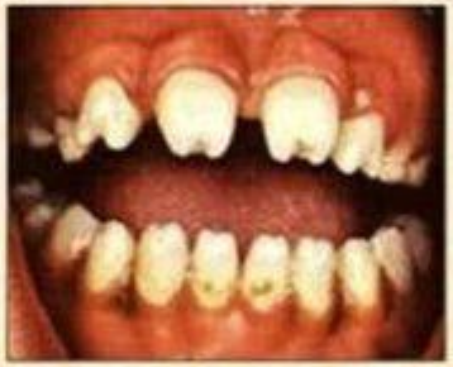
Petechial rash

Necrotizing funisitis within the matrix of the umbilical cord

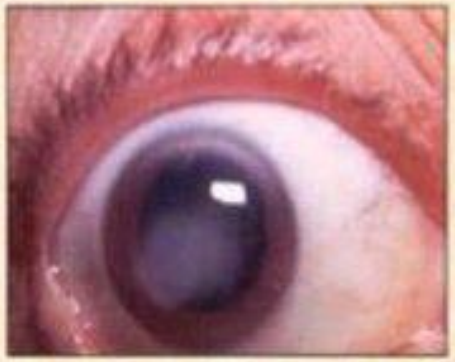
Osteitis, Metaphysitis, Periostitis Wimberger sign

HUTCHINSON'S TRIAD

Seen in late congenital syphilis



Hutchinson's teeth
- Peg-shaped, notched incisors



Interstitial keratitis

Eighth-nerve deafness

Signs & Symptoms

- Signs & symptoms of syphilis vary depending in which of the four stages (primary, secondary, tertiary, latent) it is present:

Common symptoms are:

- Fever, Malaise, Sore throat, Rashes, Head ache
- Lymphadenopathy
- Mucous patches, Perforation of palate.
- Alopecia, Weight loss
- In severe conditions it causes mental retardation, shuffle walk e.t.c.

Diagnostic Tests for Syphilis

Diagnostic Test	Method or Examination
Microscopy	Darkfield Direct fluorescent antibody staining
Culture	Not available
Serology	Nontreponemal tests: VDRL test RPR test USR test TRUST Treponemal tests: FTA-ABS TP-PA test EIA

EIA, Enzyme immunoassay; *FTA-ABS*, fluorescent treponemal antibody-absorption; *RPR*, rapid plasma reagain; *TP-PA*, *Treponema pallidum* particle agglutination; *TRUST*, toluidine red unheated serum test; *USR*, unheated serum reagin; *VDRL*, Veneral Disease Research Laboratory.

Laboratory Diagnosis

- Identification of *Treponema pallidum* in lesions
  - Darkfield microscopy
  - Direct fluorescent antibody - *T. pallidum* (DFA-TP)
- Serologic tests
  - Nontreponemal tests (qualitative and quantitative)
  - Treponemal tests (qualitative)

Darkfield Microscopy

What to look for:

T. pallidum morphology and motility

Advantage:

- Definitive immediate diagnosis

Disadvantages:

- Requires specialized equipment and an experienced microscopist
- Possible confusion with other pathogenic and nonpathogenic spirochetes
- Must be performed immediately
- Generally not recommended on oral lesions

Syphilis Serology

Non-treponemal tests

- VDRL (Venereal Disease Research Laboratory)
- RPR (Rapid Plasma Reagin)
- TRUST (Toluidine Red Unheated Serum Test)
- USR (Unheated Serum Reagin)

Treponemal tests

- TP-PA (Treponema Pallidum Particle Agglutination)
- FTA-abs (Fluorescent Treponemal Antibody -Absorbed)
- EIA (Enzyme Immunoassay)

Nontreponemal Serologic Tests

Principles

- Measure antibody directed against a cardiolipin-lecithin-cholesterol antigen
- Not specific for *T. pallidum*
- Titers usually correlate with disease activity and results are reported quantitatively, may be reactive in life

Treponemal Serologic Tests

Principles

- Measure antibody directed against *T. pallidum* antigens
- Qualitative, usually reactive in life

TREPONEMA PALLIDUM AGGLUTINATINATION (TPA) TEST:

- In this test, formalin killed T.pallidum is used as an antigen.
- It is mixed with patients serum & incubated.
- After incubation it is examined under dark ground microscope.
- Agglutination indicates positive test.

TREPONEMA PALLIDUM IMMOBILISATION (TPI) TEST:

- Patients serum is incubated anaerobically with treponemal suspension.

Treatment, Prevention, and Control

- Penicillin is the drug of choice; doxycycline is administered if patient is allergic to penicillin
- Safe sex practices should be emphasized, and sexual partners of infected patients should be treated
- No vaccine is available

PROPHYLAXIS:

- There is no vaccine against syphilis. The disease can be prevented by:
- ✓ The use of mechanical barriers, like condoms.
- ✓ Avoidance of sexual contact with infected persons.
- ✓ While the WHO recommends all women to be tested at the 1st antenatal visit & again in the 3rd trimester.

NON-VENERAL SYPHILIS

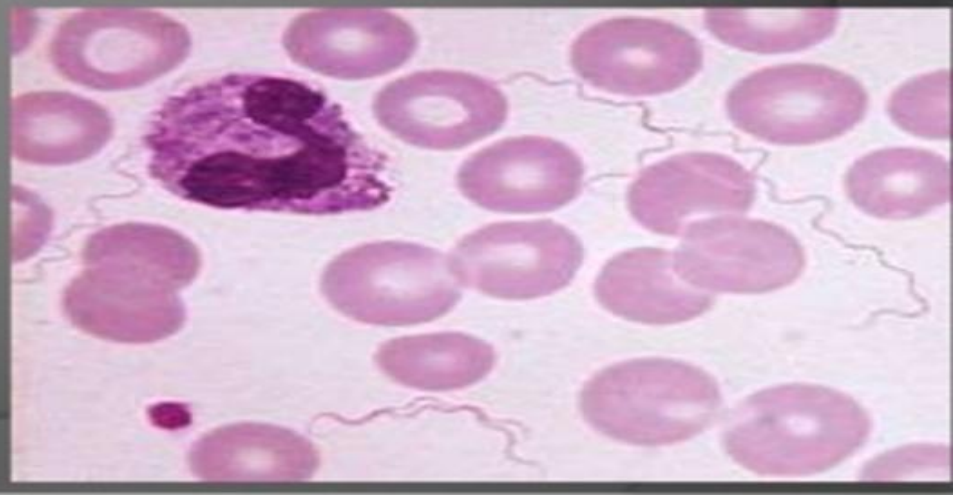
- Causative organism *is T.endemicum.*
- The disease is called by different names in different parts of the world & is more common in children of poor hygiene.
- Clinical manifestations include mucous patches in the mouth & skin eruptions,may progress to gummatous lesions on skin ,bone & nasopharynx.
- Congenital syphilis are not common.

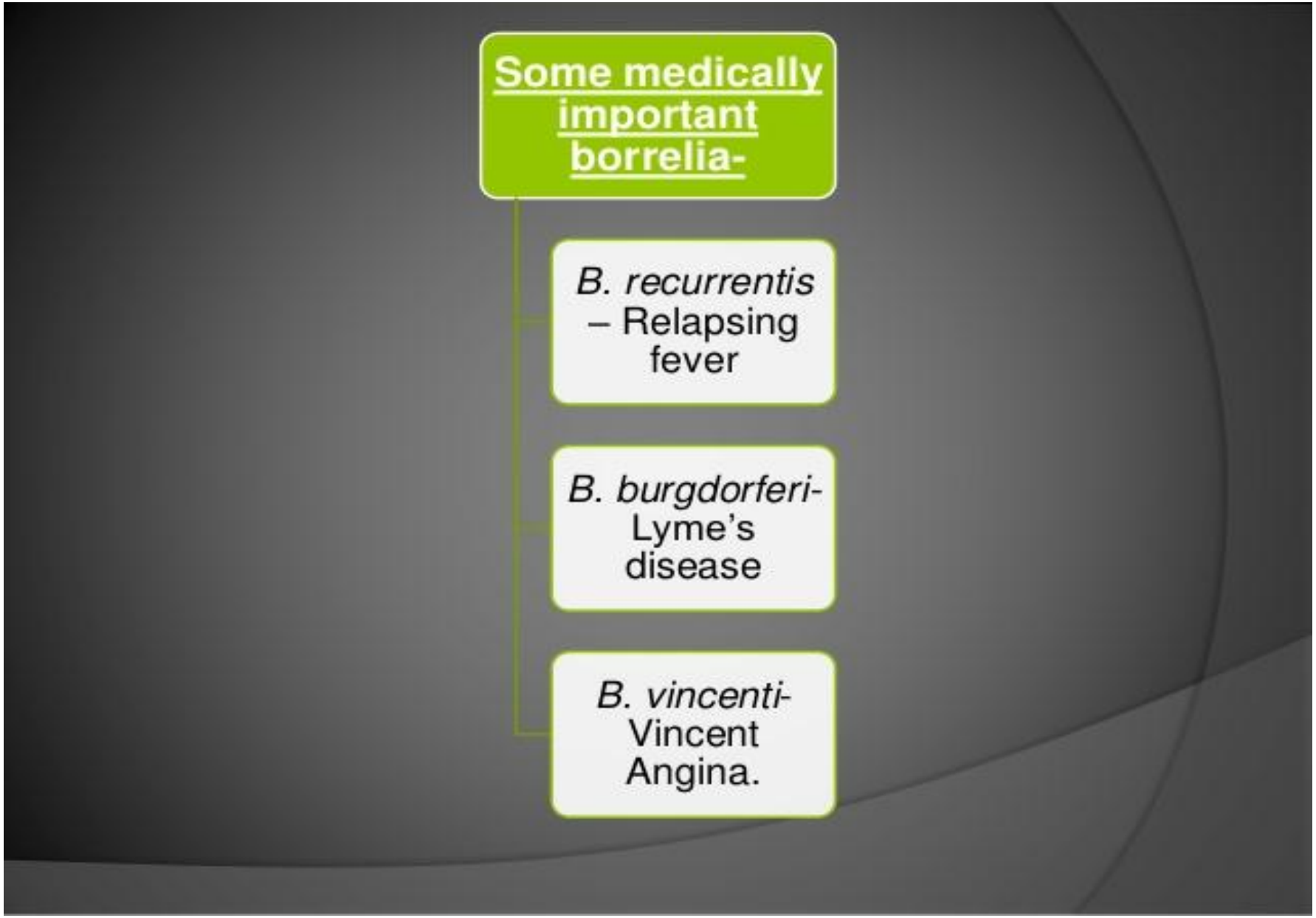
- Laboratory diagnosis & treatment of endemic syphilis are similar to those of veneral syphilis.

BORRELIA

Introduction

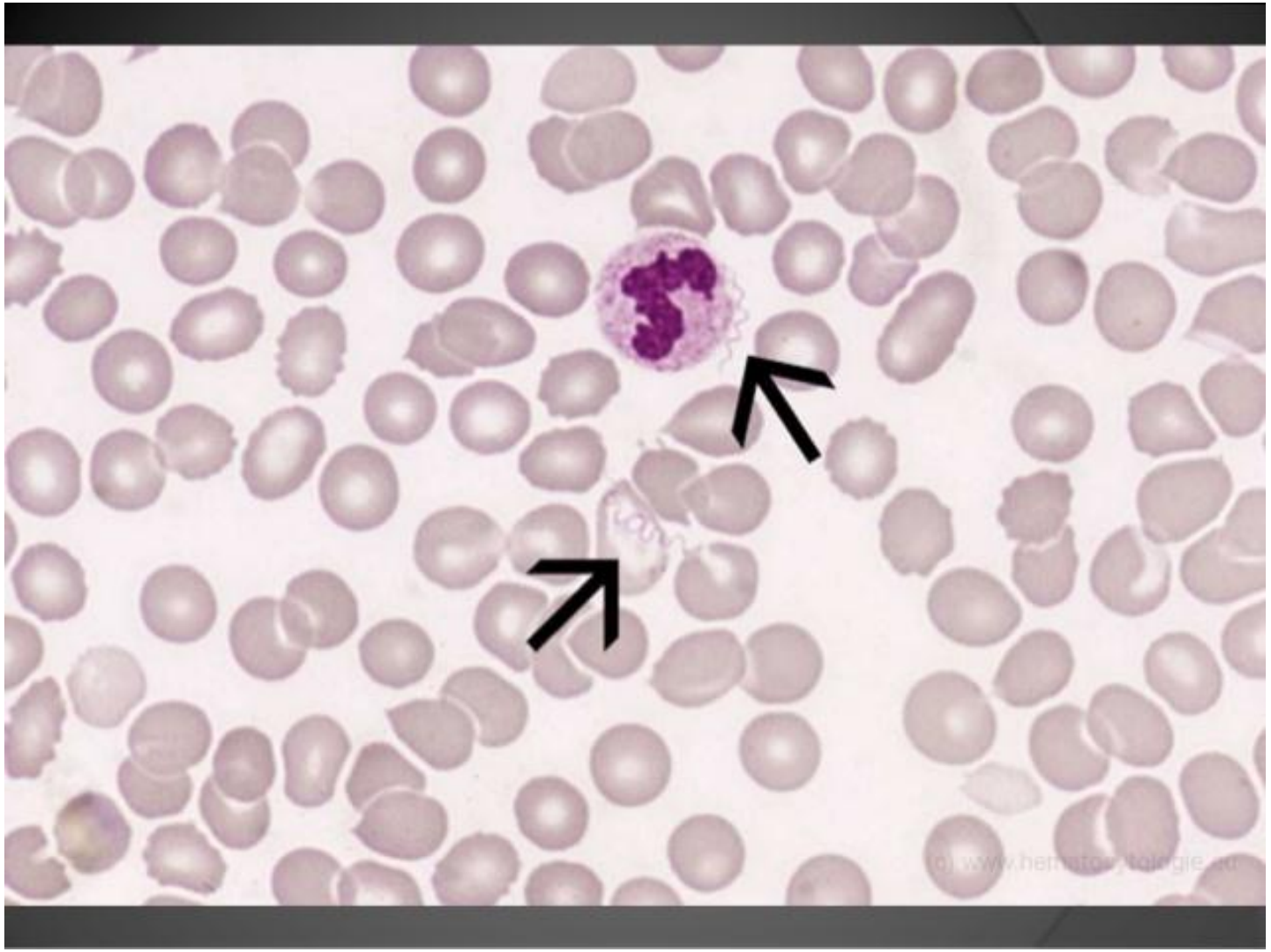
- Borrelia spp are large, motile, refractile spirochetes with irregular wide open coils.
- Measuring about 0.2-0.3um in diam. & 3-20um in length.
- 3-10 loose coils with 15-29 periplasmic flagella.
- Gram negative & stained well with Giemsa stain.




Some medically important borrelia-
B. recurrentis – Relapsing fever
B. burgdorferi- Lyme's disease
B. vincenti- Vincent Angina.

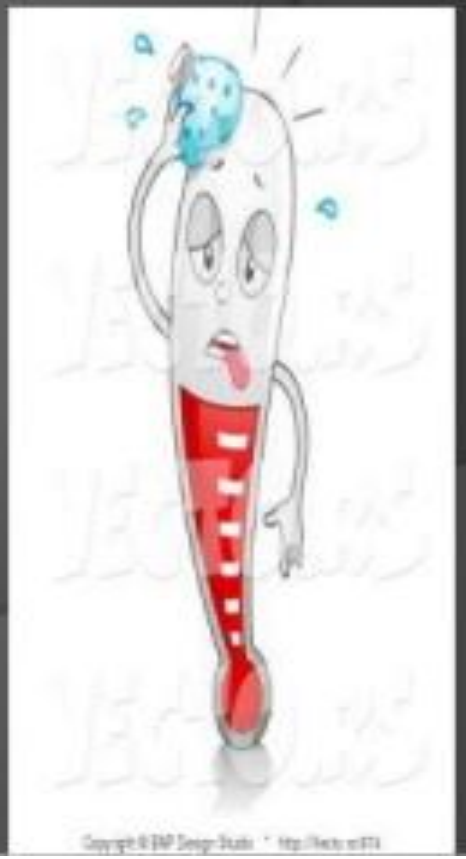
- ***Borrelia recurrentis-***
- Morphology-
  - Irregular spiral with one or both ends pointed.
  - Possesses 5-10 loose spiral coils at interval of about 2mm
- Cultural characteristics-
  - Microaerophilic, temp- 28-30 ℃
  - Cultivation is difficult but can be cultivated on 'modified Kelly's medium'
  - Grows well on CAM of chick embryos.
  - Inoculated in mice & rats intraperitoneally.

BORRELIA



Clinical features-

- Onset is typically abrupt (I.P.- 2-10 days)
- **High fever** (40 ℃) (borrelia are demonstrable)
- Shaking chills, delirium, severe muscle aches, pain in bone & joints
- Hepatosplenomegaly
- Neurologic complications
- Fever subsides in 3-5 days
- **Afebrile period** (4-10 days)(disappearence)
- **Relapse**(reappearence)
- 3-10 relapses
- Disease subsides



- **Epidemology-**
  - Poverty, overcrowding & lack of personal hygiene
  - Epidemic were common during war & in jails
  - Louse infestation is severe than tick
  - In lice borrelia does not get shed in saliva
  - No transovarial tansmission in lice.
  - Indian tick vectors- *Ornithodorus tholozoni, crossi, lahorensis.*

Relapsing Fever

- ***Relapsing fever:*** An acute, infectious, bacterial (spirochete) disease characterized by alternating febrile periods and non febrile periods.
- It is also known as recurrent fever or tick fever.

Types of Relapsing Fever

- There are 2 types of relapsing fever:
    - o Louse-borne relapsing fever
    - o Tick born relapsing fever

Transmission

Louse-borne Relapsing Fever

• Louse-borne relapsing fever is transmitted by the human head – Pediculus capitis and the common body louse; Pediculus corporis.

• Louse-borne relapsing fever is transmitted from person to person by the human louse.

• Both types of relapsing fever are caused by spirochaetes of the genus Borrelia; louse - borne carry Borrelia recurrentis.

• The spirochaetes are taken up when the louse feeds on the blood of an infected person.

• They then multiply within the body of the louse but are not present in the saliva or coxal fluid.

• This louse only infects another person when it is crushed on the body near the bite wound. The organisms are not transmitted to the offspring of the lice.

• It tends to occur in epidemics.

Tick Born Relapsing Fever

• Tick born relapsing fever is transmitted by soft ticks called Ornithodorus moubata

• Tick-borne relapsing fever is transmitted when the tick sucks blood from an infected person and the spirochaetes are taken up and multiply in the body of the tick

• Ticks carry Borrelia duttoni

• The spirochetes pass into the ovary of the tick and the offspring of an infected tick are automatically infected without themselves having sucked infectious blood i.e. transovarian or vertical transmission

• Ticks remain infectious for the rest of its life

• In this way, a house once inhabited by infectious ticks can remain dangerous for many years if no intervention

• Within one week after sucking infected blood spirochaetes appear in the tick's salivary glands and in the coxal fluid ready to be transmitted to a new host

cont...

The organisms can either be injected directly when the tick feeds on the host, or they invade the body through intact mucous membrane. (e.g., in laboratory infections: Duttoni, the discoverer of the disease died from it)

- In humans, the spirochaetes can cross the placenta from mother to foetus

- This may result in abortion, stillbirth, premature delivery or congenital infection in the newborn

Clinical Features of Relapsing Fever

SYMPTOMS

- Fever
- Headaches
- Arthralgia/myalgia
- Dry cough
- Epistaxis/gum bleeding

SIGNS

- Temperature
- Tachycardia
- Hepatomegaly
- Splenomegaly
- Petichea/ Subconjunctival bleeding
- Jaundice
- Confusion/Meningism

Relapsing Fever

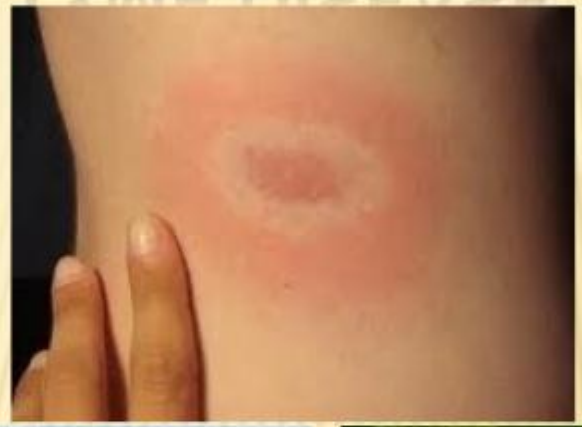
Characteristic	Louseborn	Tickborn
Epidemology	Epidemic	Usually endemic
Agent	*B. Recurrentis*	*B. hermesii, B. turicatae, B. parkeri*
Route of entry	Crushing & rubbing on abraded skin	Through bite
Shedding in saliva & discharges	No	Yes
Transovarial transmission	No	Yes
Clinical features	More severe	Less severe

Lab diagnosis-

- *Borrelia* can be found in blood during fever
- Drop of blood- Dark ground OR Phase contrast microscopy
- Blood smears- Giemsa/Leishman/dilute Carbol fuchsin
- Inoculation of 1-2 ml blood into white mice & smear is prepared from blood collected from tail of vein after 2 days, observed daily for 2 weeks.
- Fluoroscent procedures
- Serology & cultures are unreliable.
- False positive reaction for syphilis(VDRL)

- **Prophylaxis-**
  - Prevention of louse infestation using insecticides.
  - Identification & avoidance of tick infested places
- **Treatment-**
  - Tetracyclines, chloramphenicol, penicillin, erythromycin are effective.

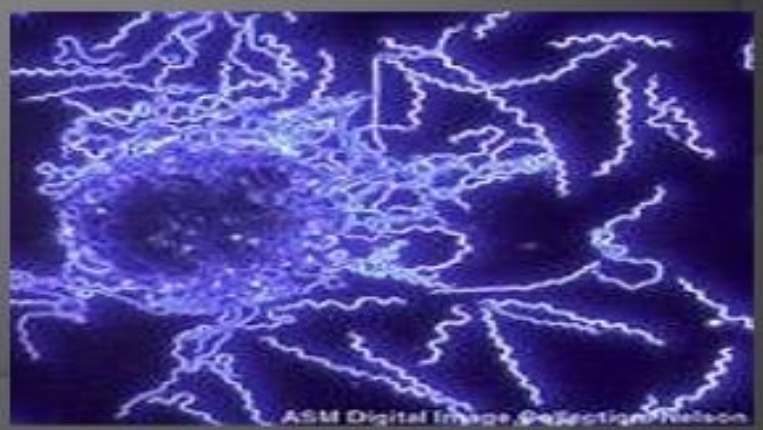
LYME DISEASE

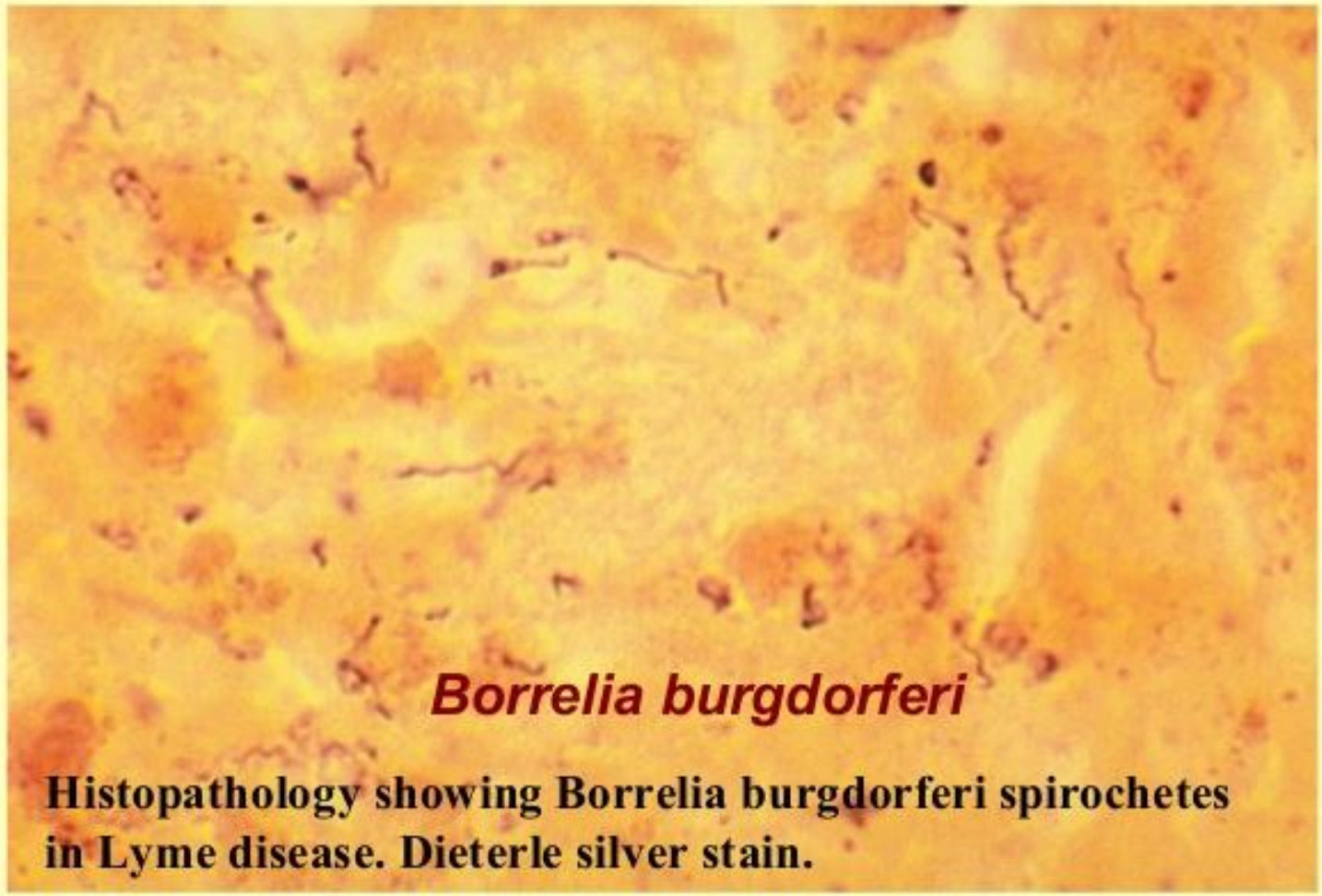


Lyme's Disease

- Identified in 1975 in Lyme , Connecticut , USA.
- Is a most common vector born disease in USA
- Causitive agent- *Borrelia burgdorferi*

  -B.garinii, B.afzeli





Histopathology showing Borrelia burgdorferi spirochetes in Lyme disease. Dieterle silver stain.

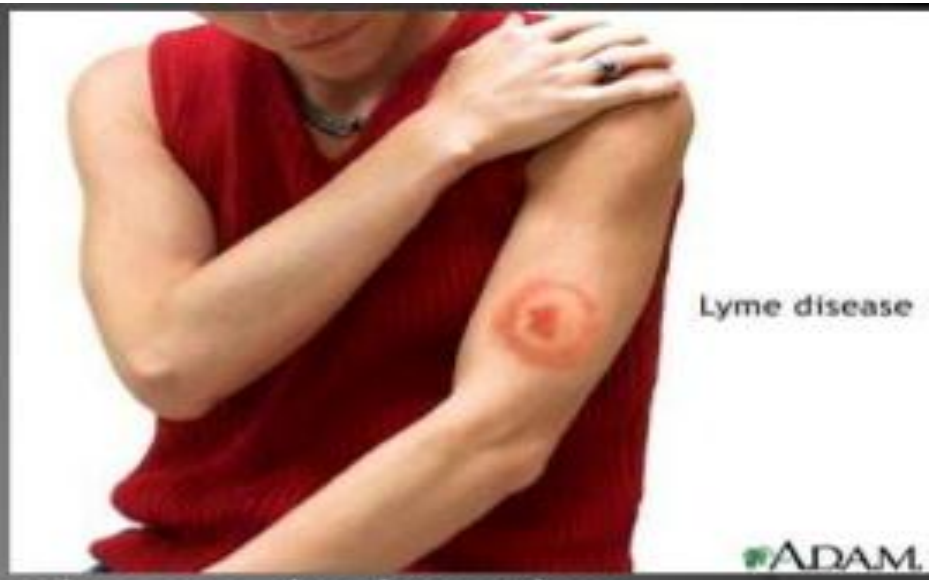
- **Epidemology-**
- Vector- Ixodid tick

- Borrelia grows mainly in midgut of the tick.
- Infection occurs by regurgitation of the gut content during biting.
- Most commonly found in North eastern states in USA.
- No vertical transmission in ticks.
- Most effective tick stage of transmission is - nymph

- **Clinical disease-**
- I.P.-3-30 days.
- Three stages-

1) Localized infection-

-'Erythema chronicum migrans'.



Lyme disease

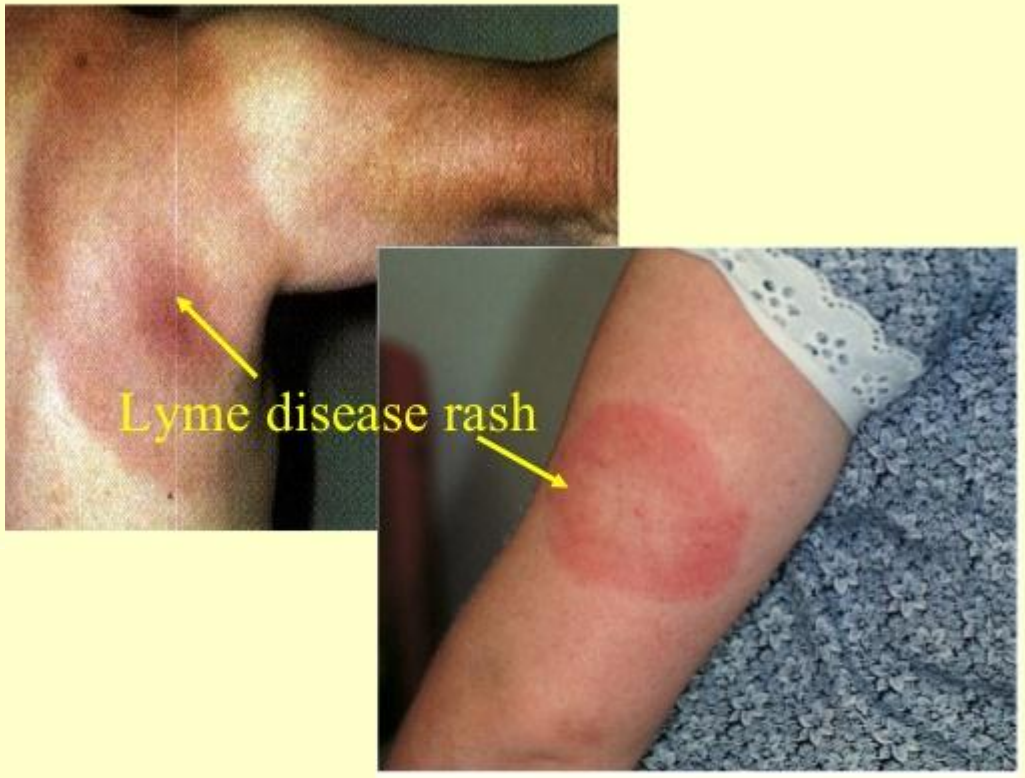
-macule at the site of bite with redness, induration.

2) Disseminated infection-

-fever, headache, myalgia , arthralgia, lymphadenopathy.

-Most common lesions are meningitis & arthritis.


Hard tick

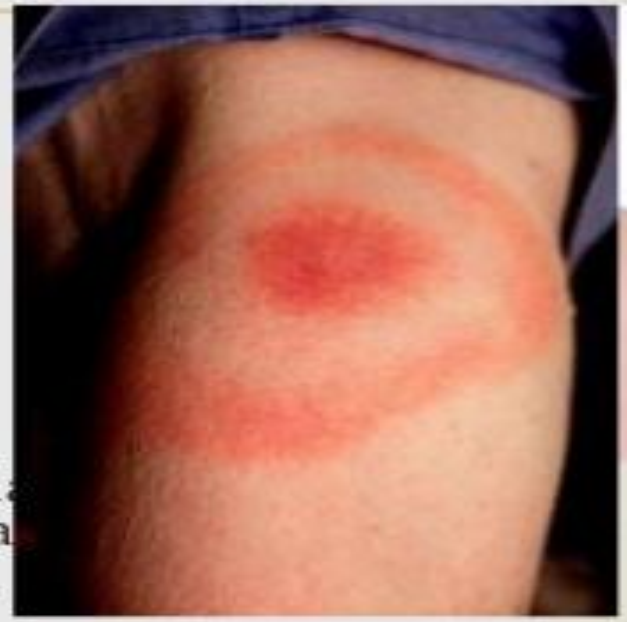

Lyme disease rash

Typical symptoms

- Fever
- Headache
- Fatigue
- Erythema migrans

Erythema migrans (EM) or "bull's-eye" rash

- Erythema migrans Rash occurs in approximately 70 to 80 percent of infected persons and begins at the site of a tick bite



- Rash gradually expands over a period of several da reach up to 12 inches (30 cm) across. Parts of the ra it enlarges, resulting in a "bull's-eye" appearance.
- Rash usually feels warm to the touch but is rarely itchy or painful.
- EM rash may appear on any area of the body.

Lab diagnosis-

- Culture- modified Kelly's medium
  -Most effective in early Lyme's disease
- Morphologic detection- silver impregnation method
  - Insensitive method.
- Molecular detection- more sensitive method
- Serologic detection- diagnostic method of choice.
  -EIA, Immunofluoroscence, Immunoblot tech.
- Cross reactions-
  -specific treponemal Ag, HIV, EBV, ricketssial infections.

- **Treatment-**
  - Doxycycline, amoxycilline & cefuroxime

LEPTOSPIRA INTERROGANS

TAXONOMY

Classification

Phylum: Spirochaetes

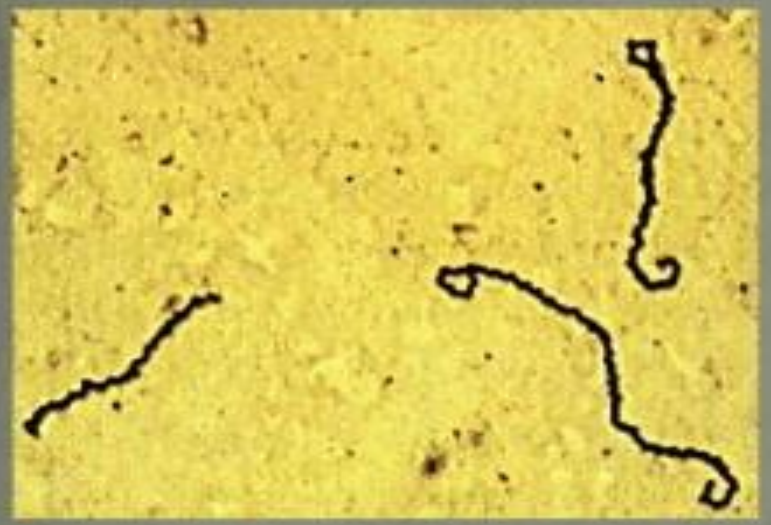
Class: Spirochaetes

Order: Spirochaetales

Species: *Leptospira*

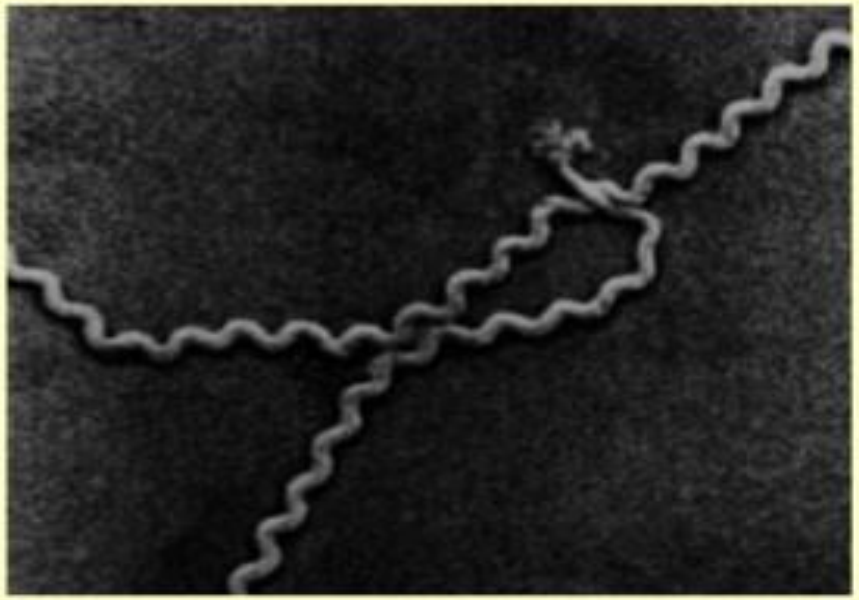
Family: Leptospiraceae

PHYSIOLOGY AND STRUCTURE



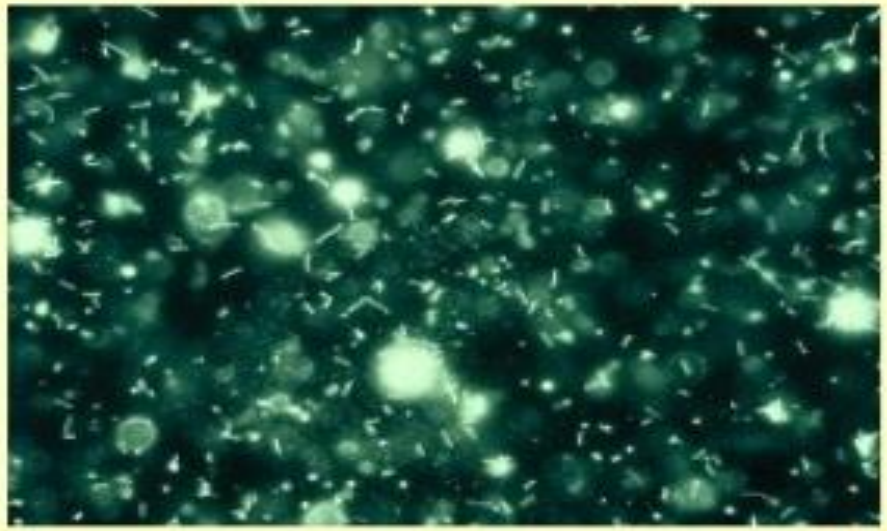
- Thin, coiled spirochetes (.1 ×6 to 20 μm)that grow slowly in culture.
- Temperature(28°C to 30°C)
- Gram-negative spirochetes
- Obligate aerobes and Characteristic hooked ends .
- Pathogenic strains: Leptospira interrogans.
- Non pathogenic strains: Leptospira biflexa.

Leptospira under the Microscope



Long, Thin, Highly Coiled

Dark Field Microscopy FL



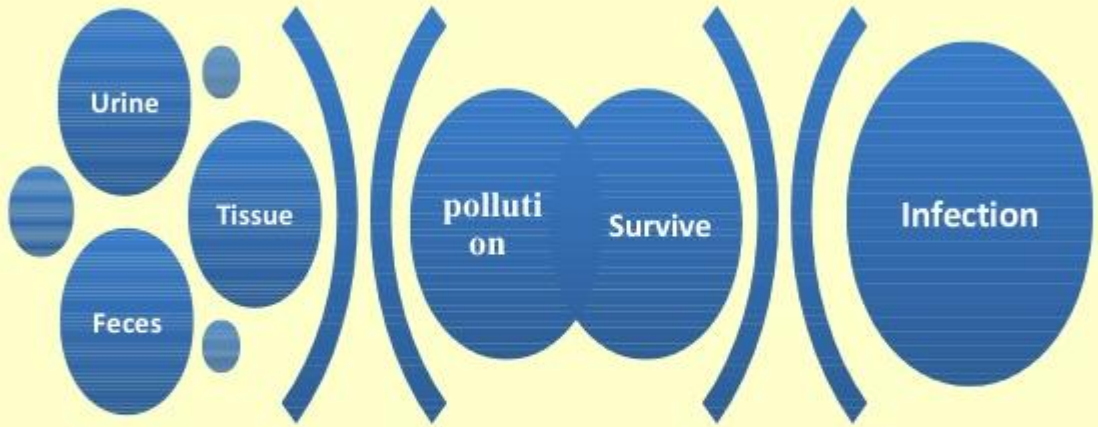
VIRULENCE FACTOR

- Direct invasion and replication in tissues (i.e.) virulence unknown.
- Immune complex produces renal disease.

EPIDERMIOLOGY

- Mainly a zoonotic disease
  - Transmitted to humans from a variety of wild and domesticated animal hosts
  - Most common reservoirs rodents (rats), dogs, farm animals and wild animals
- Organism can penetrate the skin through minor breaks in the epidermis or with intact mucus membranes
- Indirect contact (soil, water, feed) with infected urine from an animal with leptospiruria
- Occupational disease of animal handling and more common during warm months

Transmission



Human infection is accidental

No human to human transmission

PATHOGENESIS OF LEPTOSPIROSIS

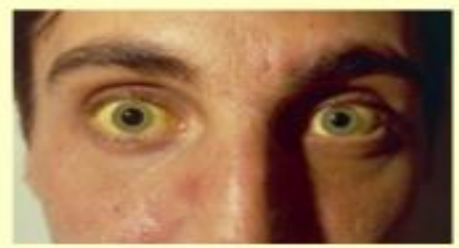
- Leptospirosis, also called Weil's disease in humans
- Its directly invaded and replicated in tissues
- Characterized by an acute febrile jaundice and glumerulonephritis
- Incubation period usually 10-12 days with flu-like illness usually progressing through two clinical stages:
  i. Leptospiremia develops rapidly after infection (usually lasts about 7 days) without local lesion
  ii. Infects the kidneys and organisms are shed in the urine (leptospiruria) with renal failure and death not uncommon
- Hepatic injury & meningeal irritation is common

CLINICAL DISEASES

- Mild virus-like syndrome
- (Anicteric leptospirosis) Systemic with aseptic meningitis
- (Icteric leptospirosis) Overwhelming disease (Weil's disease)
  - ✓Vascular collapse
  - ✓Thrombocytopenia
  - ✓Hemorrhage
  - ✓Hepatic and renal dysfunction

NOTE: Icteric refers to jaundice (yellowing of skin and mucus membranes by deposition of bile) and liver involvement

Leptospirosis



Microbiological Diagnosis

Specimens :

- **Blood (the first week of illness)**
- **Urine (the second week of illness)**
- **CSF (the patient with meningeal irritation sign)**

Etiological [ˌi:tɪ'ɒlədʒɪ] examinations

- △ **Direct microscopy**
  - ◎ **Dark-ground microscopy**
  - ◎ **Silver stain**
  - ◎ **Fluoresent antibody staining**
- △ **Culture isolation and identification : Korthof liquid medium**
- △ **Animal test**
- △ **Molecular diagnostics**

Serological examinations

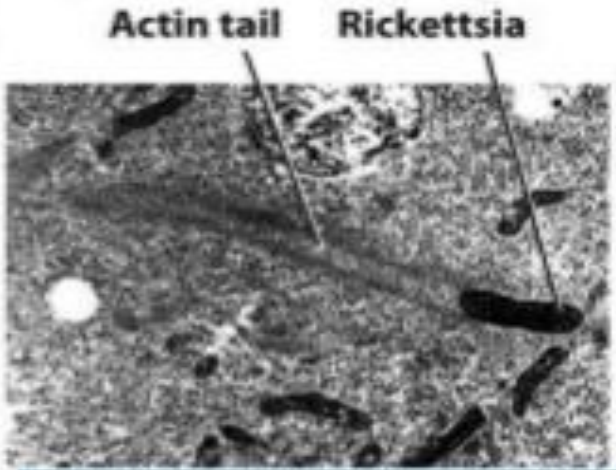
Paired serum

- △ **Microscopic agglutination test （MAT ）**
- △ **Indirect agglutination test**
- △ **Complement fixation test**
- △ **IFA**
- △ **ELISA**

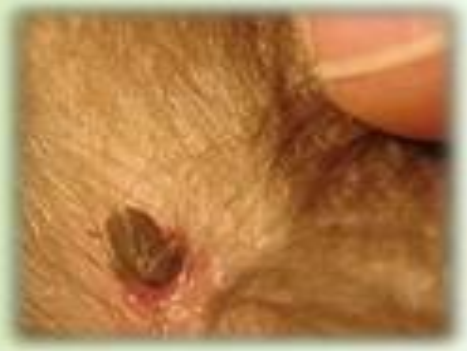
RICKETTSIA

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



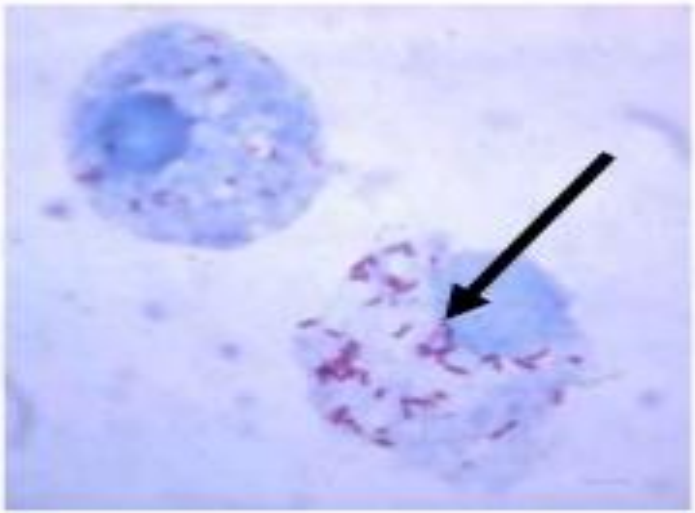
Rickettsia rickettsii



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

Introduction

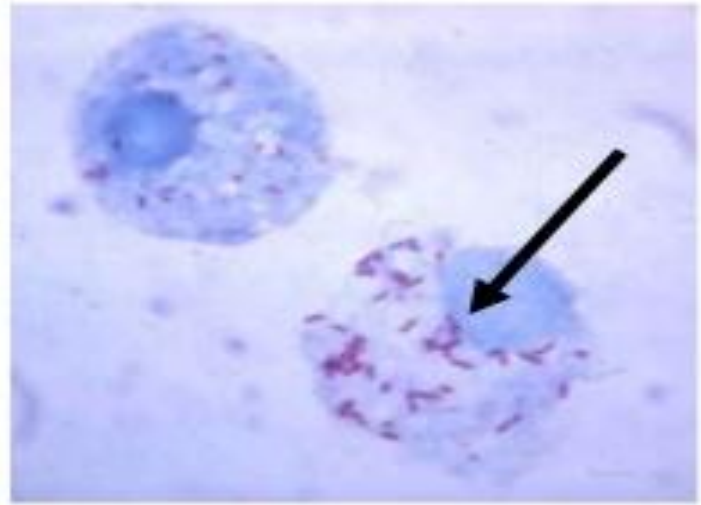
- Obligate intracellular parasite
- Gram negative pleomorphic rods
- Parasite of arthropods – fleas, lice, ticks and mites.
- No Human to human transmission.
- Despite the similar name, *Rickettsia* bacteria do not cause rickets, which is a result of vitamin D deficiency.
- In the past, positioned somewhere between viruses and true bacteria.



Rickettsia inside the host cell

Rickettsial characteristics:

- Obligate intracellular parasite.
- Gram negative pleomorphic bacteria.
- Most are zoonoses spread to humans by arthropods (except Q fever).
- Cannot grow in culture media, but cultivable only in living tissue.
- No human to human transmission.



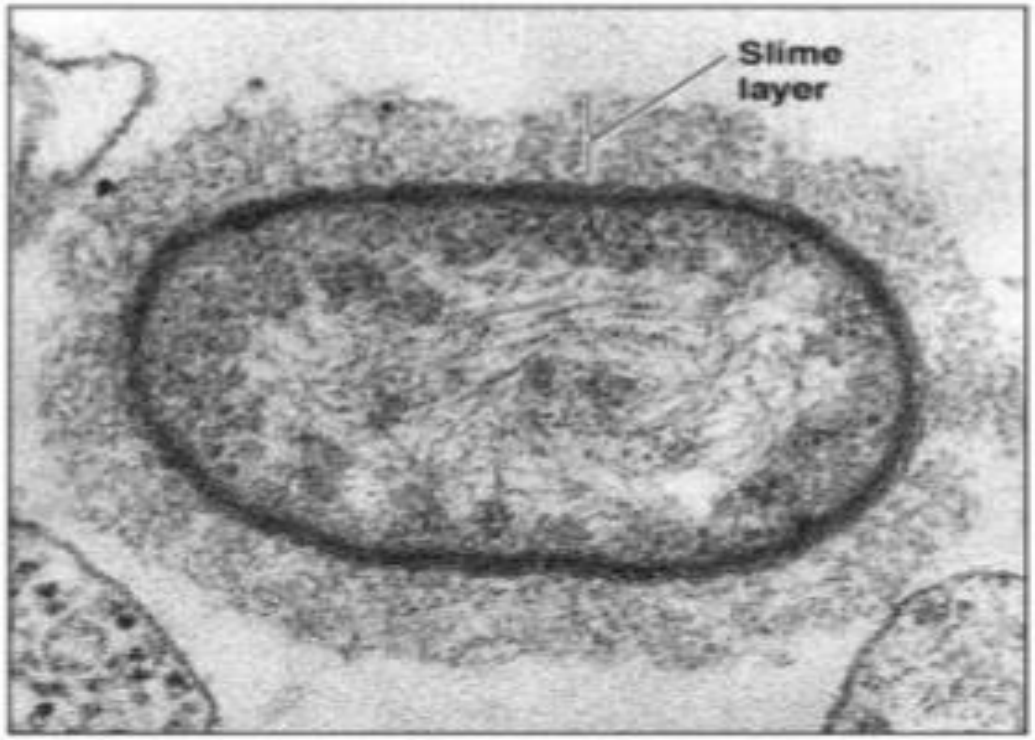
Rickettsia inside the host cell

TICK **FLEA** **LICE** **MITE**

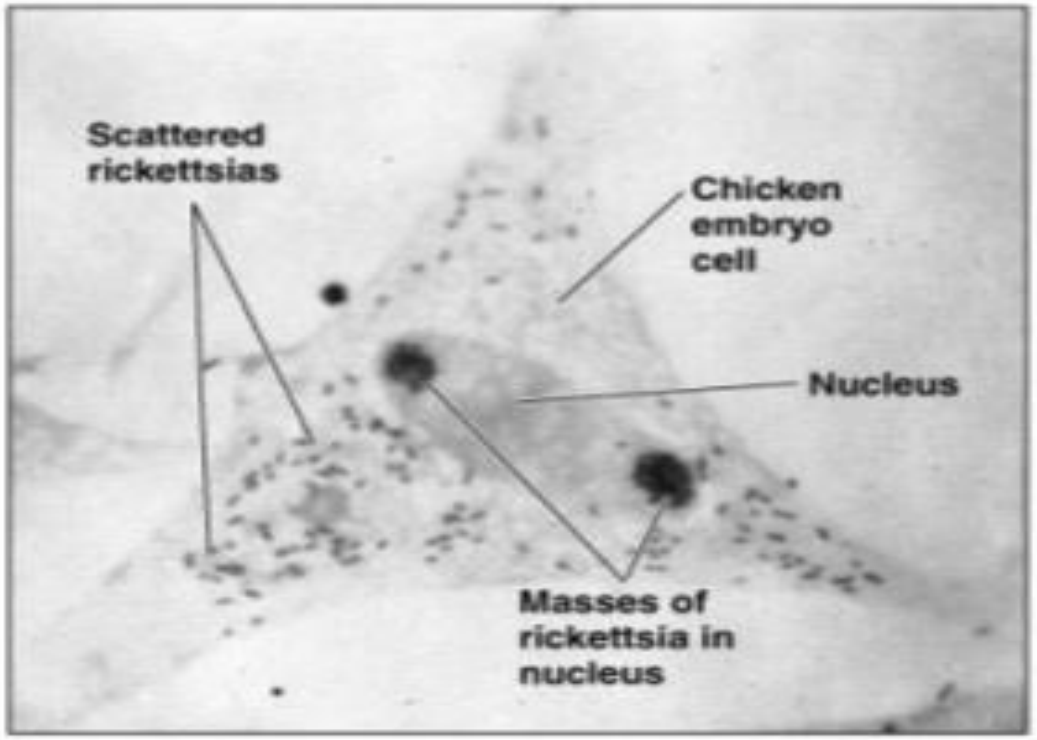
General characteristics

- 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

Microscopic figure



(a) Rickettsia



(b) Rickettsias in chicken embryo cell

Rickettsial species and its disease

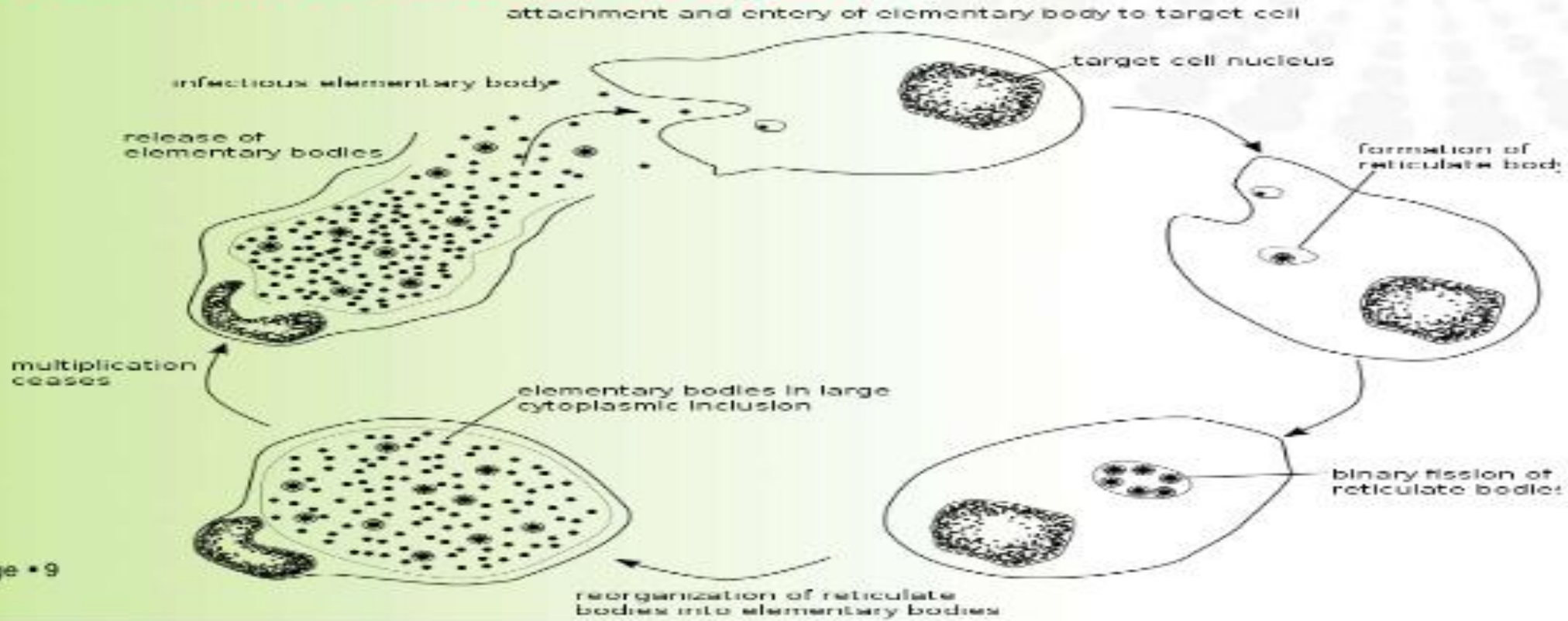
Species	Disease	Reservoir
R. prowazekii	Epidemic typhus, Brill-Zinsser disease	Human body louse
R. typhi	Endemic typhus	Rat flea
R. rickettsii	Rocky-Mountain spotted fever	Ticks
R. conori	Boutonneuse fever	Ticks
R. australis	Australian tick typhus	Ticks
R. siberica	Siberian tick typhus	Ticks
R. akari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



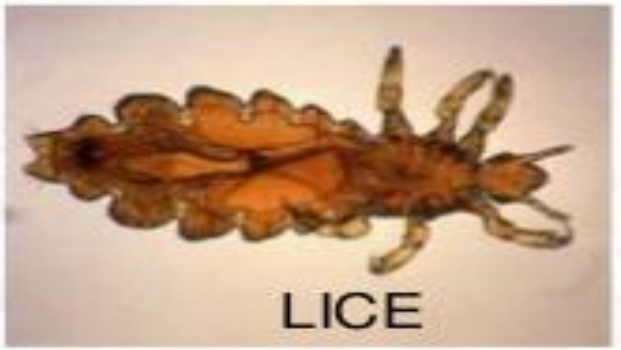
Rickettsial infections: Classification

- **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
- 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.


LICE

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.

Laboratory Diagnosis

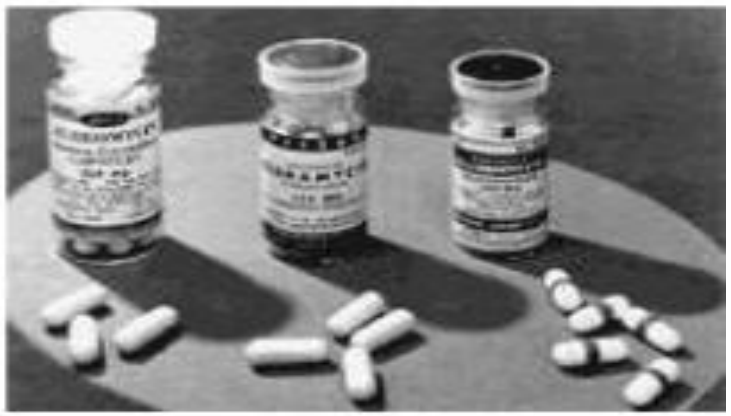
- Culture & isolation
- Serologic test

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*)

Treatment

- Adequate antibiotic therapy initiated early in the first week of illness is highly effective and is associated with the best outcome.
- Fever usually subsides within 24-72 hours after starting antibiotic therapy. If fever fails to subside with the use of a suitable antibiotic, the diagnosis of rickettsial disease should be reconsidered.
- Doxycycline is the drug of choice; it is preferred over other tetracyclines for treatment of rickettsial infections.
- Chloramphenicol may be used as an alternative.
- Recent data from Europe suggest that fluoroquinolones, such as ciprofloxacin and ofloxacin, may be effective in the treatment of certain rickettsial disease.



Q FEVER (QUERY FEVER)

Q fever

Etiology: *Coxiella burnetti*

Vector : None

Reservoir: Cattle, sheep, goat

MOT: ingestion of dust containing organisms or aerosols excreted in urine, feces, milk etc.

I.P:- 2-3 wks

C/F:- resembles influenza or non bacterial pneumonia

Individuals at risk : food handlers, veterinarians

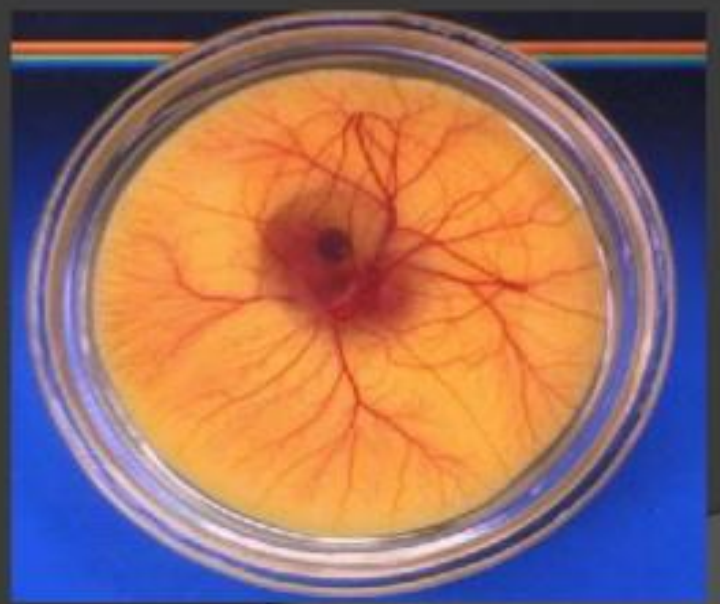
Infective endocarditis occasionally in chronic Q fever

Introduction

- ***Q Fever*** *is a disease caused by infection with Coxiella burnetii.*
- *Coxiella burnetii Obligate intracellular, gram negative bacterium*
- Q stands for Query or Queensland
- Origin of disease unknown
- First reported cases were in Queensland, Australia
- Distributed globally
- Found in many species of animals

Culture

- Grows well in yolk sac of chick embryos and in various cell cultures .



structure

- shows phase variation .
- phase – I ,II .
- phase – I :- autoagglutinable

  more immunogenic activity due to periodate sensitive trichloracetic acid-soluble surface carbohydrate .
- Phase – II :- more suitable for complement fixation test (CFT) .
- both phase I ,II elicit good Ab response .

Resistance

- Resistant to physical and chemical agents
- Can survive in dust and aerosols
- Inactivated by 2% formaldehyde

  5% H_2O_2

  1% Lysol .
- Resistant to heat, drying and disinfectants
- Air samples test positive for 2+ weeks
- Soil samples test positive for 150+ days
- Spore formation

Primary Reservoir

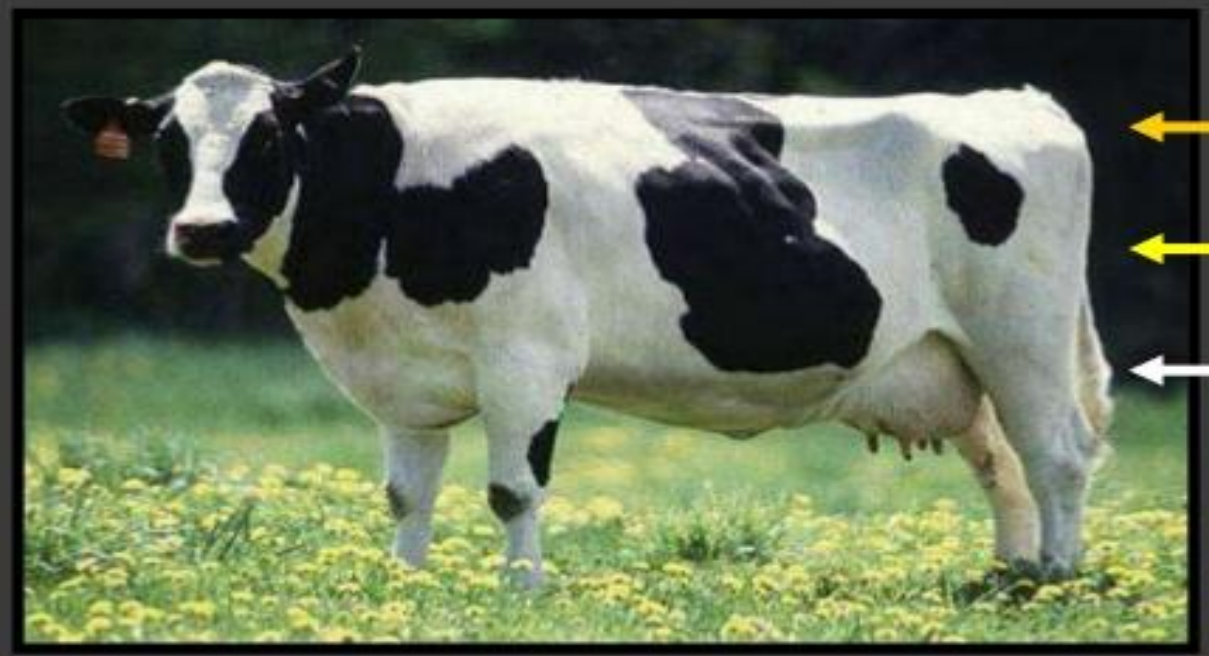
Goats

Cattle

Sheep

* All eukaryotes can be infected

Bacteria is excreted in:



of infected animals

Transmission

- Most common route is inhalation of aerosols
- Contaminated dust, manure, birthing products
- Tick bites (rare)
- Person-to-person (rare)
  - Transplacental (congenital)
  - Blood transfusions
  - Bone marrow transplants
  - Intradermal inoculation
  - Possibly sexually transmitted

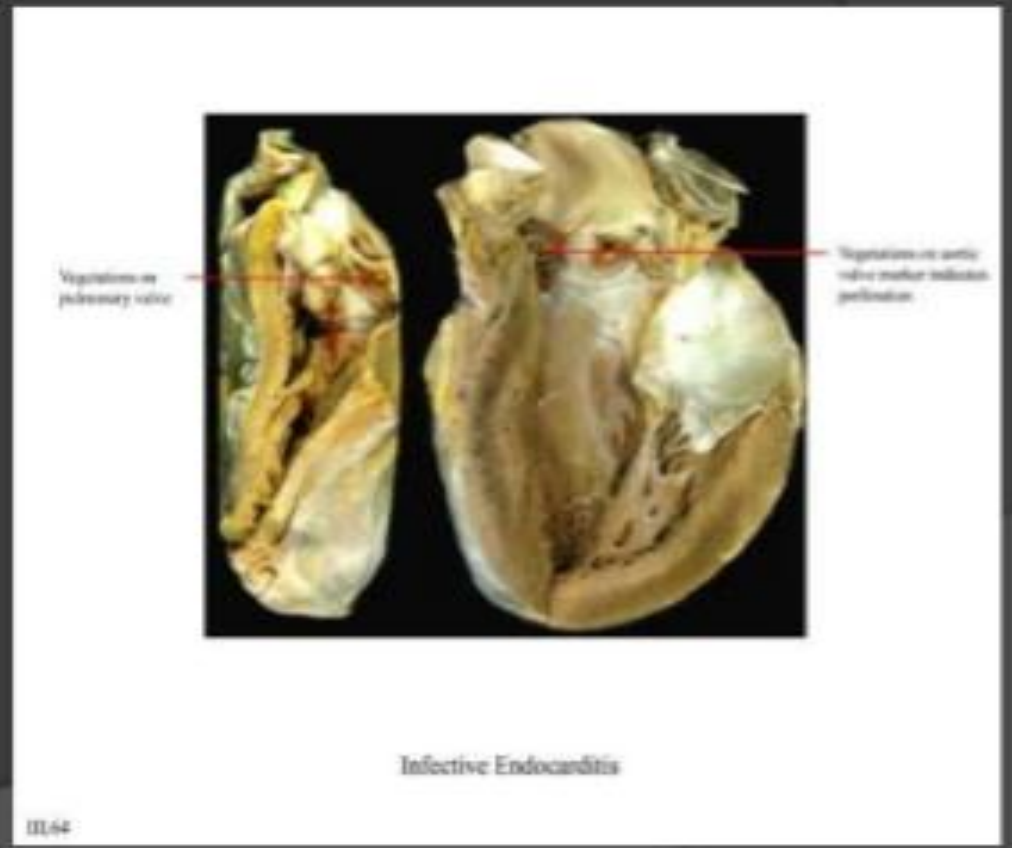
Symptoms

Acute Q fever

- Self-limiting, flu-like disease
- Fever, nausea, headaches, vomiting, chest/abdominal pain
- Pneumonia & granulomatous hepatitis
- Other signs (< 1%)
  - Myocarditis, pericarditis, meningoencephalitis
- Death: 1-2%

Chronic Q fever (> 6 months)

- Endocarditis & meningoencephalitis
- Pre-existing disease
- 1-5% of those infected
  - Prior heart disease,
  - pregnant women,
  - immunocompromised
- Other
  - Osteomyelitis
  - Granulomatous hepatitis
  - Cirrhosis



LAB DIAGNOSIS

Hard to diagnose because:

- Asymptomatic in most cases
- Looks like other disease (Flu or cold)
- Serology continues to be best method
- PCR, ELISA and other methods
- WEIL – FELIX test is negative .
- Bio safety level 3 (BSL-3) facility

Treatment

- Once infected, humans can have life-long immunity

Acute Q fever treated with:

Doxycycline (100 – 200 mg/day)

Chloramphenicol (Adult : 50 – 100 mg/kg/day

Child : 25 – 50mg/kg/day)

Erythromycin (Adult : 1-2 g/day up to 4gm/day

Child : 30 -50 mg/day up to 1g/day)

Timethoprim/sulfamethoxazole (160/800 mg)

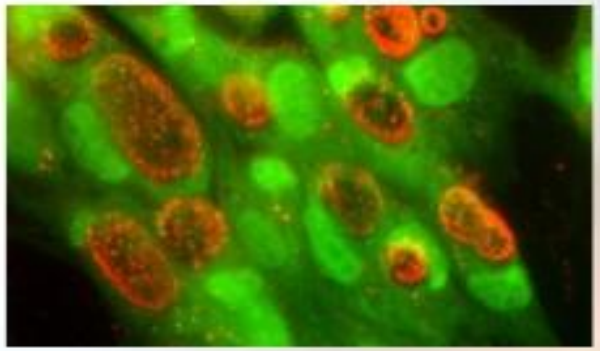
Fluoroquinolones:-

Ciprofloxacin, Gemifloxacin,

Levofloxacin, Moxifloxacin

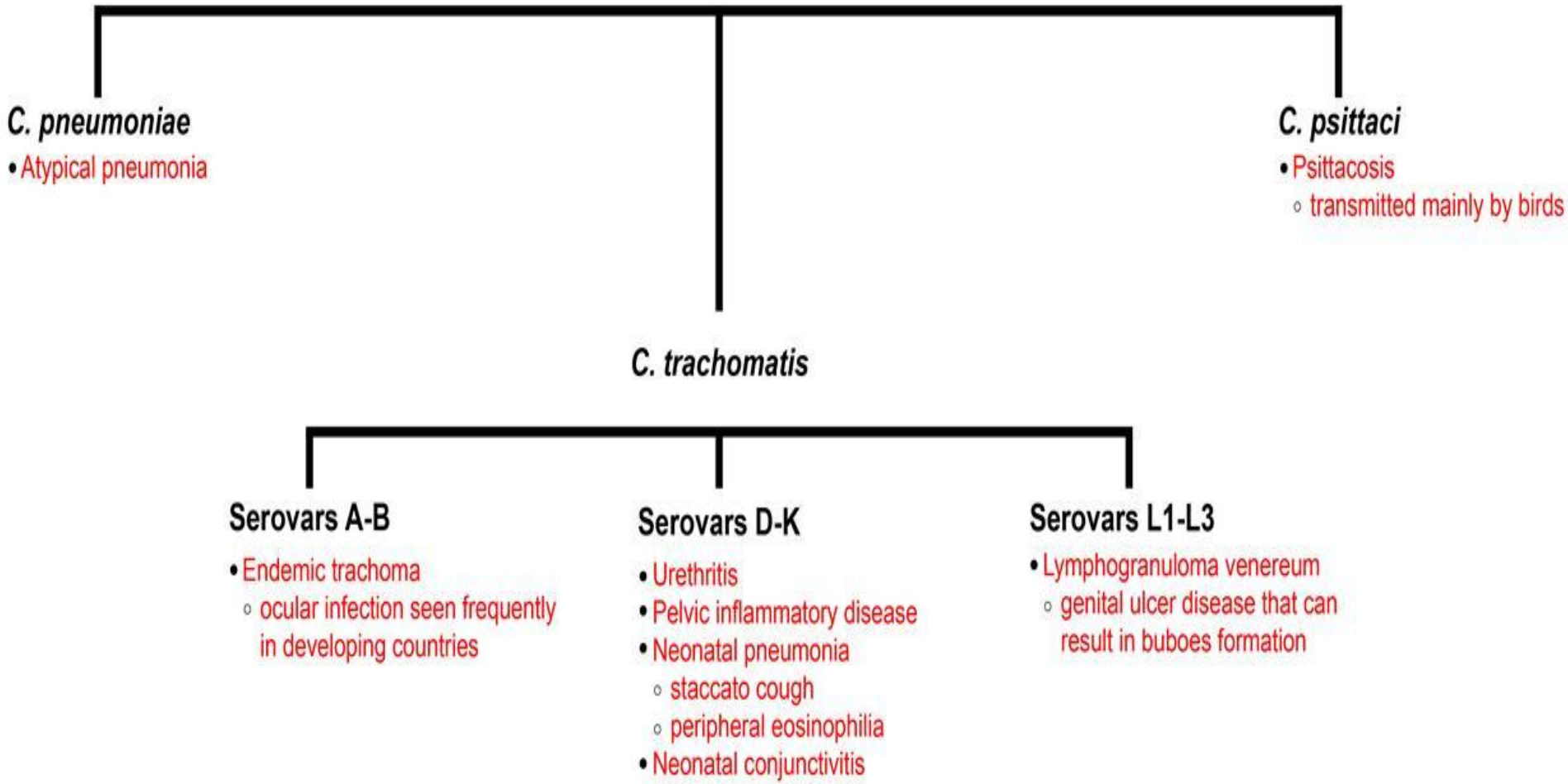
Norfloxacin, Ofloxacin

Chlamydia



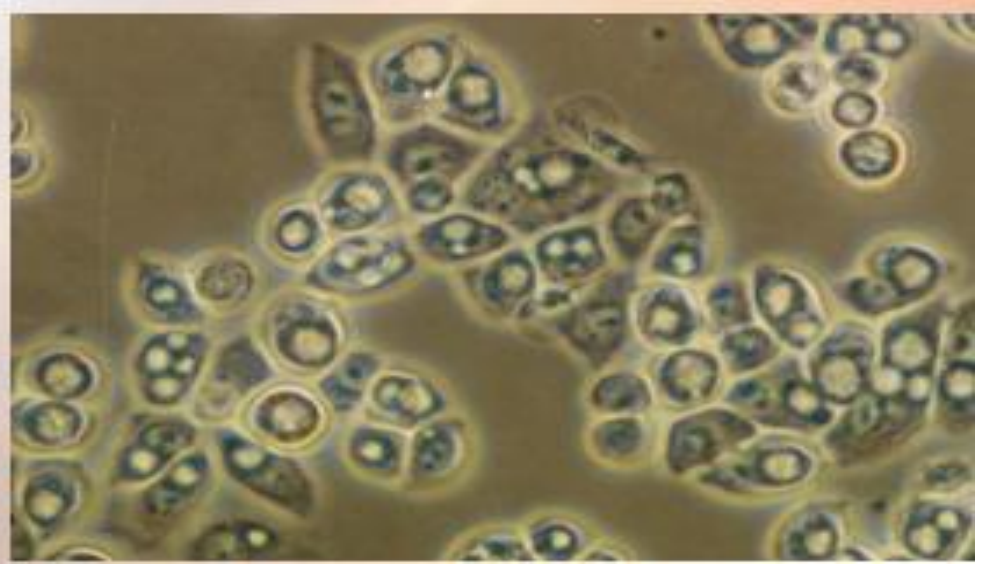
Chlamydia

Obligate intracellular organism



Characteristics - Chlamydia

- Family Chlamydiaceae
- Obligate intracellular bacteria
- Rod-shaped or coccoid
- Aerobic
- Gram negative but difficult to stain
- Cell Wall – lipopolysaccharides form the outer membrane, not peptidoglycan
- Infect columnar epithelial cells
- Forms elementary bodies (EB)
- Non-motile
- 37°C



Developmental cycle

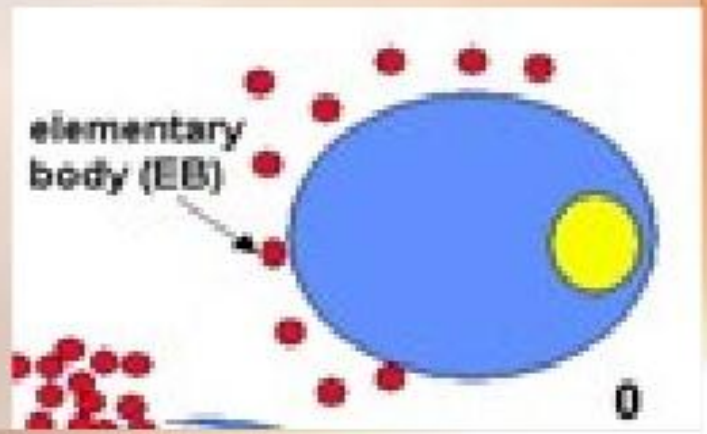
- Morphologically distinct infectious and replicative forms
- Stage 1. Attachment of elementary bodies (EBs)

  Infectious form

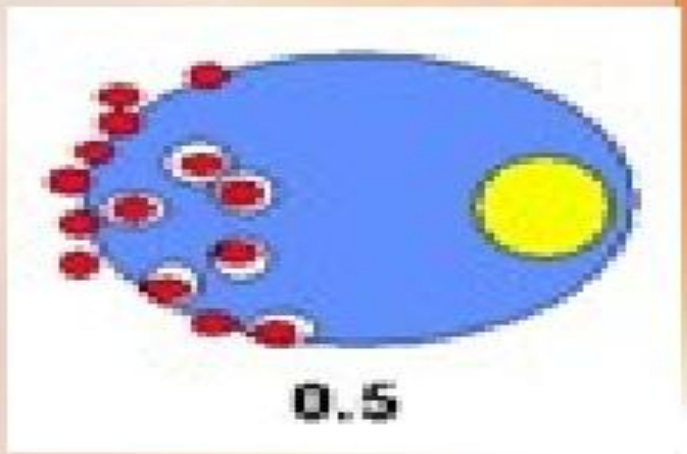
  - found in secretions
  - relatively resistant to environment

  Usual target cell

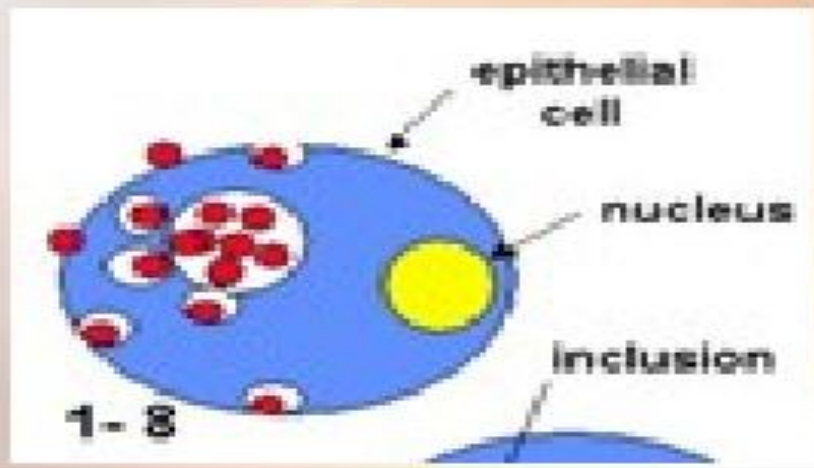
  - columnar epithelial cells of mucous membranes
  - not normally phagocytic



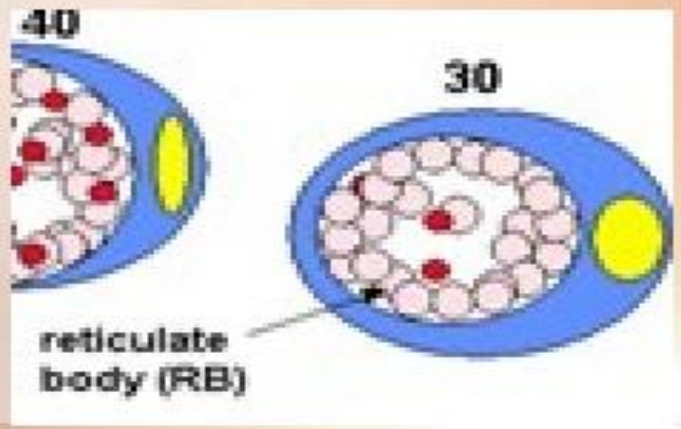
- **Stage 2. Entry of EBs**
- Chlamydia-specific receptor mediated **endocytosis**
- EBs enter cell within a phagosome
  - All development occurs here until rupture
  - Called an inclusion when visible



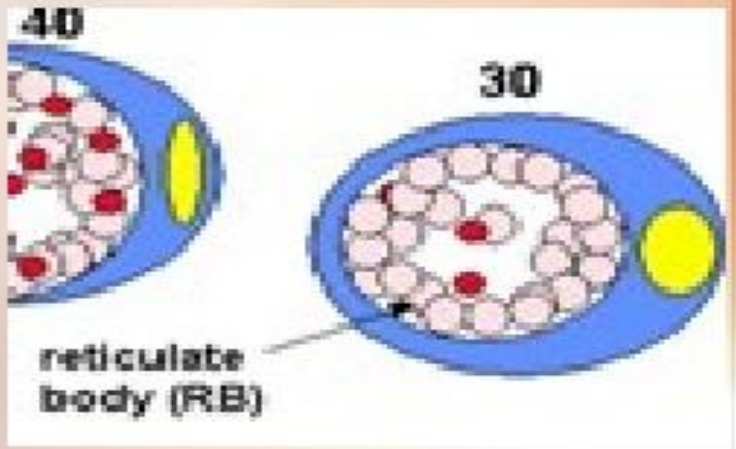
- **Stage 3. EBs change morphologically to reticulate bodies (RBs)**
  - 8 hours after entry
  - RB more permeable
    - **metabolically active**
  - not infectious at this stage



- **Stage 4. Replication**
  - RBs divide by binary fission for 20-24 hours

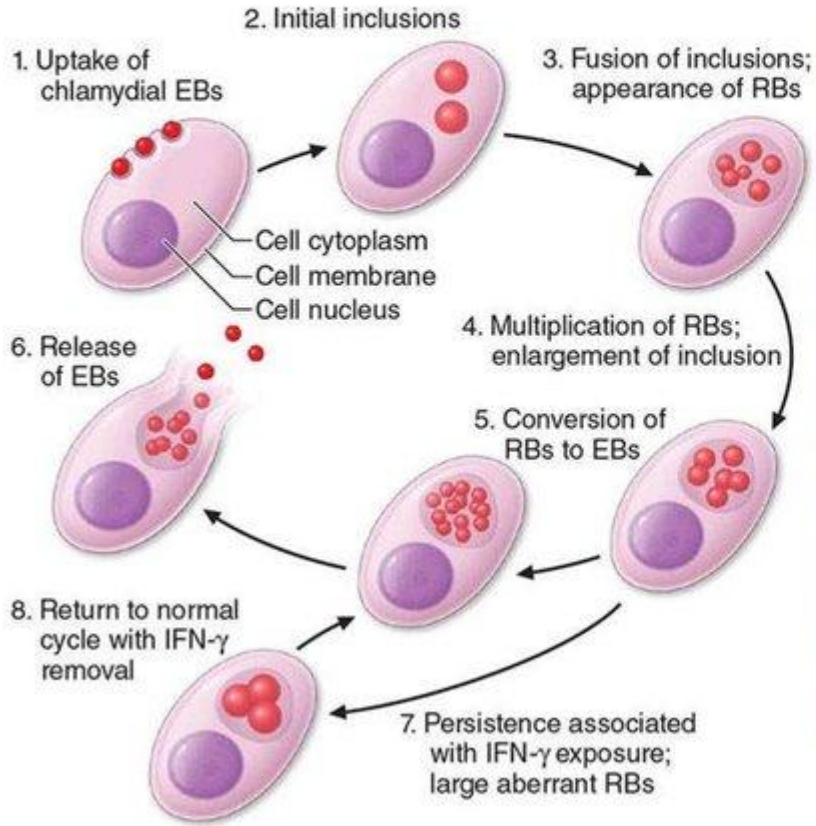


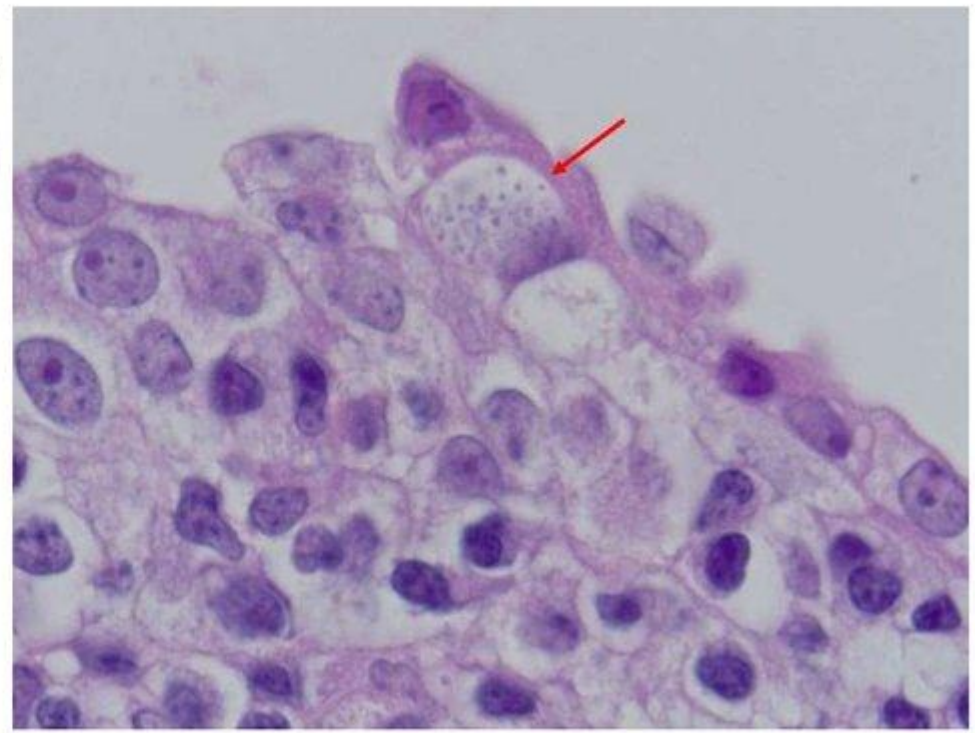
- **Stage 5. RBs change back to EB form**
  - DNA condenses
  - decreases in size
  - cell wall becomes more resistant
  - mature inclusion can contain hundreds of organisms



- **Stage 6. Release of infectious EBs**
  - both cell and inclusions lyse

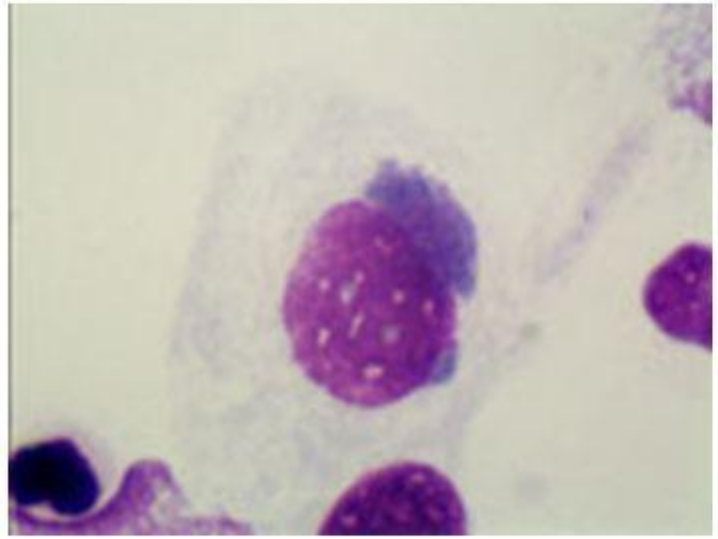
CHLAMYDIA TRACHOMATIS: MORPHOLOGY



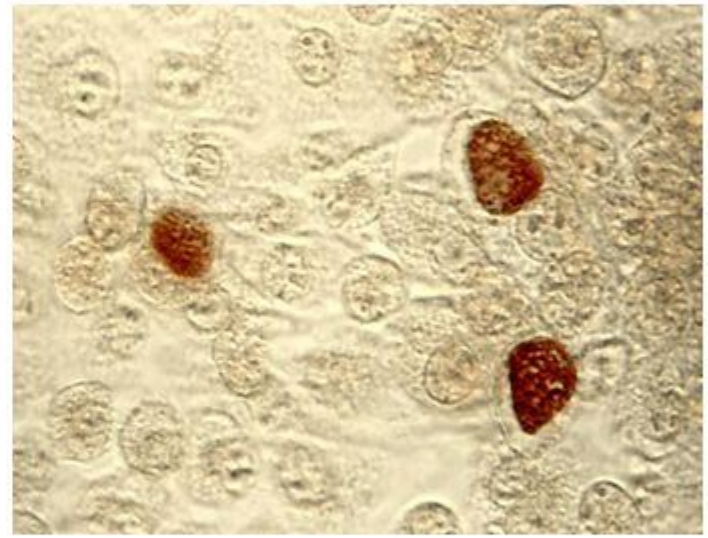


Chlamydia trachomatis

With Giema stain

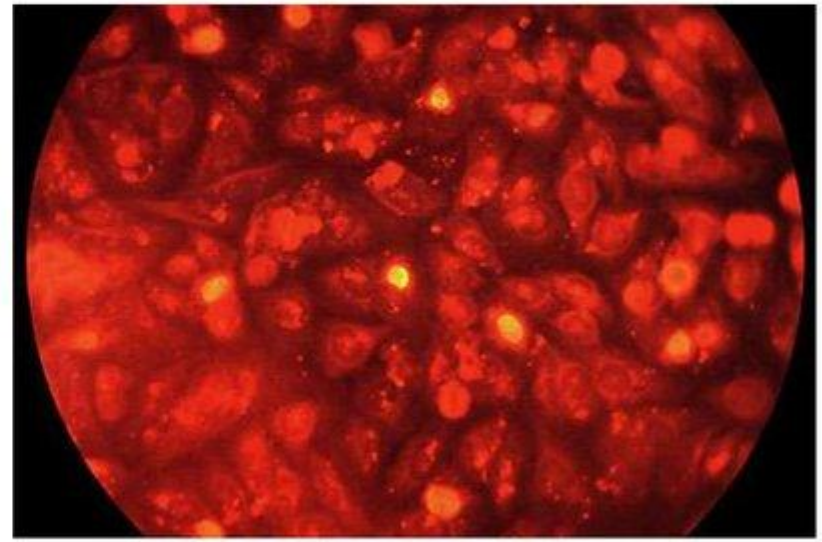


With Iodine stain

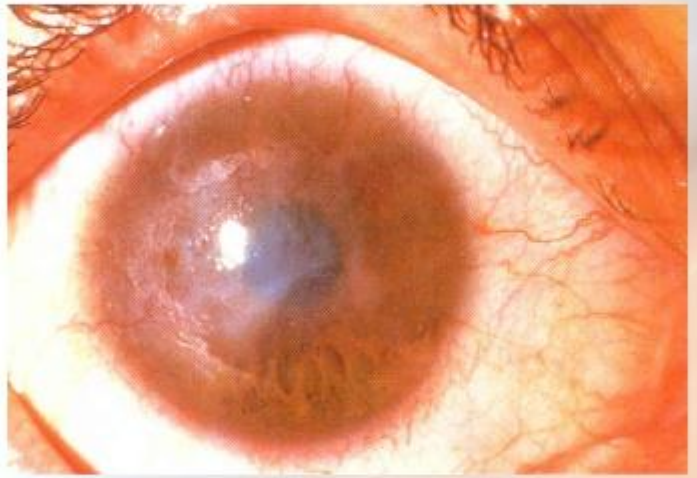


Giemsa stain of Chlamydia inclusion bodies (purple "caps" on epithelial cell).

CHLAMYDIA TRACHOMATIS: CULTURE

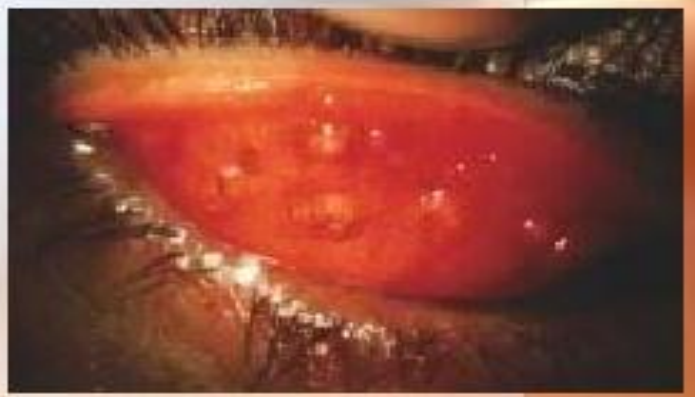


Trachoma



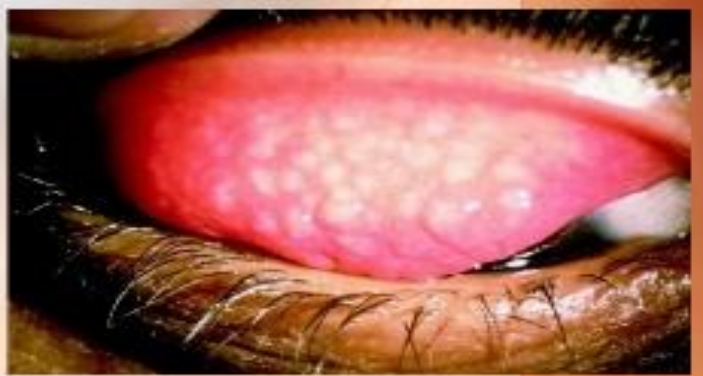
Trachoma

- **Largest single cause of preventable blindness in the world**
  - 500 million cases worldwide
  - 5.9 million blind
  - Primarily in developing countries
- **Caused by serovars A, B, Ba, C**
- **Clinical manifestations**
  - Chronic follicular kerato-conjunctivitis
    - With conjunctival scarring and pannus formation



Pathogenesis

- **Caused by repeated infections**
  - Causes inflammation of the eye
  - Eyelids turn inwards so the eye lashes rub against the cornea causing scar tissue to form irreversible blindness
  - 1st infection in childhood
    - subsequently persistent infection or many re-infections
    - final stages 15-20 years later
  - Sensitivity to products of the organism causes most of the pathogenesis



- **Treatment**
  - Azithromycin
  - Topical doxycycline
  - Improved sanitation
  - Surgical intervention

STDs

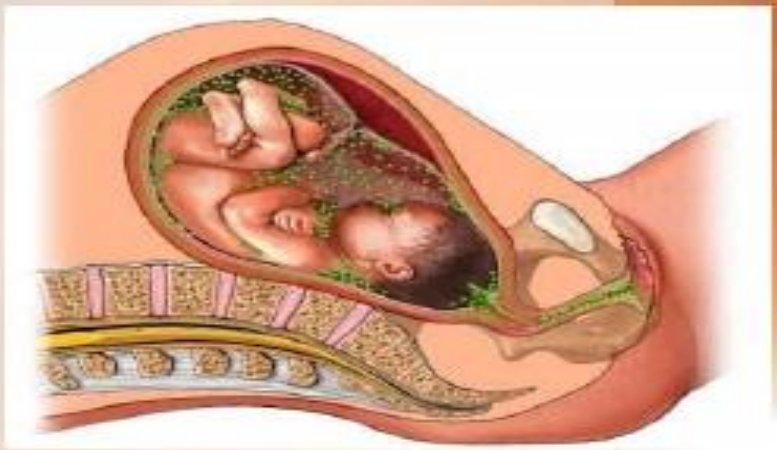
Serovars D-K

Epidemiology of STDs

- Incidence
  - 4-6 million cases/year in US
  - Increasing in some locations
- Only reservoir is human
  - Person to person transmission
  - Asymptomatic carriers important in transmission

Pathogenicity

- **Chlamydia (STD)**
  - Transmitted through direct contact between infected membranes
  - If left untreated, common cause of infertility
  - Newborns can contract the disease from infected mothers



Infections in male

- **Urethritis**
  - 50-75% symptomatic
    - urethral discharge
    - pyuria
    - itching
    - dysuria
  - 25-50% asymptomatic
- **Inclusion conjunctivitis**
- **Epididymitis**
  - acute and unilateral inflammation of epididymis
  - may result in decreased fertility
- **Proctitis**

Male

- Discharge from penis
- Dysuria
- Pain, blood, discharge from rectum
- Signs and symptoms 1-3 weeks after exposure

Infections in female

- Urethritis (60-80%)
  - dysuria
  - pyuria
- Proctitis
  - inflammation of the rectum
- Inclusion conjunctivitis
- Peri-hepatitis
  - infection of liver capsule

Infections in neonate / infant

- **Acquired** from direct contact with infected cervical secretions of mother at delivery
- **Inclusion conjunctivitis**
  - most common cause of neonatal conjunctivitis (2-6% infants)
  - acute mucopurulent eye discharge
  - systemic therapy with erythromycin
- **Pneumonia**
  - 33-50% of all cases of interstitial pneumonia
  - failure to thrive

Complications

- **Reiter's syndrome**
  - A reactive arthritis secondary to an immune-mediated response
    - o It may present as asymmetric polyarthritis, urethritis, inflammatory eye disease, mouth ulcers
    - o 80% of affected patients are HLA-B27 positive
- **Deeper pelvic complications in the female**
  - o PID
  - o Potential infertility
  - o Spread to the newborn during parturition


Lymphogranuloma venereum (LGV)
serovars L_1, L_2 and L_3
•Climatic or tropical bubo
•Lymphogranuloma inguinale

Stages of infection (untreated)

- **Primary stage**
  - 3 to 30 days after incubation
  - Small **painless papule** which may ulcerate at site of inoculation: self limiting
- **Secondary stage**
  - **Inguinal lymph nodes** (more common in males)
    - Painful lymphadenopathy
  - Necrosis in lymph nodes – may enlarge to form abscess
  - Acute haemorrhagic proctitis (10cm of the anorectal canal)
  - Fever, myalgia and headaches
- **Tertiary stage**
  - **Chronic inflammatory lesions** typical of chlamydial infection
  - Scarring in genital tract
  - Fibrosis, lymphatic obstruction, elephantiasis
  - Rectal strictures and fistulae

PSITTACOSIS

Chlamydophila psittaci

Etiology: Bacterial

Chlamydophila psittaci

Formerly known as Chlamydia psittaci

Also known as
Psittacosis, Parrot Fever and Ornithosis

Morbidity +
Mortality ++

- Gram Negative
- Coccoid
- Resistant to Drying
- Remains viable on surfaces for 2-3 weeks
- Survives in turkey carcass for over 1 year
- Obligate intracellular bacterium

Transmission

Risk of Psittacosis is highest among:

Bird Owners
Veterinarians
Laboratory Workers
Pet Shop Employees
Poultry Workers
(including workers in processing plants)

- Inhalation of dried bird droppings of infected birds
- Handling the feathers or tissues of infected birds
- Mouth-to-beak contact

- **Diagnosis of *Chlamydia psittaci* infection**
- Primarily **clinical** diagnosis
  - Acute onset febrile LRTI with hepato-splenomegaly, history of exposure to birds
  - Symptoms
    - fever, headache, malaise, muscle aches, dry hacking cough, bilateral interstitial pneumonia
  - Occasional systemic symptoms
    - myocarditis, encephalitis, hepatitis

Diagnosis

- **Cytological diagnosis**
  - By using Giemsa, iodine or papanicolaou stains to detect chlamydial inclusions in epithelial cells
- **Isolation in cell culture**
  - It is gold standard, but slow process and expensive
    - collect material containing columnar epithelial cells from urethra or cervix
  - Culture on monolayer of McCoy cell lines
    - incubation is for 40-72 hours
  - Stain with fluorescein-conjugated anti-Chlamydia monoclonal antibody after 48 hours and look for characteristic inclusions

Diagnosis

- **Antigen detection and nucleic acid hybridization**
  - DFA staining (direct fluorescent antibody)
  - ELISA
  - Detection of chlamydial ribosomal RNA by hybridization with DNA probe
- **Amplification techniques**
  - PCR - LCR
- **Serology**
  - ICT
  - CFT
  - Micro-immunofluorescence test

Treatment

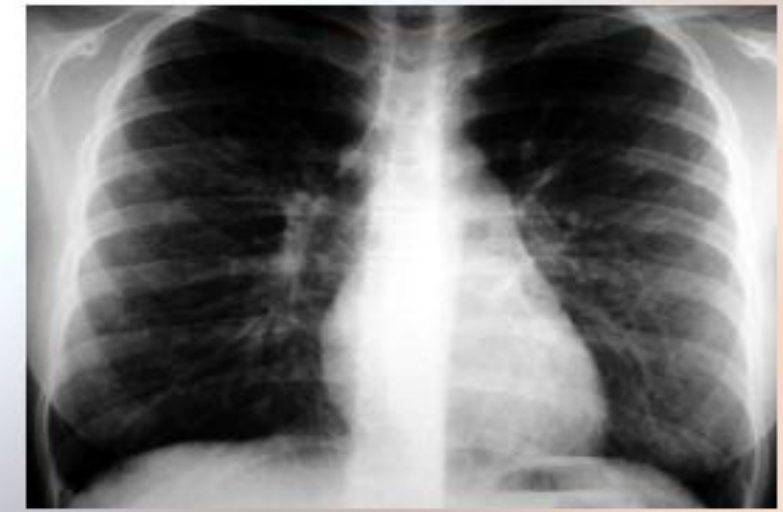
- **Azithromycin**
  - treatment of choice
  - effective against *C. trachomatis* and *N. gonorrhoeae*
  - well tolerated
- **Tetracycline**, especially **doxycycline**
- **Erythromycin** for pregnant women and children
- No drug resistant *C. trachomatis*

Prevention

- No vaccine
- Safe sex
  - Barrier contraceptive
- Topical antimicrobials
  - Silver nitrate not effective
  - topical erythromycin
- Regular Screening
- Educational programs

Chlamydia pneumoniae

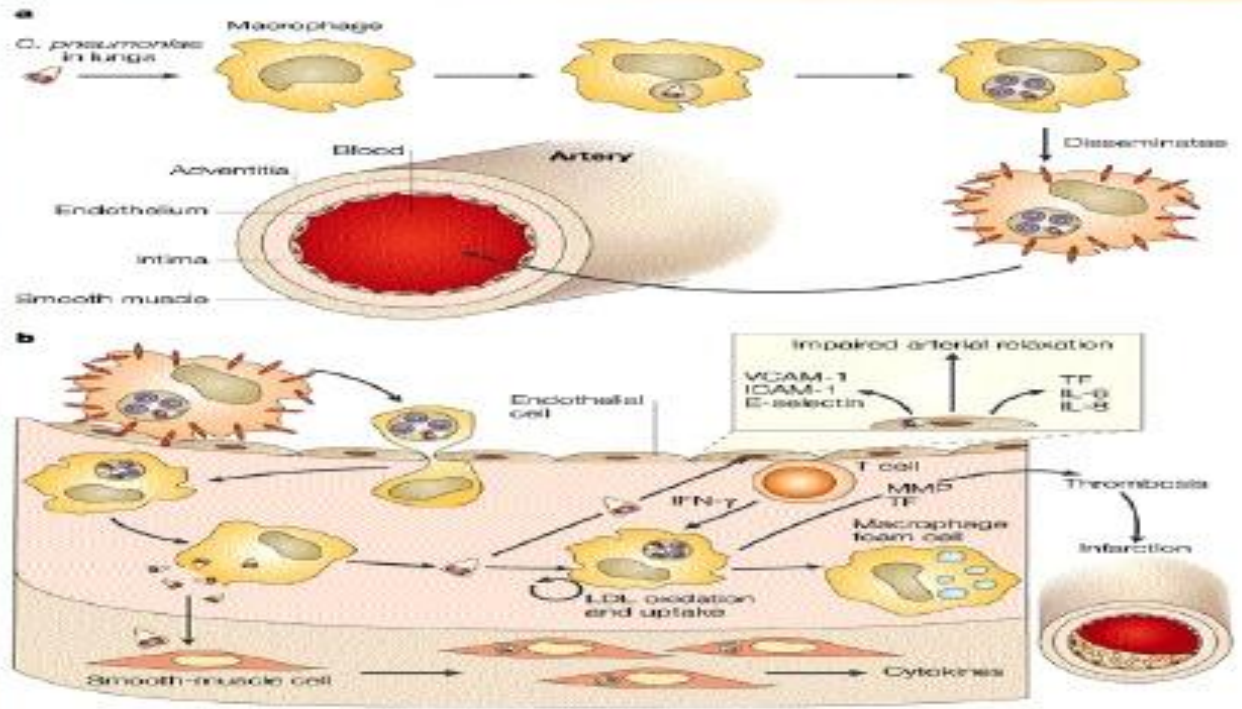
- *Chlamydia pneumophila*
- **Pneumonia**, bronchitis, sinusitis, pharyngitis, laryngitis
- Variety of mild respiratory infections w/ fever, cough



Epidemiology

- Person to person spread by respiratory droplets
  - no animal reservoirs known
- May be common infection among children 5-14 years old
    - 10% of pneumonia
    - 5% of bronchitis
  - 50% antibody prevalence in adults

- Infects the **lungs**
  - Majority of humans are infected
  - All effects of disease not known
    - Asthma, chronic bronchitis?
- Also, it has recently been linked to **heart disease and atherosclerosis**
  - organisms found in valve lesions



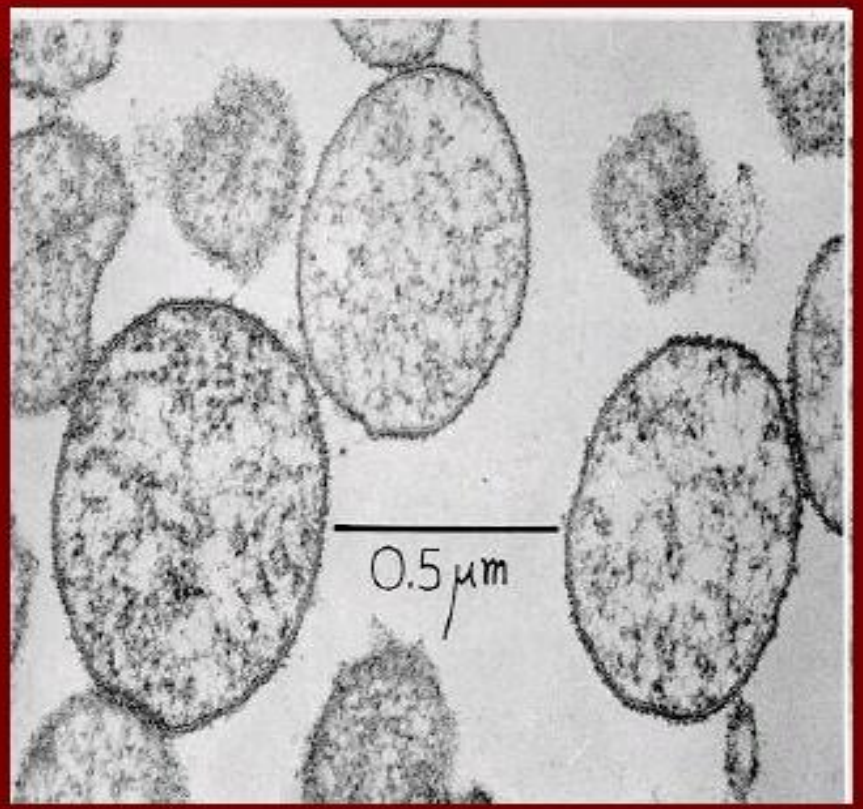
Mycoplasma

Kingdom	:	Bacteria
Division	:	Firmicutes
Class	:	Mollicutes
Order	:	Mycoplasmatales
Family	:	Mycoplasmataceae
Genus	:	*Mycoplasma*

Mycoplasmataceae

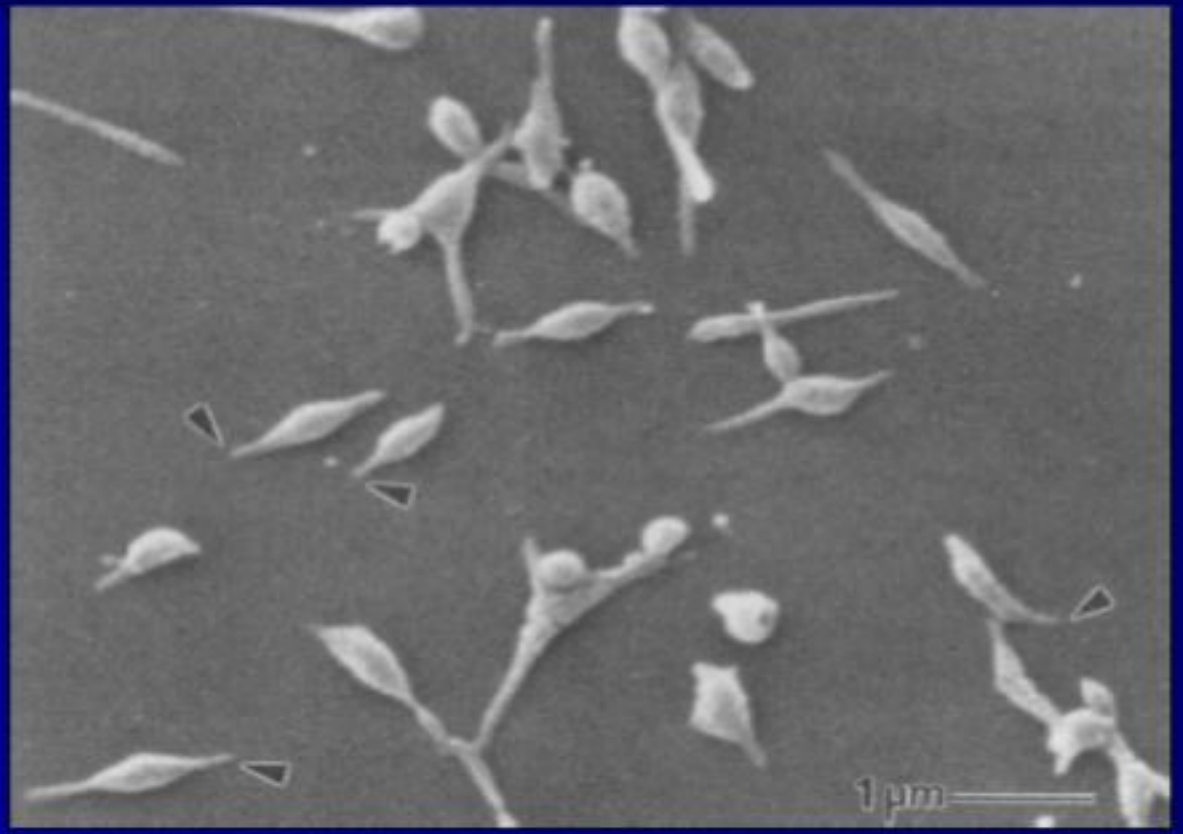
- Two genera
  - Mycoplasma
  - Ureaplasma
- Smallest free living bacteria
- Do not have a cell wall
  - Resistant to cell wall acting antibiotics
- Cell membrane contains sterols
- Slow growing & Facultative anaerobe
- Do not stain by ordinary methods
- Morphology : Highly pleomorhic

Mycoplasma are cell wall deficient microorganisms



- Cross-section of **Mycoplasma** bacteria, a common cause of atypical pneumonia. This bacteria is unusual in that it lacks a cell wall.

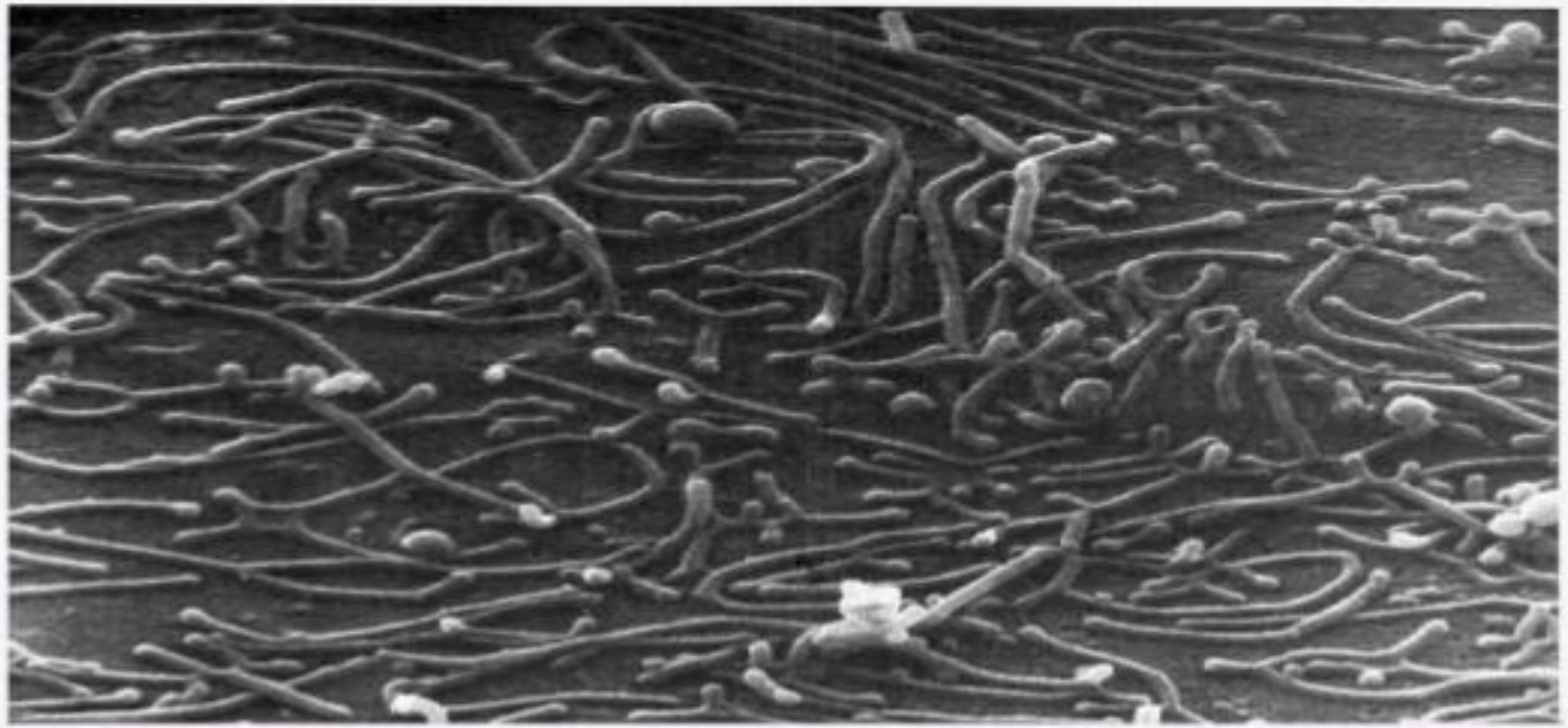
Mycoplasma (Scanning EM)



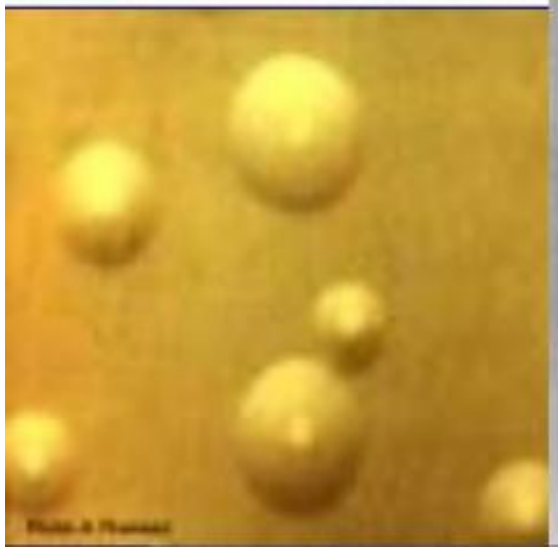
Morphology

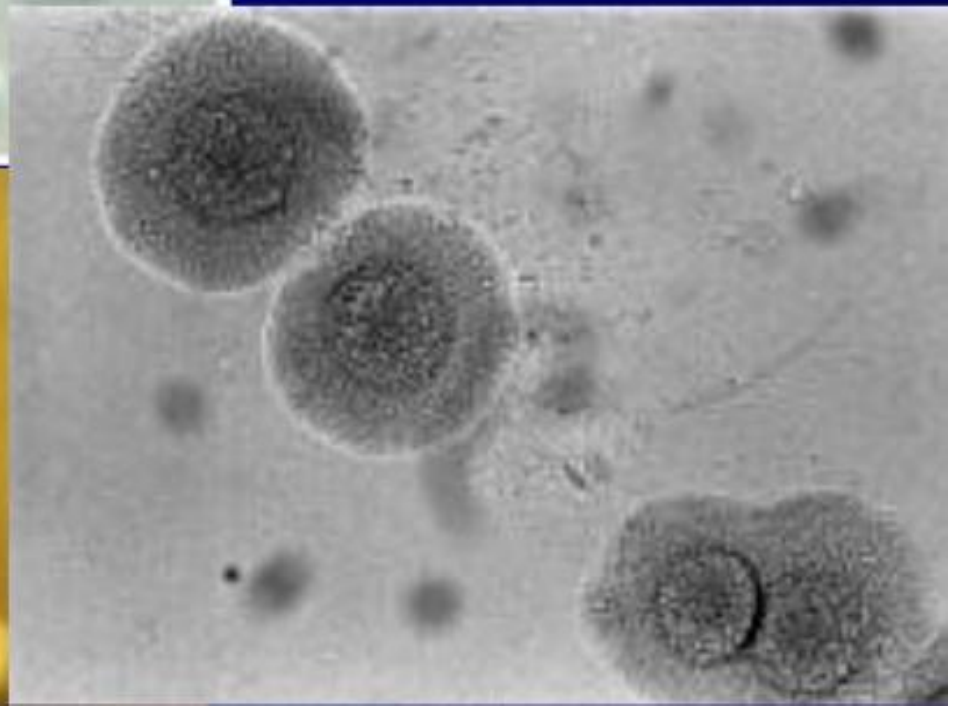
Size :- 300nm to 0.2nm

Shape :- Coccoidal,Diploform,Filamentus,Spiral shape &Teardrop shape.

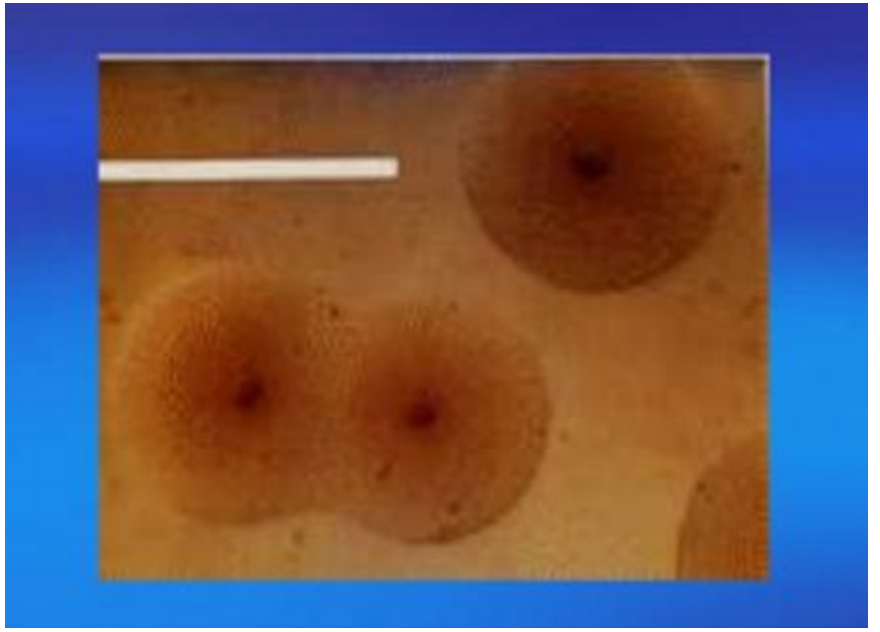


Mycoplams spp -Colonies





"fried egg" colonial morphology.



Important human pathogens

- Mycoplasma pneumoniae
  - Eaton's agent
  - Pleuropneumonia like organism (PPLO)
- Mycoplasma hominis
- Mycoplasma genitalium
- Ureaplasma urealyticum

Differentiation of Species

- *M. pneumoniae* – *ferments* glucose
- *M. hominis* – Hydrolyses Arginine
- *U. urealyticum* – Hydrolyses Urea
- *M. genitalium* - difficult to culture

Diseases Caused by Mycoplasma

Organism	Disease
M. pneumoniae	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia, (chronic asthma??)
M. hominis	Pyleonephritis, pelvic inflammatory disease, postpartum fever
M. genitalium	Nongonococcal urethritis
U. urealyticum	Nongonococcal urethritis, (pneumonia and chronic lung disease in premature infants??)

Mycoplasma pneumoniae

- Strict human pathogen
- Attaches by P1 pili to ciliary base on epithelial cell
- Disease spreads by inhalation of aerosols
- Spreads among close contacts
- Disease seen worldwide

Mycoplasma pneumoniae

- Diseases
  - Upper respiratory tract diseases
  - Tracheobronchitis
  - Primary atypical pneumonia
  - Complications
    - neurological abnormalities,
    - myo/pericarditis
    - hemolytic anemia

Atypical pneumonia

M. pneumoniae

- Incubation - 2-3 weeks
- Fever, headache and malaise
- Persistent non-productive cough
- Respiratory symptoms
  - **Radiological signs precede symptoms**
- Organisms persist
- Slow resolution
- Rarely fatal

LAB Diagnosis- M pneuminiae

Specimen

- Respiratory secretions
- Serum

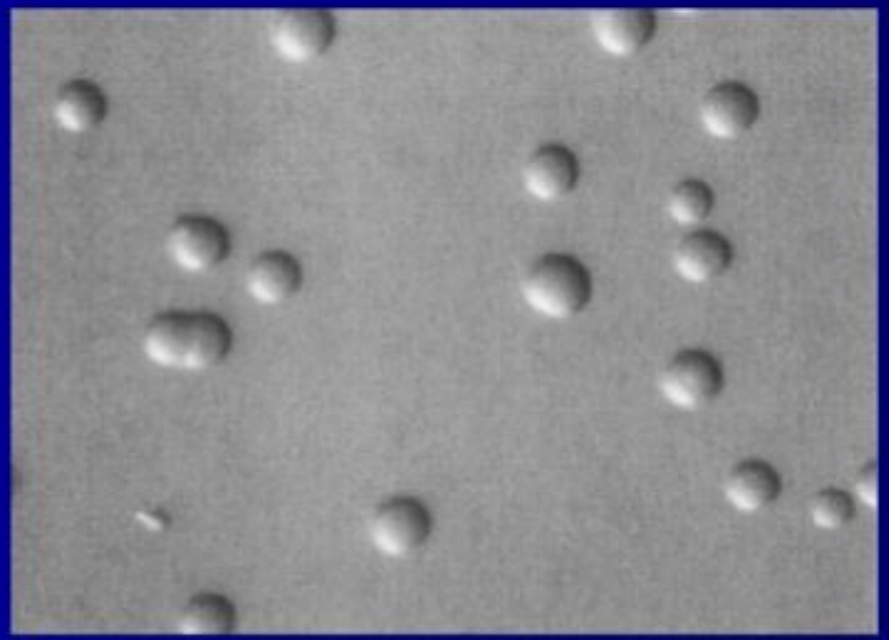
Laboratory Diagnosis - *M. pneumoniae*

- Microscopy
  - Difficult to stain
  - Can help eliminate other organisms
- Culture (definitive diagnosis)
  - Sputum or throat washings
  - Special transport medium needed
    - High index of suspicion - *M. pneumoniae*
  - May take 2-3 weeks

Culture media used

- PPLO broth and agar
- Serum is necessary for growth
- In broth, growth indicated by pH change due to carbohydrate metabolism
- Colonies best seen under microscope – Diene's staining technique

M pneumoniae colonies



Serology

- Very useful as culture is technically demanding
- Specific and nonspecific serological tests
- Specific tests
  - Complement Fixation, ELISA, IFA
- Non specific tests
  - Streptococcus MG agglutination test
  - Cold agglutinin test with human O RBCs. Significant titer is > 1:128.

Treatment - *M. pneumoniae*

- Treatment
  - Tetracycline or erythromycin
  - Can't use cell wall synthesis inhibitors

Genital mycoplasma

- M.hominis
- M.genitalium
- Ureaplasma urealyticum

M. hominis, *M. genitalium* and *U. urealyticum*

- Laboratory diagnosis
  - Culture (except *M. genitalium*)
  - Fried egg appearence of colonies on PPLO agar

Genital Mycoplasmosis -Treatment

- Tetracycline or
- Erythromycin