AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

# Lesson 9.

## Microbiology diagnosis of diseases, caused by

## *Spirochetes* and *Rickettsiae*

**FACULTY: General Medicine**
**SUBJECT: Medical microbiology - 2**

# Discussed questions:

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

- *Treponemas*. The causative agent of syphilis, morpho-biological characteristics, antigen structure, pathogenicity factors, pathogenesis. The causative agents of syphilis-like diseases (frambezia, pinta). Microbiological diagnostics: microscopic and serological methods (research of treponemal and non-treponemal antibodies (RPR, DIFR, TPHA, etc.) and their diagnostic significance. Principles of treatment of syphilis.

- *Borrelia*. The causative agents, morpho-biological characteristics, pathogenicity factors, pathogenesis. Microbiological diagnosis. The causative agent of Lyme disease, the pathogenesis of the disease. Microbiological diagnosis.

- *Leptospirosis* causative agent, morpho-biological characteristics, classification. Source of infection, ways of infection, 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.

# Purpose of the lesson:

- Students will learn the morpho-biological features of pathogenic spirochetes (causitive agents of syphilis, relapsing fever, Lyme disease and leptospirosis) and rickettsiae (causitive agents of spotted fever group diseases, scrub fever, monocytic and granulocytic ehrlichiosis and Q-fever), disease pathogenesis, microbiological to familiarize with the principles of diagnosis, specific treatment and prevention.

# SPIROCHETES

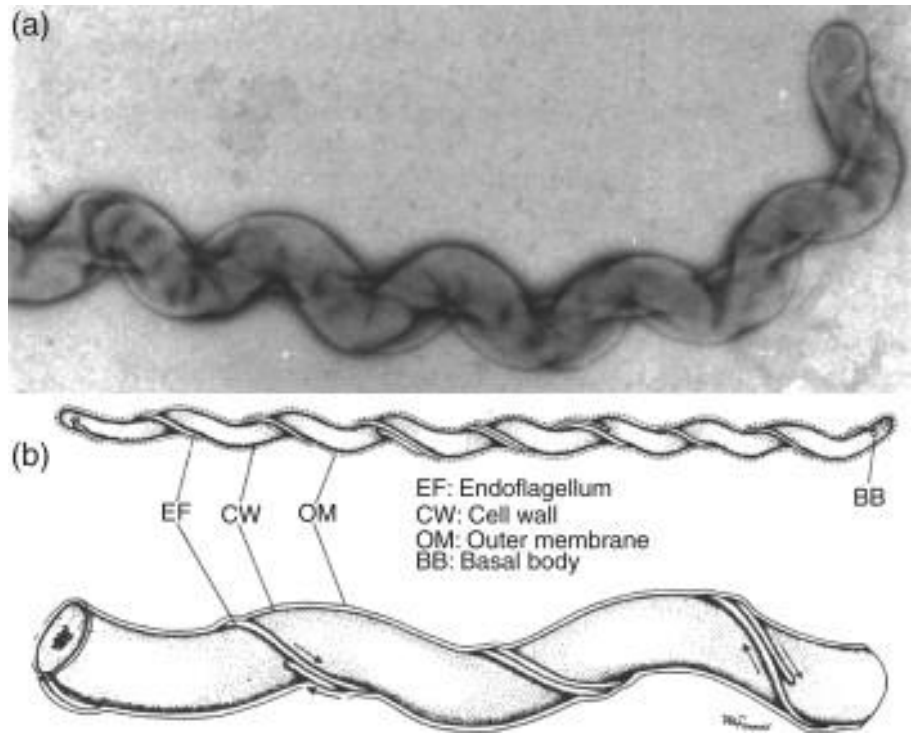
- Spirochetes (also spelled spirochetes) belong to a phylum of distinctive diderm (double membrane) bacteria, most of which have long, helically coiled (corkscrew-shaped) cells.
- Spirochetes are chemoheterotrophic in nature, with lengths between 5 and 250 μm and diameters around 0.1–0.6 μm.
- Spirochetes are distinguished from other bacterial phyla by the location of their flagella, sometimes called axial filaments, which run lengthwise between the bacterial inner membrane and outer membrane in periplasmic space. These cause a twisting motion which allows the spirochete to move about.

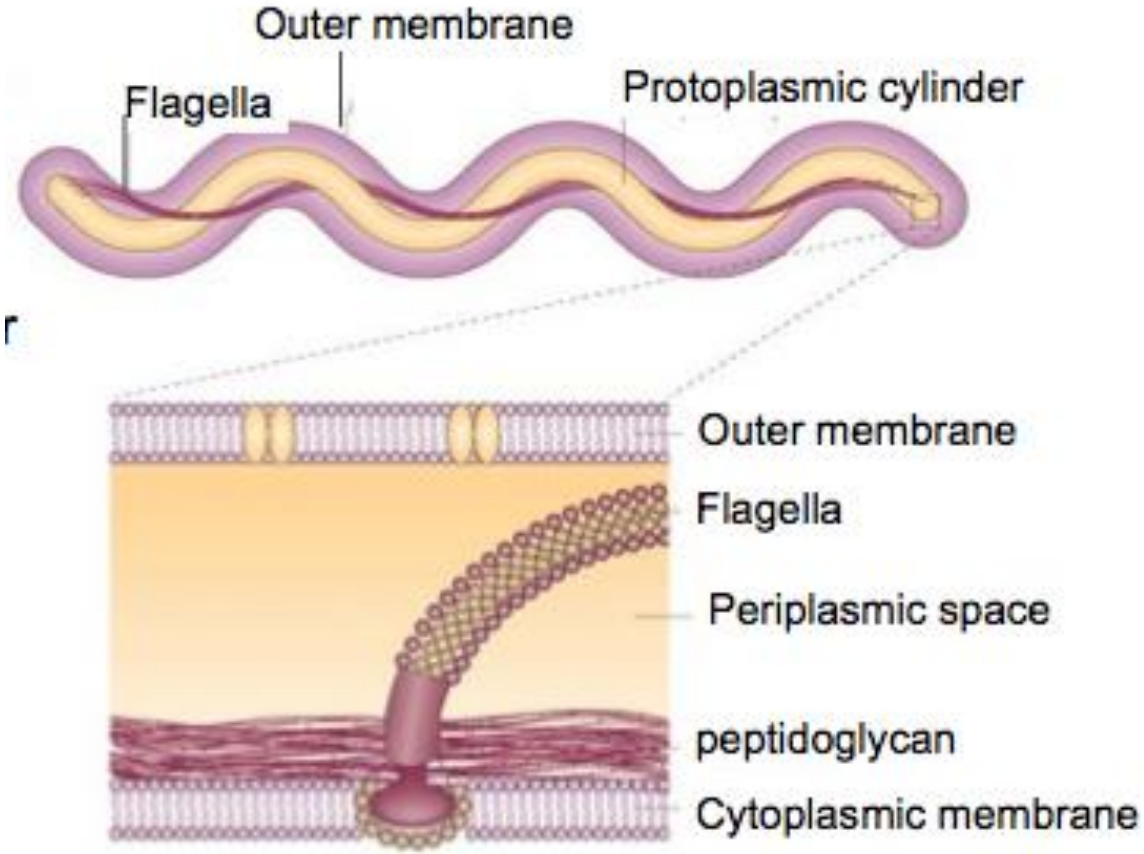
# Spirochetes

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

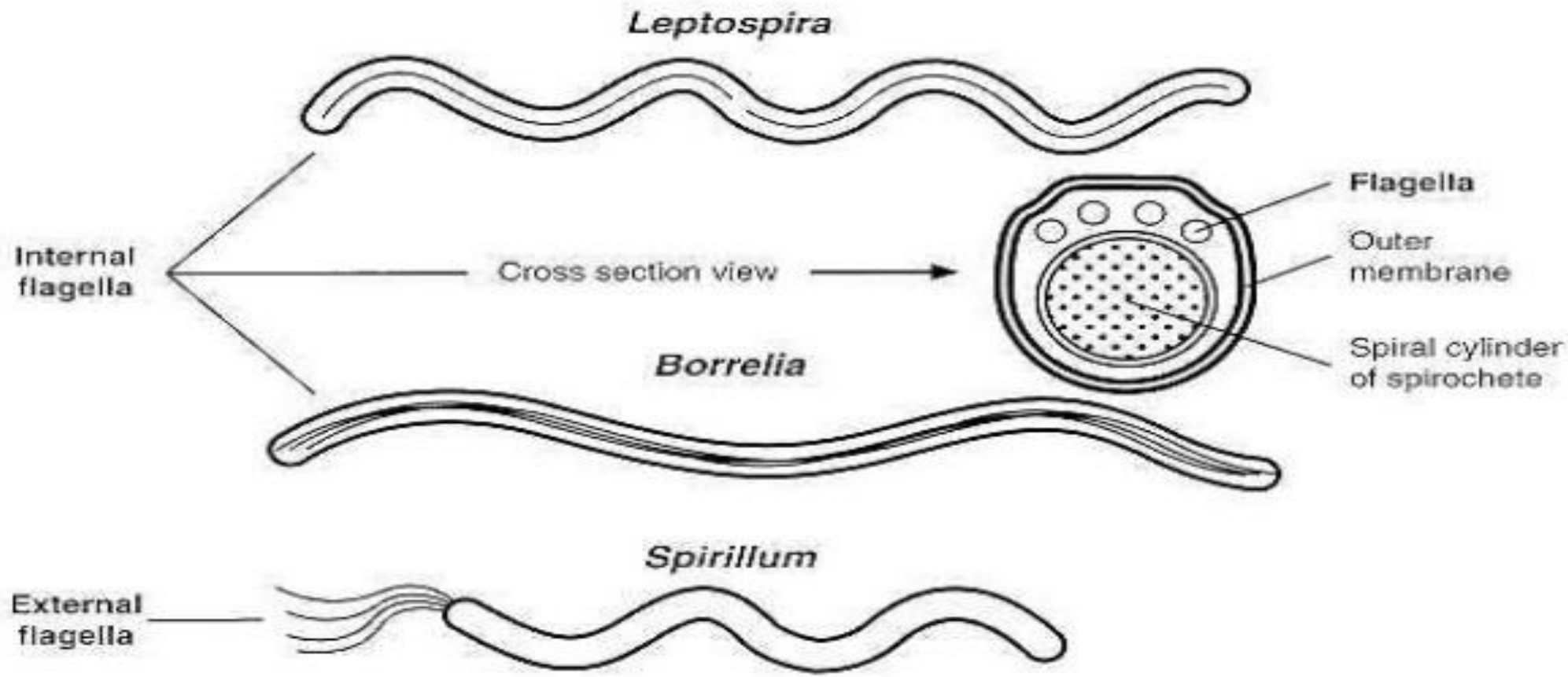
spirochetes contain – **endoflegalla** which are polar flagella along the helical protoplasmic cylinder and situated between the outer membrane and cell wall





# Comparative Morphology of Spirochetes



# Human pathogens

Genera Treponema

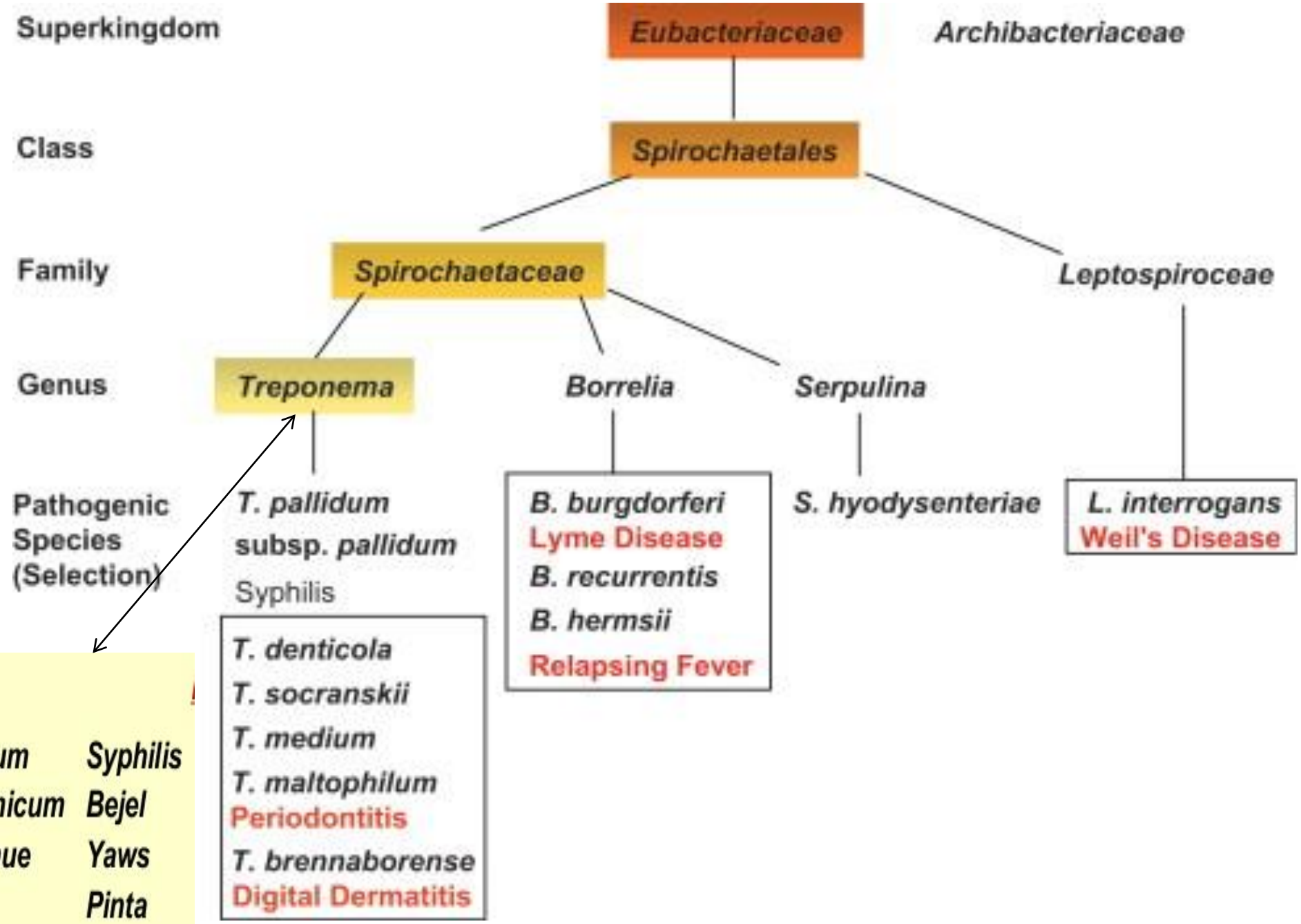
Borreilia

Leptospira

*Borrelia* and *Brachyspira*

*Leptospira*

*Treponema*




| Genus | Species | |
|---|---|---|
| Treponema | pallidum ssp. pallidum | Syphilis |
| | pallidum ssp. endemicum | Bejel |
| | pallidum ssp. pertenue | Yaws |
| | carateum | Pinta |

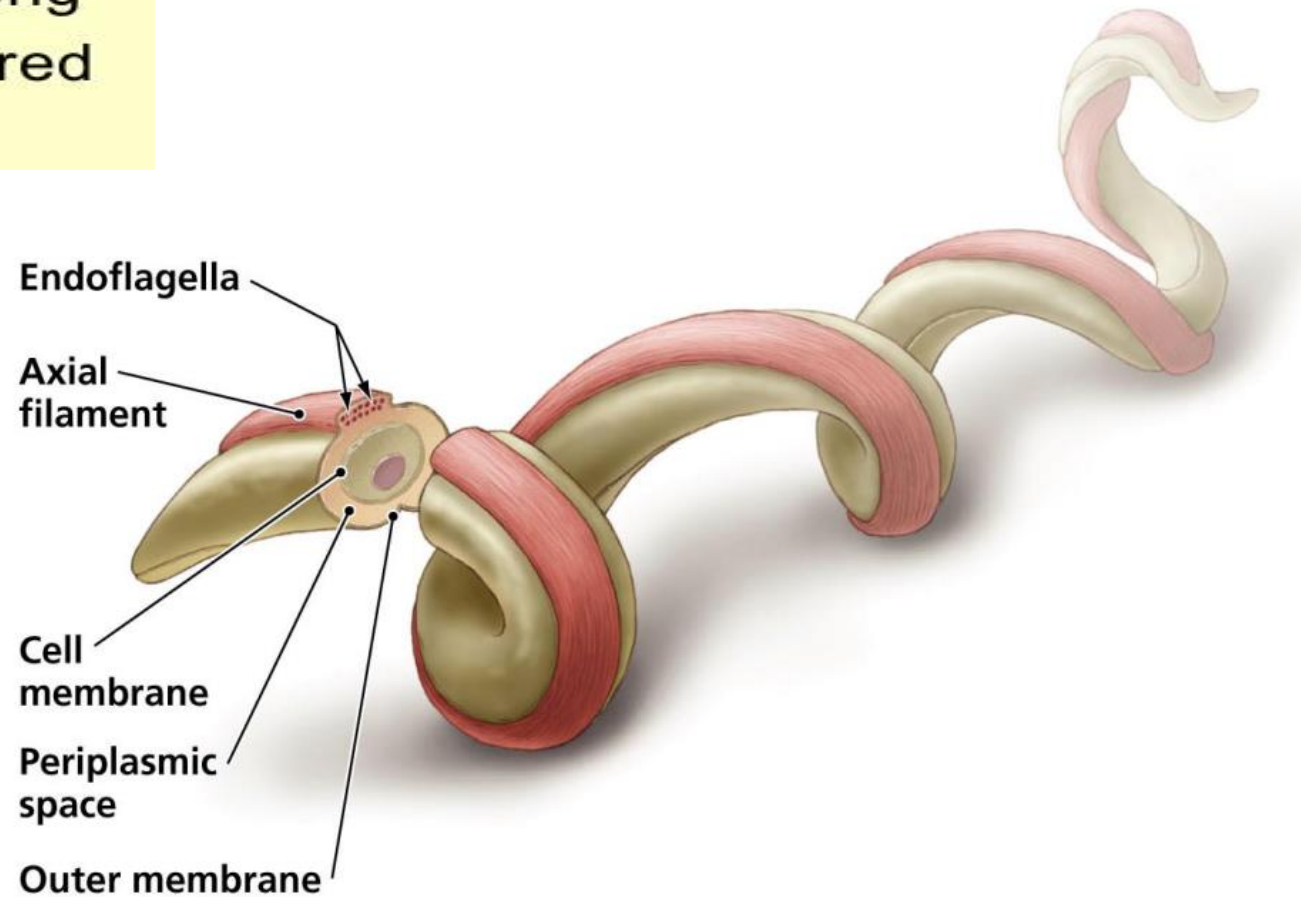
# *Treponema* - Taxonomy

- (Domain): Bacteria
- (Kingdom): Spirochaetota
- (Class): Spirochaetia
- (Order): Spirochaetales
- (Family): Treponemataceae
- (Genus): *Treponema*
- (Species): ***T.pallidum***

# Treponema pallidum

## Biological Characteristics

- Gram-negative spirochetes
- Spirochete from Greek for “coiled hair”
  - Extremely thin and can be very long
- Tightly coiled helical cells with tapered ends



# Trepanoma pallidum

## Biological Characteristics

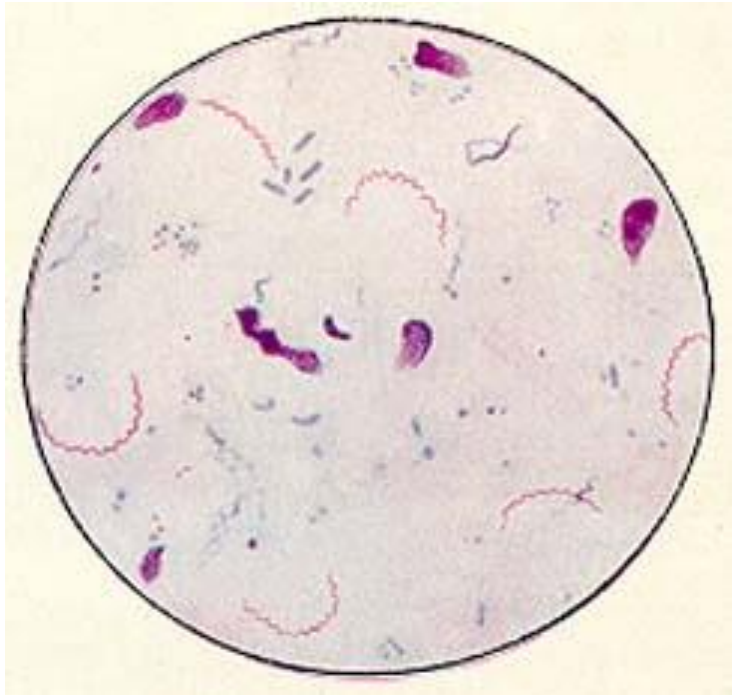
Motile, sluggish in viscous['vɪskəs]environments

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

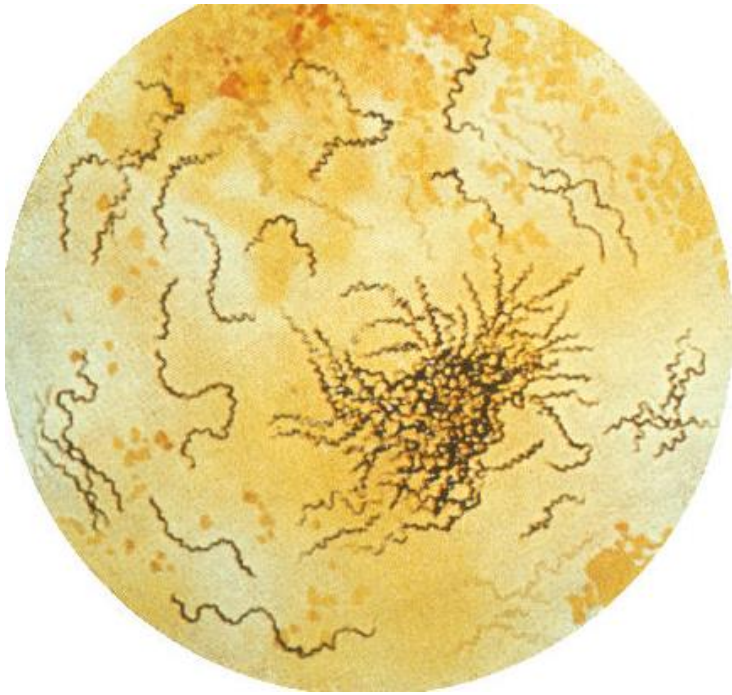
Structure

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

# *Treponema pallidum*



**Giemsa stain**

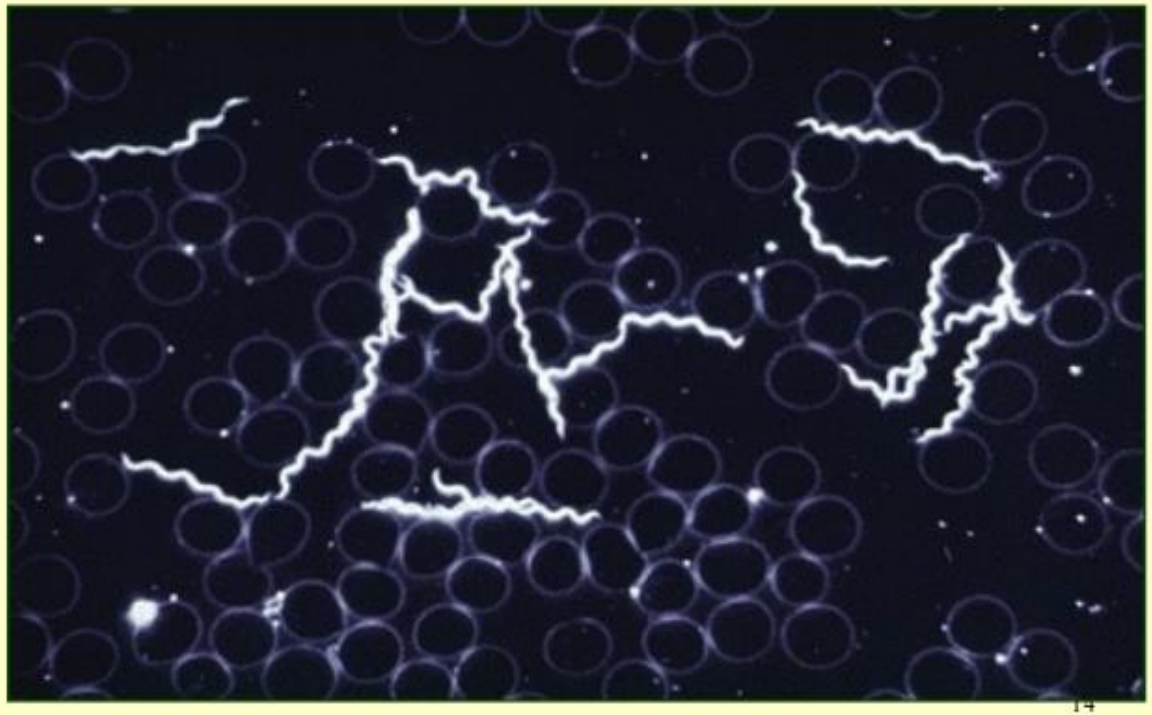


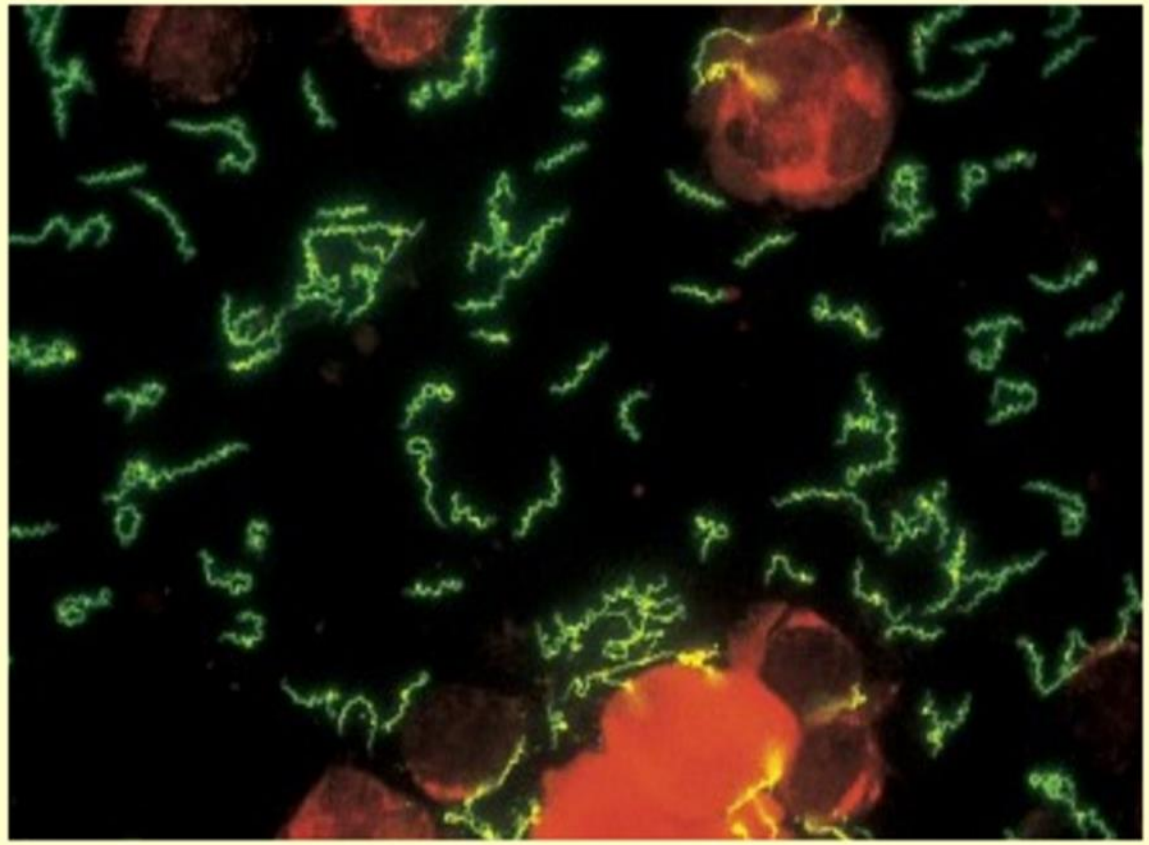
**Silver impregnation**

# 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**

# *Treponema pallidum*

dark field microscopy

# Cultural characteristics

- *T. pallidum* do not grow in artificial culture media.
- Maintained in motile and virulent form for 10-12 days in complex media under anaerobic conditions.
- **Serial testicular passage in rabbits** maintain virulent *T.pallidum* for many decades eg. Nichol's strain.
- Reiter strain (*T.phagedenix*), nonpathogenic treponeme, grows well in thioglycollate medium.

# *Treponema pallidum*
## biochemical activity

- Reuter's strain of *T.pallidum* and other non-pathogenic strains:
- they form indole, hydrogen sulfide
- they melt the gelatin
- they break down glucose, galactose, sucrose, maltose, mannitol
- some strains lyse erythrocytes

# Antigenic structure

- Induces at least three types of antibodies
- **Reagin antibodies**: react in standard or nonspecific tests for syphilis e.g. wassermann, kahn and VDRL
- Hapten extracted from beef heart is used as antigen K/a cardiolipin chemically diphosphatidyl glycerol, also detected in T. pallidum
- **Group antigen**: found in pathogenic and non pathogenic treponemes
- **Polysaccharide antigen**: species specific, demonstrated by specific T. pallidum tests

## TABLE 44-2 Virulence factors of *Treponema pallidum*

| Virulence factors | Biological functions |
|---|---|
| Outer membrane proteins | Promote adherence of *T. pallidum* to the surface of host cells |
| Enzyme hyaluronidase | Facilitates perivascular infiltration |
| Fibronectin | Prevents phagocytosis of *T. pallidum* by macrophages |

# Mode of Transmission

- Direct sexual contact (90 – 96%)
- Blood transfusion
- Via placenta from infected pregnant mother ⇨ faetus ⇨ causes congenital syphilis.
- Contact ⇨ accidental contact E.g. *Medical personnel*.

**Source** of T. pallidum: Primary and secondary syphilis lesions.


## CLASSIFICATION

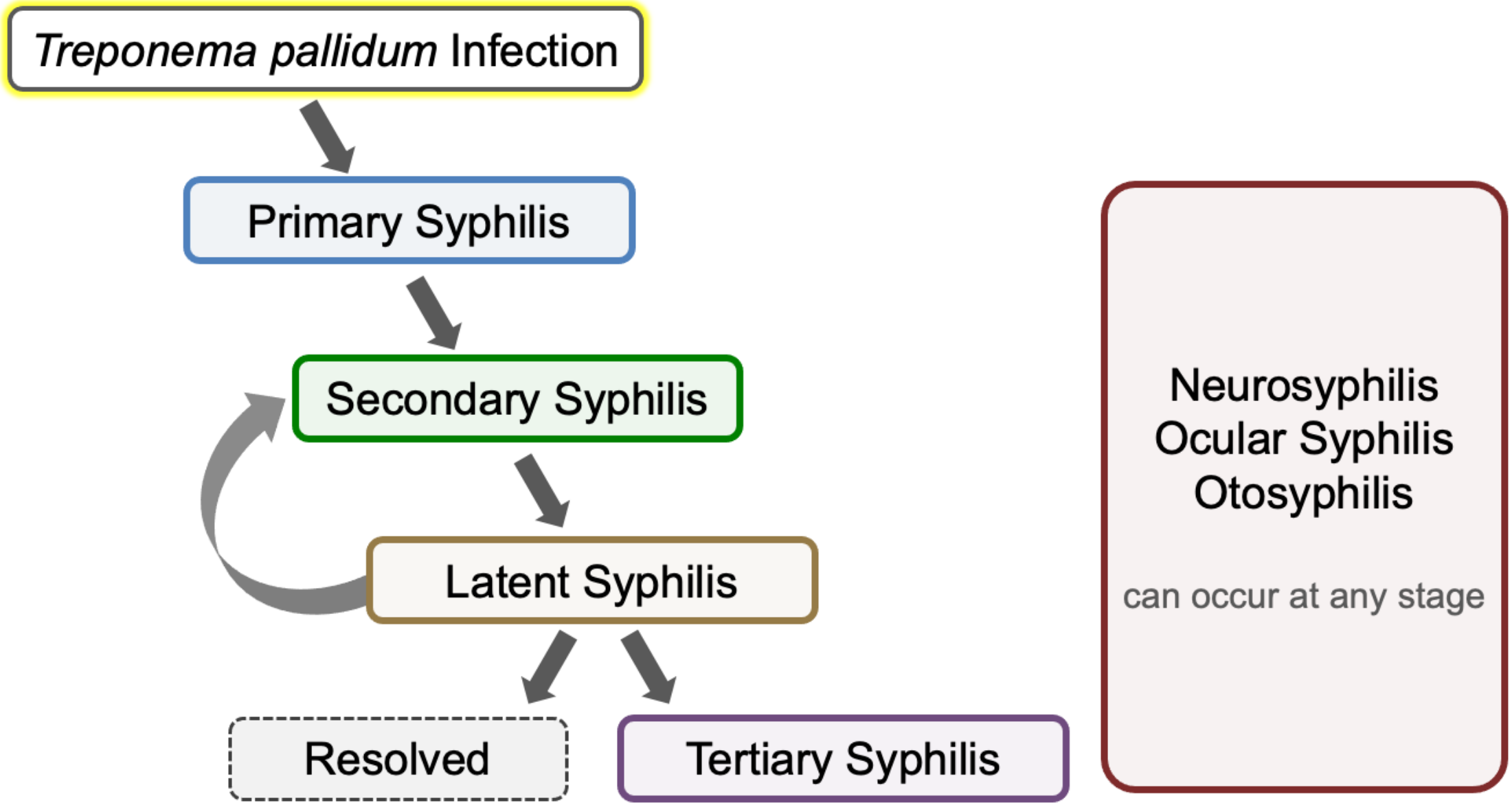
Syphilis is majorly classified in to two types namely:

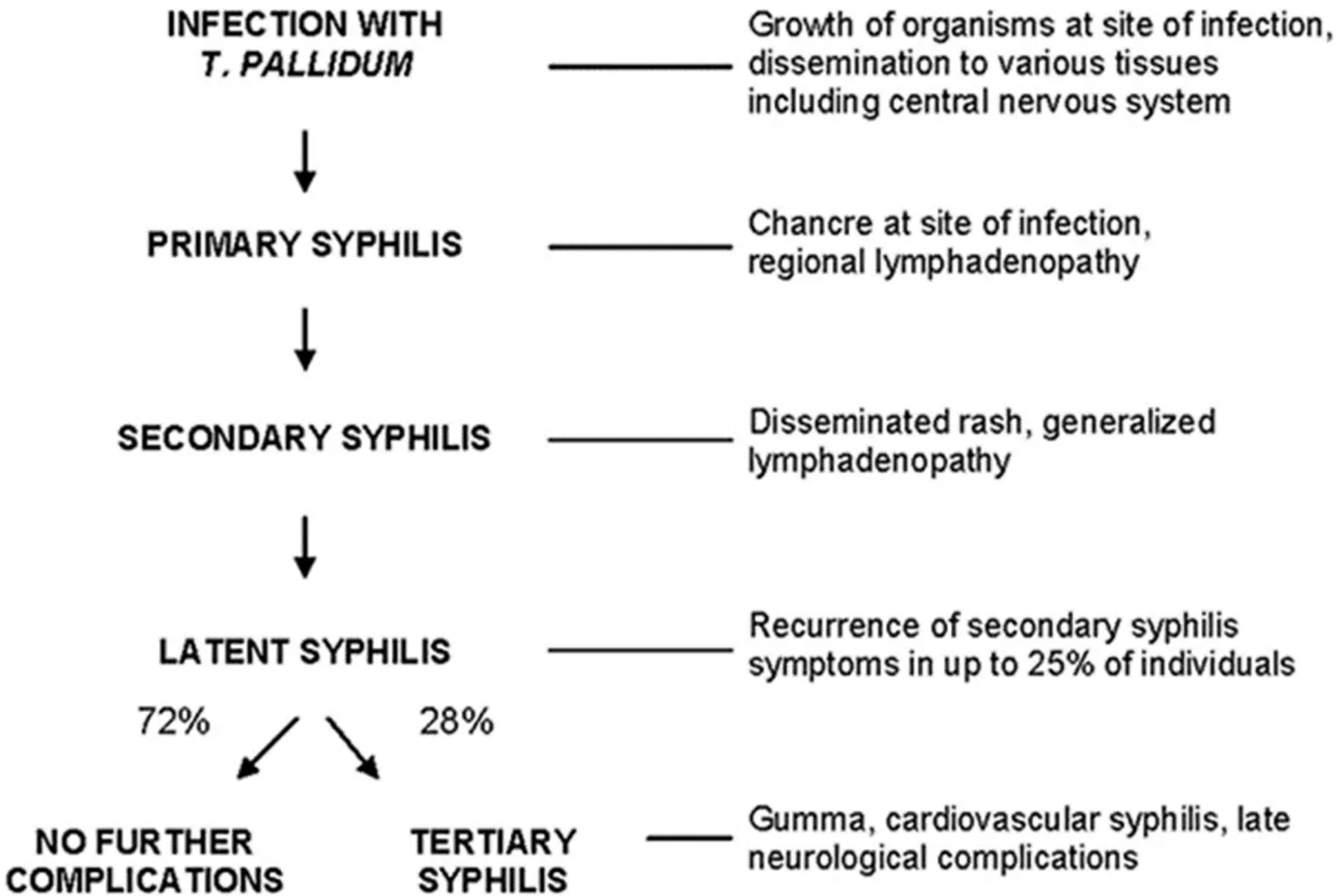
- Veneral syphilis.
- Non-veneral syphilis.

## VENERAL SYPHILIS:

The disease falls in to 3 stages namely:

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


*Treponema pallidum* Infection
Primary Syphilis
Secondary Syphilis
Latent Syphilis
Resolved
Tertiary Syphilis
Neurosyphilis
Ocular Syphilis
Otosyphilis
can occur at any stage


INFECTION WITH
T. PALLIDUM
Growth of organisms at site of infection, dissemination to various tissues including central nervous system
PRIMARY SYPHILIS
Chancre at site of infection, regional lymphadenopathy
SECONDARY SYPHILIS
Disseminated rash, generalized lymphadenopathy
LATENT SYPHILIS
Recurrence of secondary syphilis symptoms in up to 25% of individuals
72%
28%
NO FURTHER COMPLICATIONS
TERTIARY SYPHILIS
Gumma, cardiovascular syphilis, late neurological complications

# 1° and 2° Syphilis: *Pathogenesis and clinical findings*

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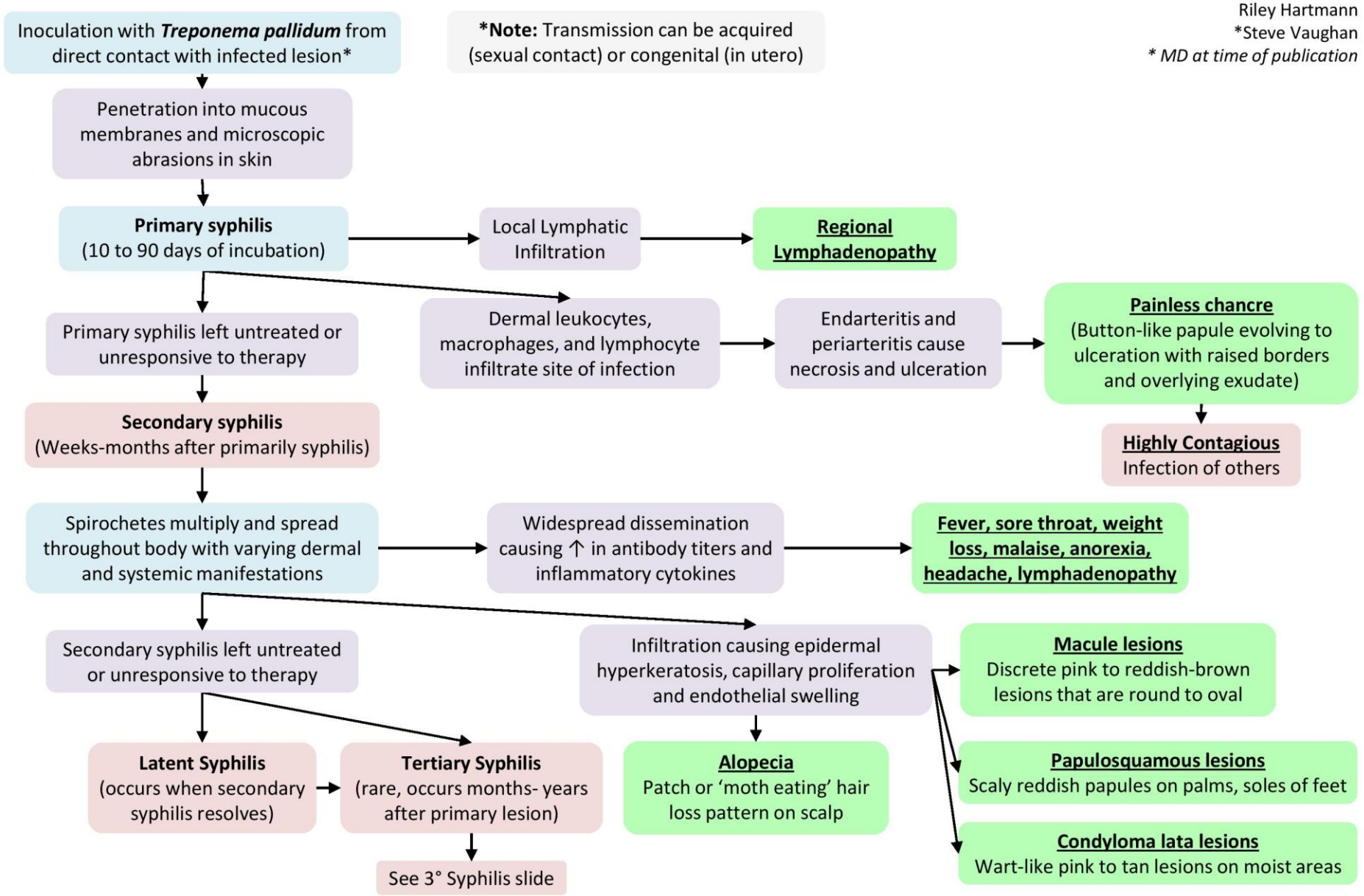


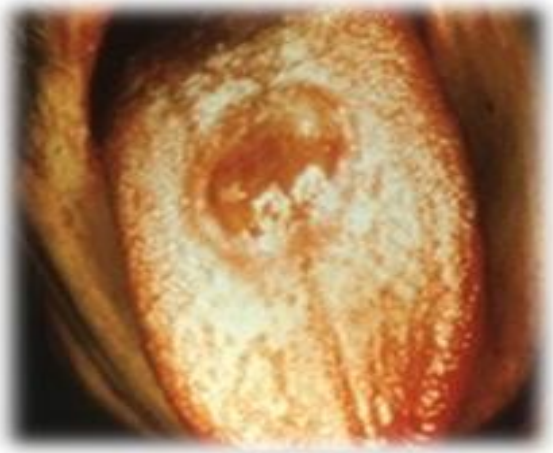

**Legend:** Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

Published **March 15, 2015** on www.thecalgaryguide.com

# 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%)

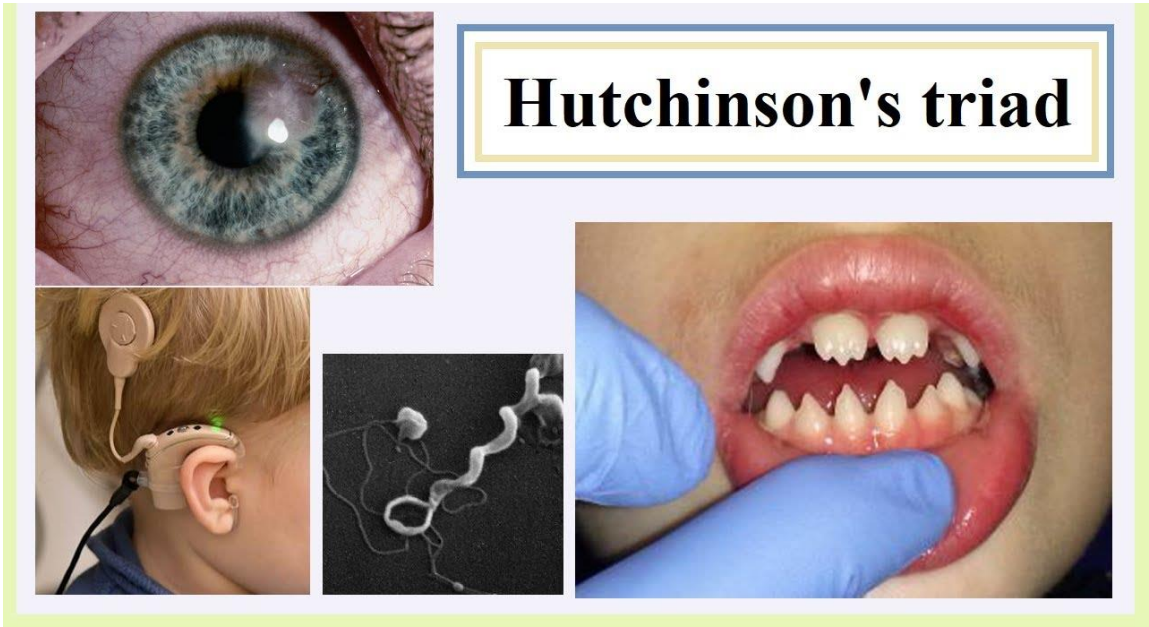
Ulcerating gumma, cardiovascular

# 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

# Congenital Syphilis

- 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



# 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.

## 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

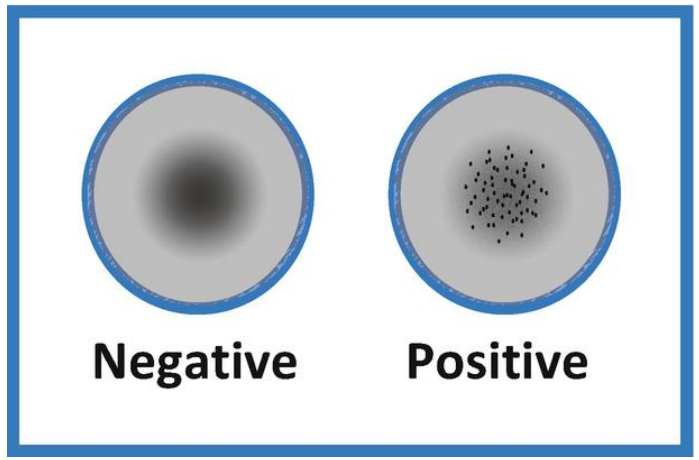
- Measure antibody directed against a cardiolipin-lecithin-cholesterol antigen
- Not specific for *T. pallidum*
- Titers usually correlate with disease activity and results are reported quantitatively, may be reactive in life

## Treponemal Serologic Tests

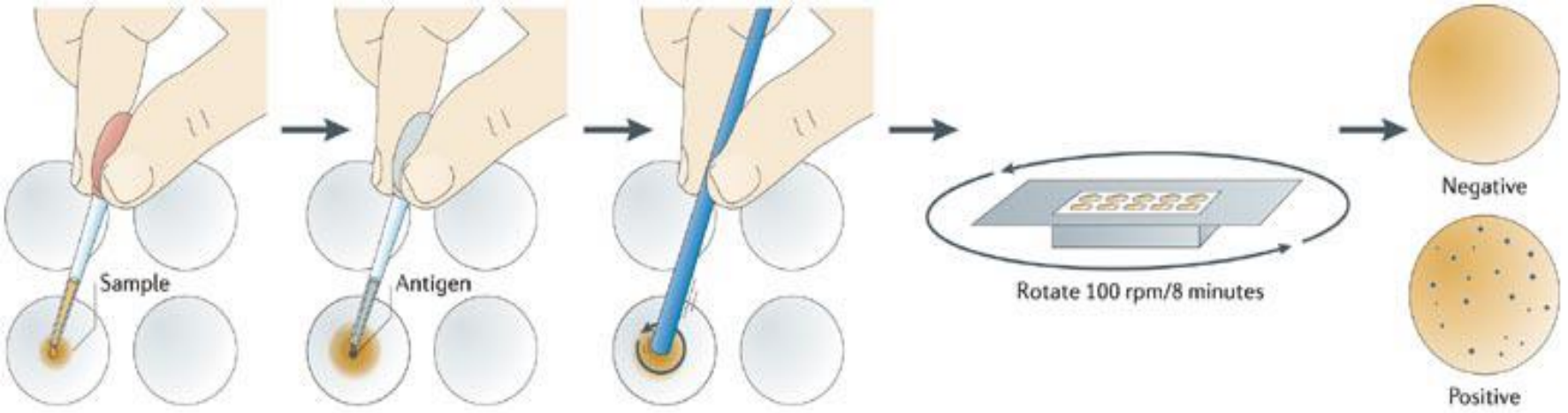
### Principles

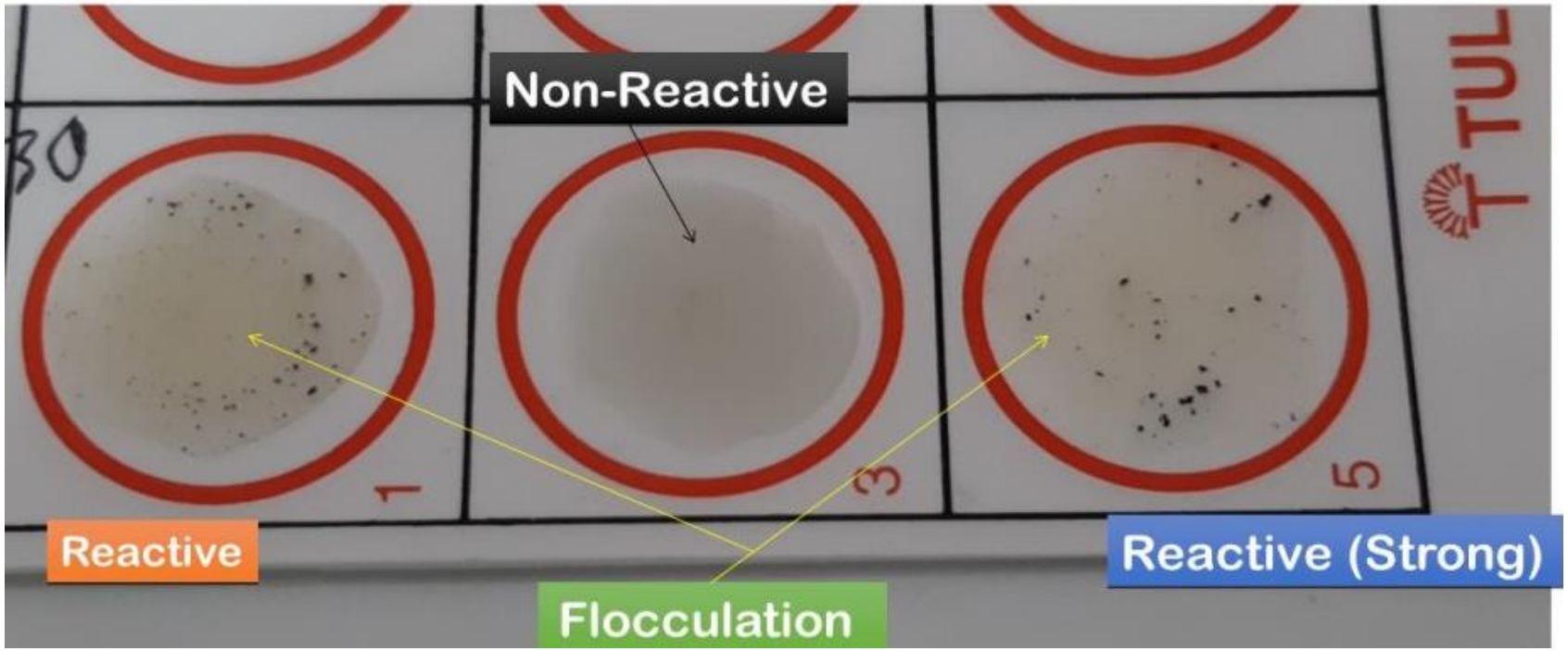
- Measure antibody directed against *T. pallidum* antigens
- Qualitative, usually reactive in life

# RPR Test

# RPR test


Sample
Antigen
Rotate 100 rpm/8 minutes
Negative
Positive


Non-Reactive
Reactive
Flocculation
Reactive (Strong)
1
3
5

## 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.

**Penicillin allergic:**

- Doxycycline 100 mg orally twice daily for 28 days
- Tetracycline 500 mg orally 4 times daily for 28 days

## Therapy for Syphilis in Pregnancy

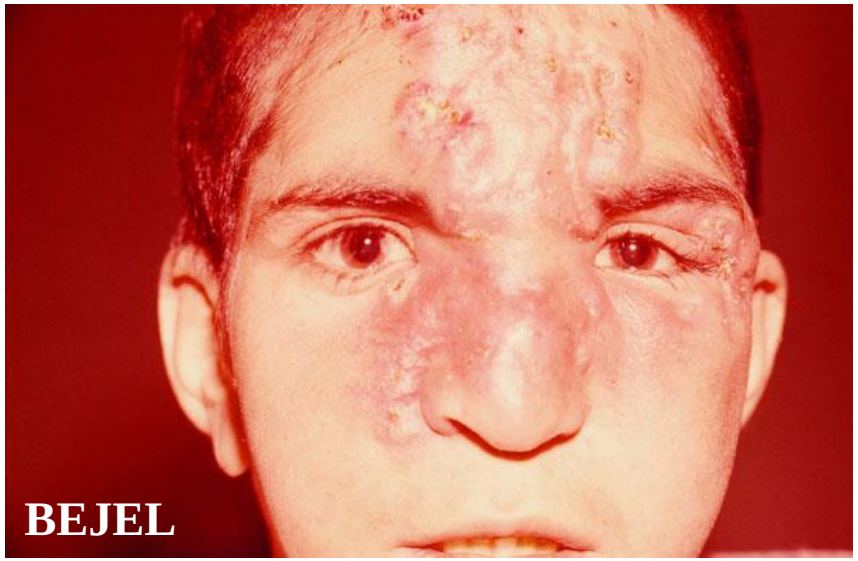
- Treat with penicillin according to stage of infection.
- Erythromycin is no longer an acceptable alternative drug in penicillin-allergic patients.

## 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 SYPHİLİS

**YAWS**


**PİNTA**

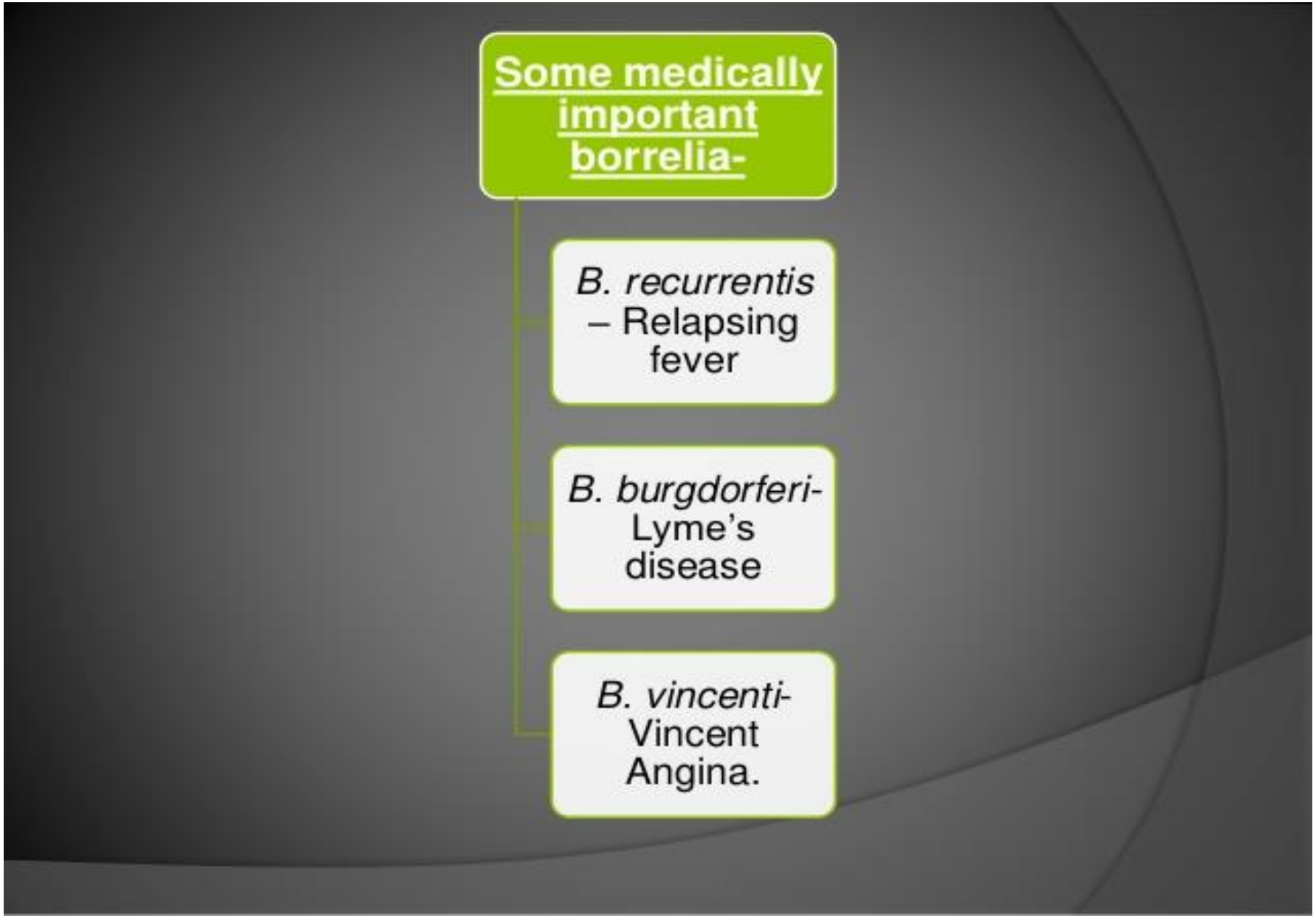
# *Borreliaceae* - Taxonomy

- (Domain): Bacteria
- (Kingdom): Spirochaetota
- (Class): Spirochaetia
- (Order): Spirochaetales
- (Family): Borreliaceae
- (Genus): Borrelia
- (Species): *B.recurrentis*, *B.duttoni*, *B.persica*, *B.burgdorferi* etc.

# BORRELIA

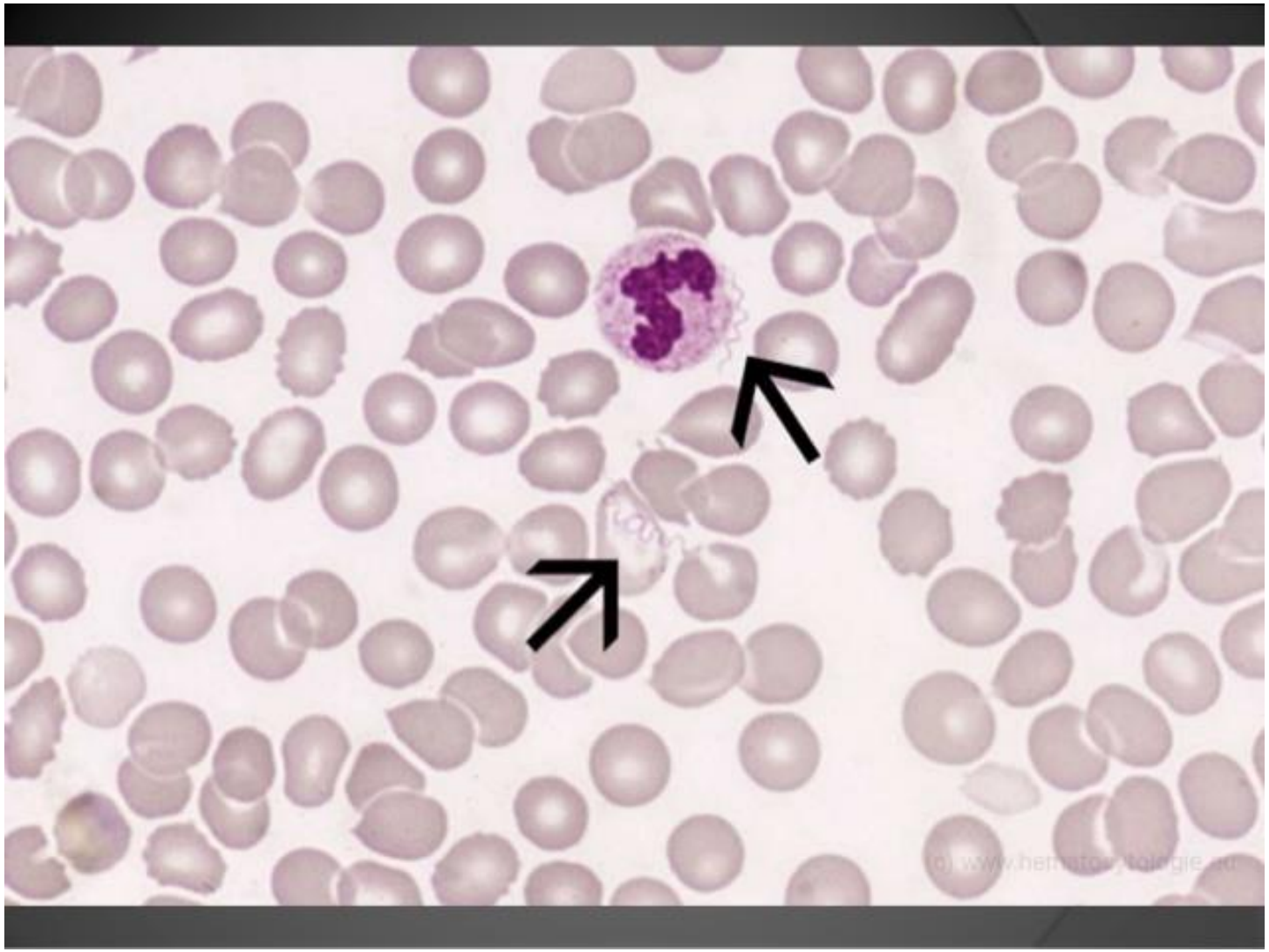
## Introduction

- 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.

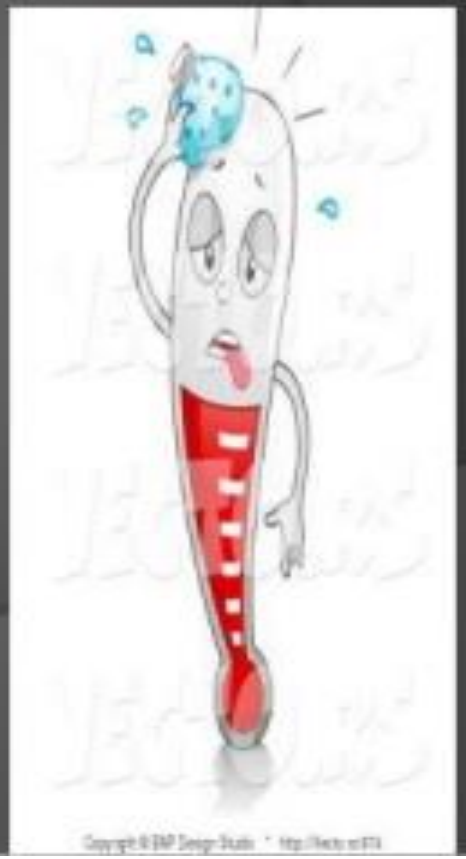
- ***Borrelia recurrentis-***
- Morphology-
  - Irregular spiral with one or both ends pointed.
  - Possesses 5-10 loose spiral coils at interval of about 2mm
- Cultural characteristics-
  - Microaerophilic, temp- 28-30 ℃
  - Cultivation is difficult but can be cultivated on 'modified Kelly's medium'
  - 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:
    - Louse-borne relapsing fever
    - 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

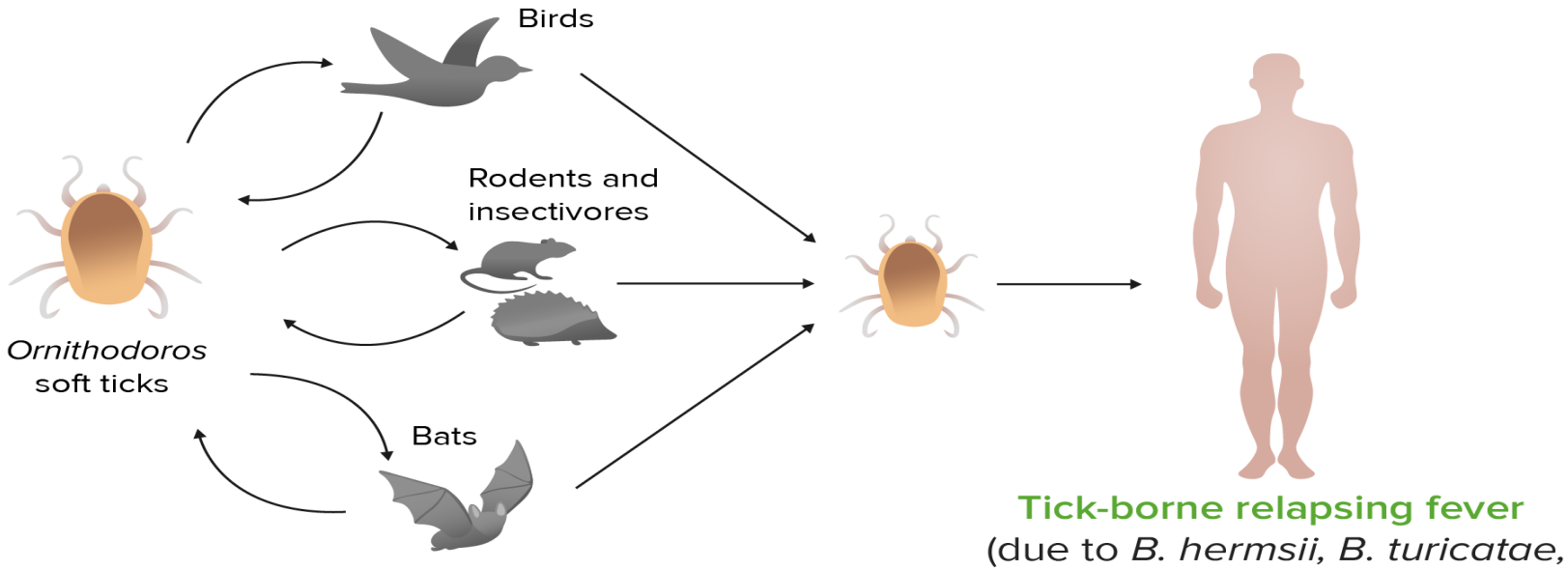
• Within one week after sucking infected blood spirochaetes appear in the tick's salivary glands and in the coxal fluid ready to be transmitted to a new host

# cont...

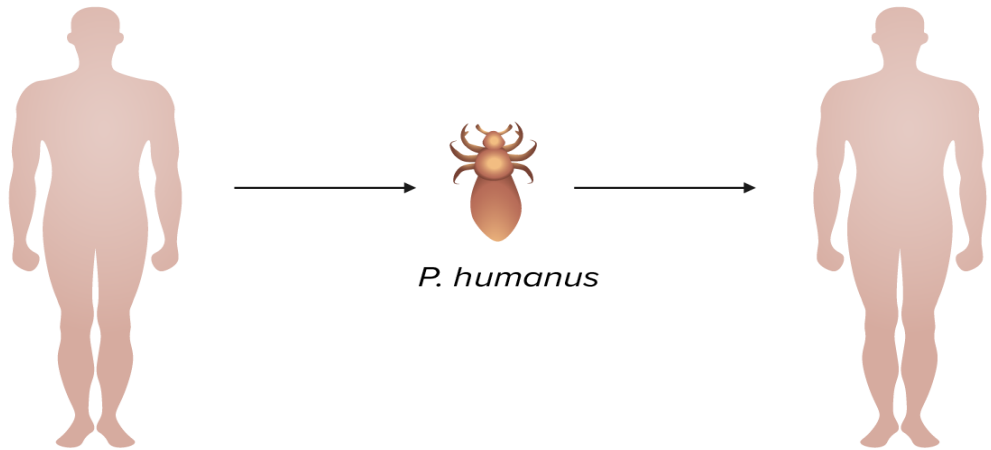
The organisms can either be injected directly when the tick feeds on the host, or they invade the body through intact mucous membrane. (e.g., in laboratory infections: Duttoni, the discoverer of the disease died from it)

- 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




**Tick-borne relapsing fever**
(due to *B. hermsii, B. turicatae, B. hispanica, B. persica)*




**Louse-borne relapsing fever**
(due to *B. recurrentis)*

# Clinical Features of Relapsing Fever

## SYMPTOMS

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

## SIGNS

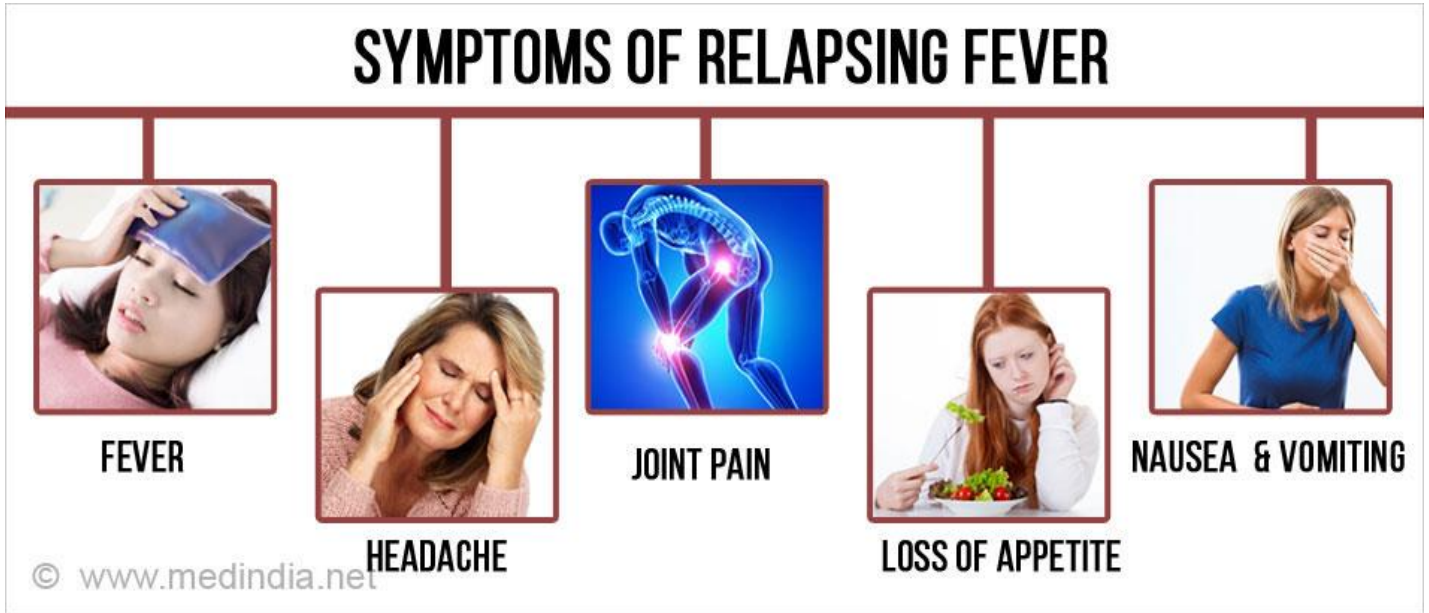
- Temperature
- Tachycardia
- Hepatomegaly
- Splenomegaly
- Petichea/ Subconjunctival bleeding
- Jaundice
- Confusion/Meningism

# Relapsing Fever


Incubation period

Symptomatic periods / Relapsing episodes (~ 3)

Afebrile (no fever) periods



# 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)

Geographical site

Blood specimens

Dissected vectors

Isolation and culture of *Borrelia*

BSK-H Medium

Dark-field Microscopy

Giemsa- stained blood smears

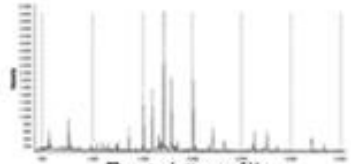
Quantitative buffy coat analysis

Immunoflourescence assay

A

Detection of *Borrelia* only

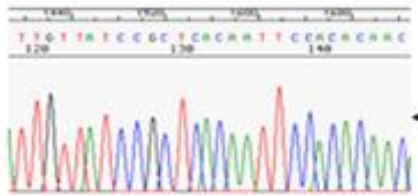
MALDI-TOF-MS



Protein profile

DNA extraction

B



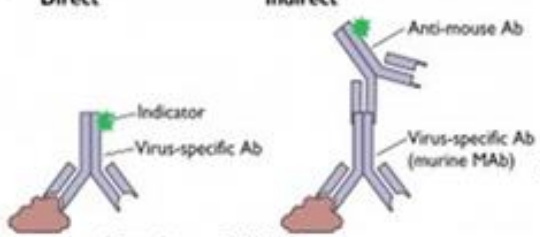
Sequencing of 16S–23S intergenic spacer, *glpQ*, 16S rRNA and *flaB* genes, Multispacer Sequence Typing

Multiplex real time PCR

Identification of species

Direct

Indirect




Serology: ELISA or Immunofluorescence assay

Direct identification in Point-Of-Care: results in 2 hours

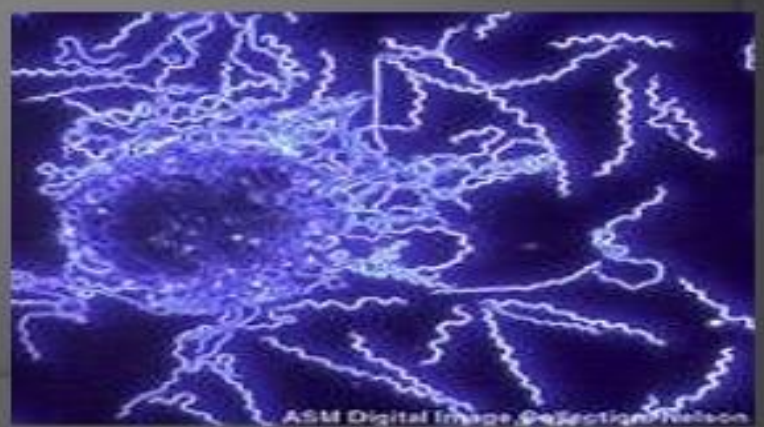
- **Prophylaxis-**
  - Prevention of louse infestation using insecticides.
  - Identification & avoidance of tick infested places
- **Treatment-**
  - Tetracyclines, chloramphenicol, penicillin, erythromycin are effective.

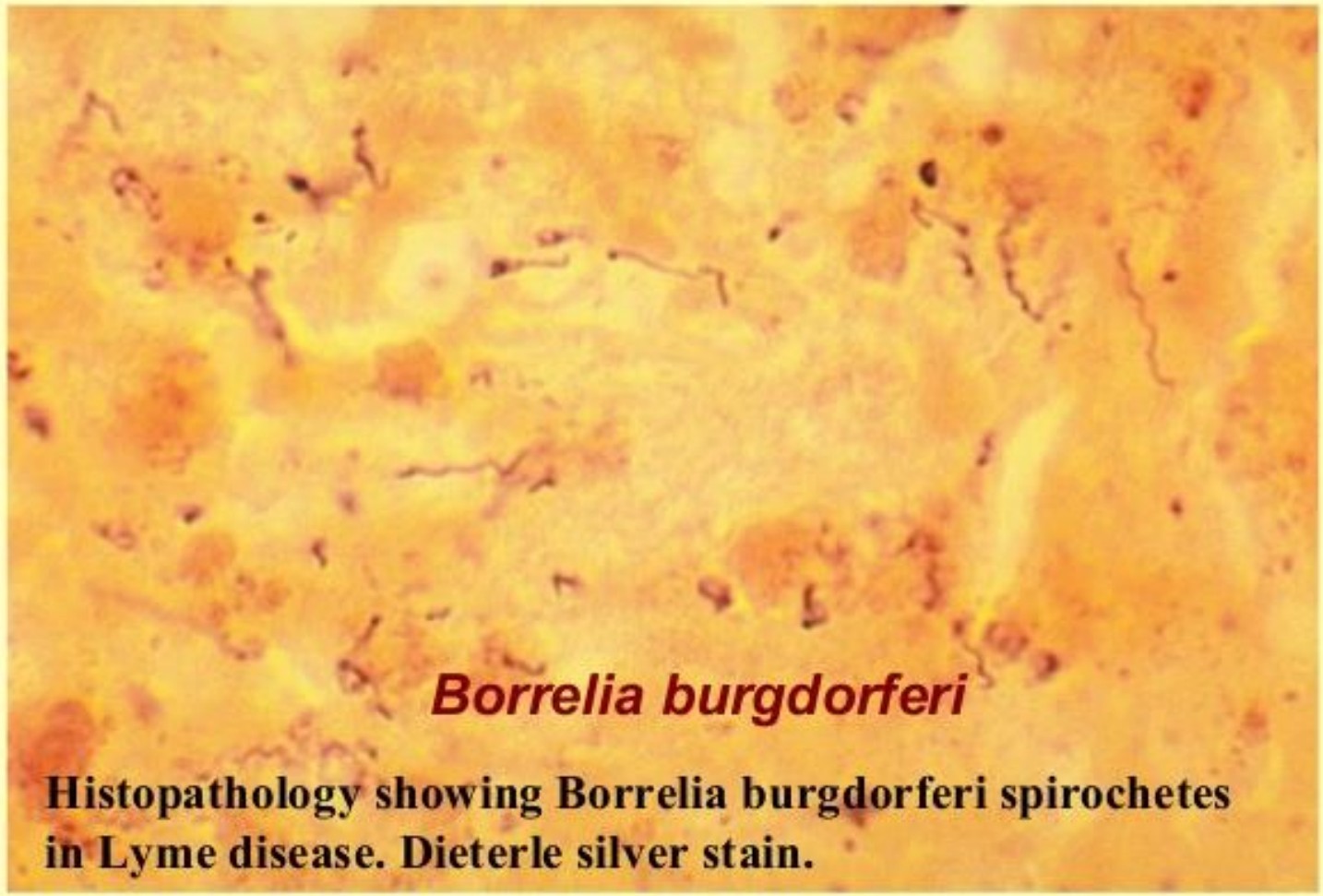
# LYME DISEASE

# 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

# Lyme Disease: *Pathogenesis and clinical findings*

**Authors**:
Victoria Chang
**Reviewers**:
Taylor Woo
Crystal Liu
Yan Yu*
Richard Haber*
** MD at time of publication*

Tick bite from *Borrelia burgdorferi* infected *Ixodes pacificus* (western black-legged tick in British Columbia, Canada)

Tick bite from *Borrelia burgdorferi* infected *Ixodes ricinus* (tick from European countries)

Tick bite from *Borrelia burgdorferi* infected *Ixodes scapularis* (Deer tick/black-legged tick in SE Canada and NE United States)

Binding of OspC (a surface protein expressed by *B. Burgdorferi*) to human plasminogen allowing the spirochete to spread from bite site to other host organs and tissues

*B. burgdorferi* spreads through skin and other tissues via bloodstream in human host.
If tick bite lasts 36-72 hours or more, this is sufficient time for ticks to transmit the infection.
(<36 hours of tick attachment results in a lower rate of infection: 1.2% -1.4%)

**Abbreviations:**
- **OspC** – outer surface protein C of *B. burgdorferi*

**Lyme Disease**

**A** vector-borne, infectious multi-system disease with highest risk in late spring and summer by the spirochete *Borrelia burgdorferi*

## Early Disease Stage (<30 days)

Macrophages and T-cells produce ↑ inflammatory (TNF-α, IFN-γ) and ↑ anti-inflammatory cytokines, causing eosinophils to concentrate adjacent to the tick bite site

Erythema migrans (a slowly expanding red skin patch with partial central clearing resulting in a "target clearing lesion" appearance) at site of tick bite

Systemic inflammatory response after dissemination of the spirochete to body tissues and organs

Flu-like symptoms (fever, chills, muscle aches, headache, fatigue, joint aches, swollen lymph nodes)

## Early Disseminated Disease Stage (<3 months)

*B. burgdorferi* attach to host cell integrins

Pro-inflammatory response with production of matrix glycosaminoglycans and extracellular matrix proteins which have an affinity to attack collagen fibrils on the heart, nerves, and joints

1. Multiple erythema migrans
2. Meningitis
3. Meningoradiculoneuritis
4. Cranial nerve palsies
5. Carditis
6. Borrelial lymphocytoma

## Late Disease Stage (>3 months)

Ongoing and repeated innate and adaptive host immune response to *B. burgdorferi*

Chronic inflammatory state results in synovial hypertrophy, vascular proliferation, and ↑ mononuclear cell infiltrate in large joints

Large joint arthritis (most commonly affecting the knees)

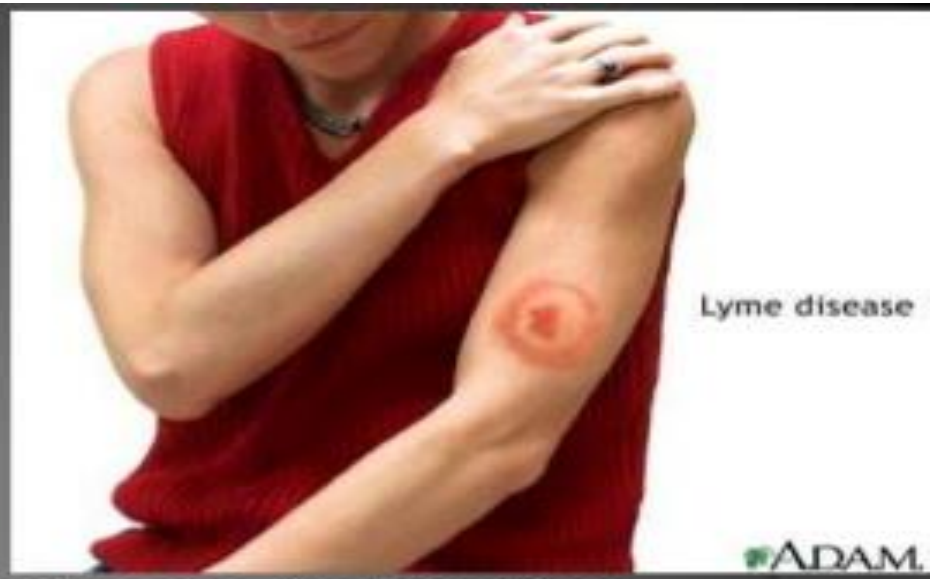
**Legend:** Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

Published **July 24, 2019** on www.thecalgaryguide.com

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

1) Localized infection-

-'Erythema chronicum migrans'.




-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.

# Lyme disease (*erythema migrans*)

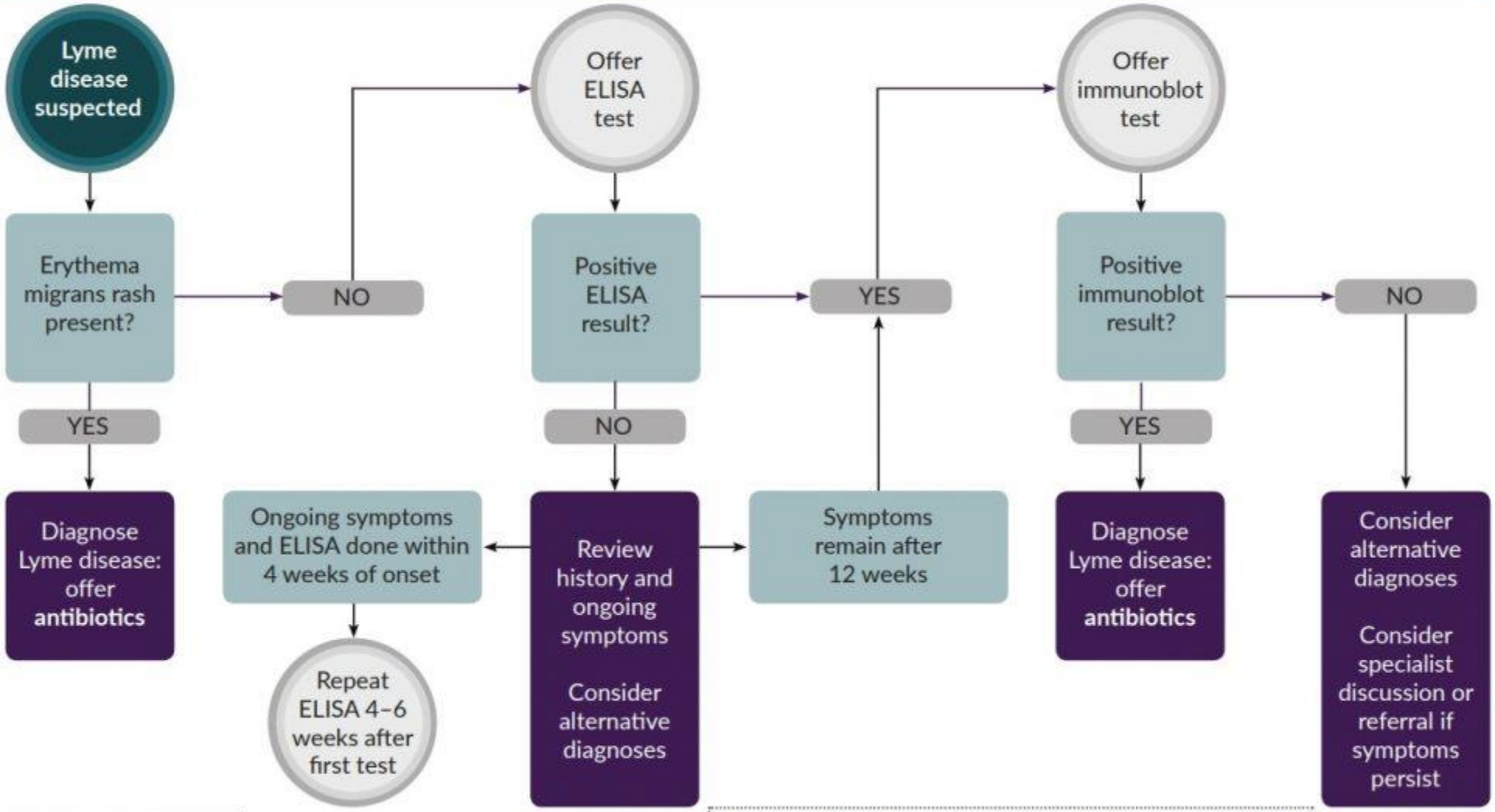

## Lab diagnosis-

- 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.

# Lyme disease: laboratory investigations and diagnosis

Use clinical presentation and laboratory testing to guide diagnosis. If there is a high clinical suspicion of Lyme disease:

- consider starting treatment while waiting for test results
- do not rule out Lyme disease even if results are negative




This is a summary of the recommendations on tests for Lyme disease from NICE's guideline on Lyme disease. The guideline also covers clinical assessment and management, including antibiotic treatment. See www.nice.org.uk/guidance/NG95

NICE National Institute for Health and Care Excellence

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

# LEPTOSPIRA INTERROGANS

## TAXONOMY

### Classification

**Phylum: Spirochaetes**

**Class: Spirochaetes**

**Order: Spirochaetales**

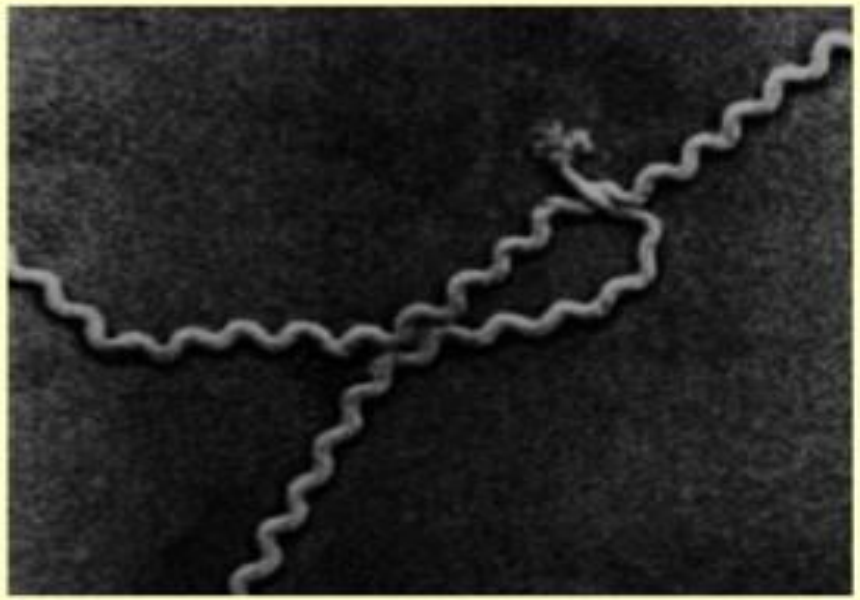
**Species: *Leptospira***

**Family: Leptospiraceae**

# PHYSIOLOGY AND STRUCTURE

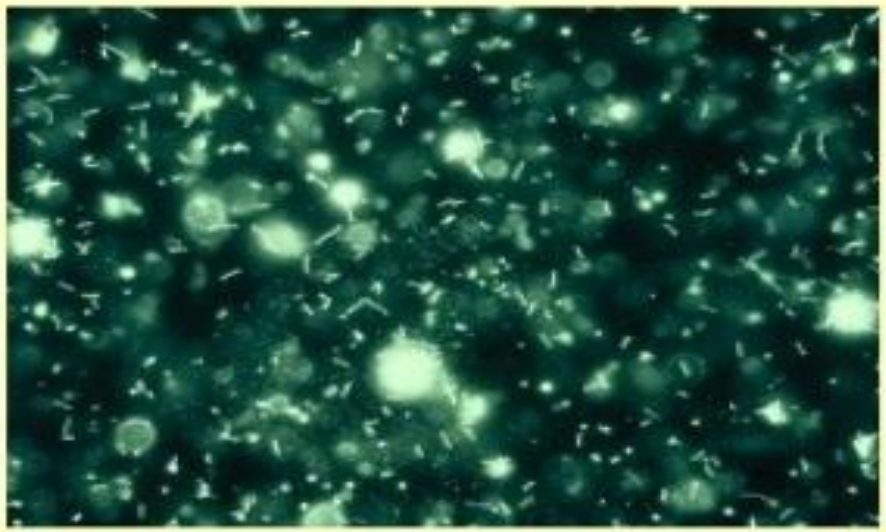
- Thin, coiled spirochetes (.1 ×6 to 20 µm)that grow slowly in culture.
- Temperature(28°C to 30°C)
- Gram-negative spirochetes
- Obligate aerobes and Characteristic hooked ends .
- Pathogenic strains: Leptospira interrogans.
- Non pathogenic strains: Leptospira biflexa.

# Leptospira under the Microscope



Long, Thin, Highly Coiled

Dark Field Microscopy FL



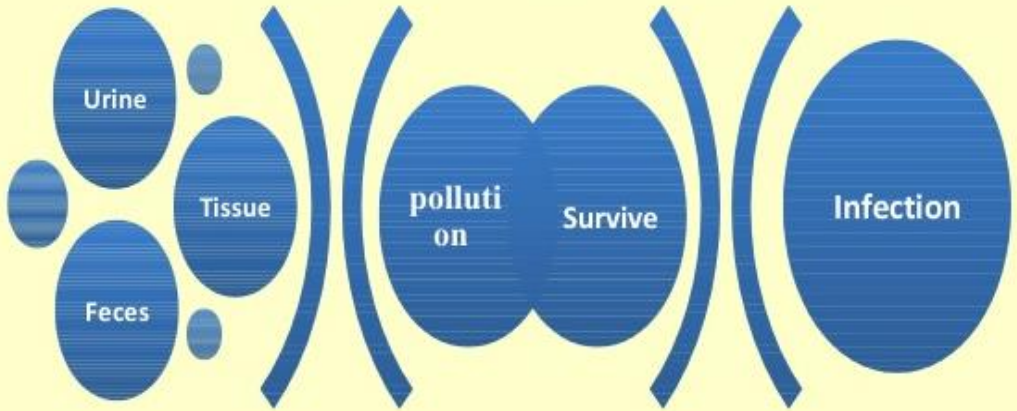
# VIRULENCE FACTOR

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

# *EPIDERMIOLOGY*

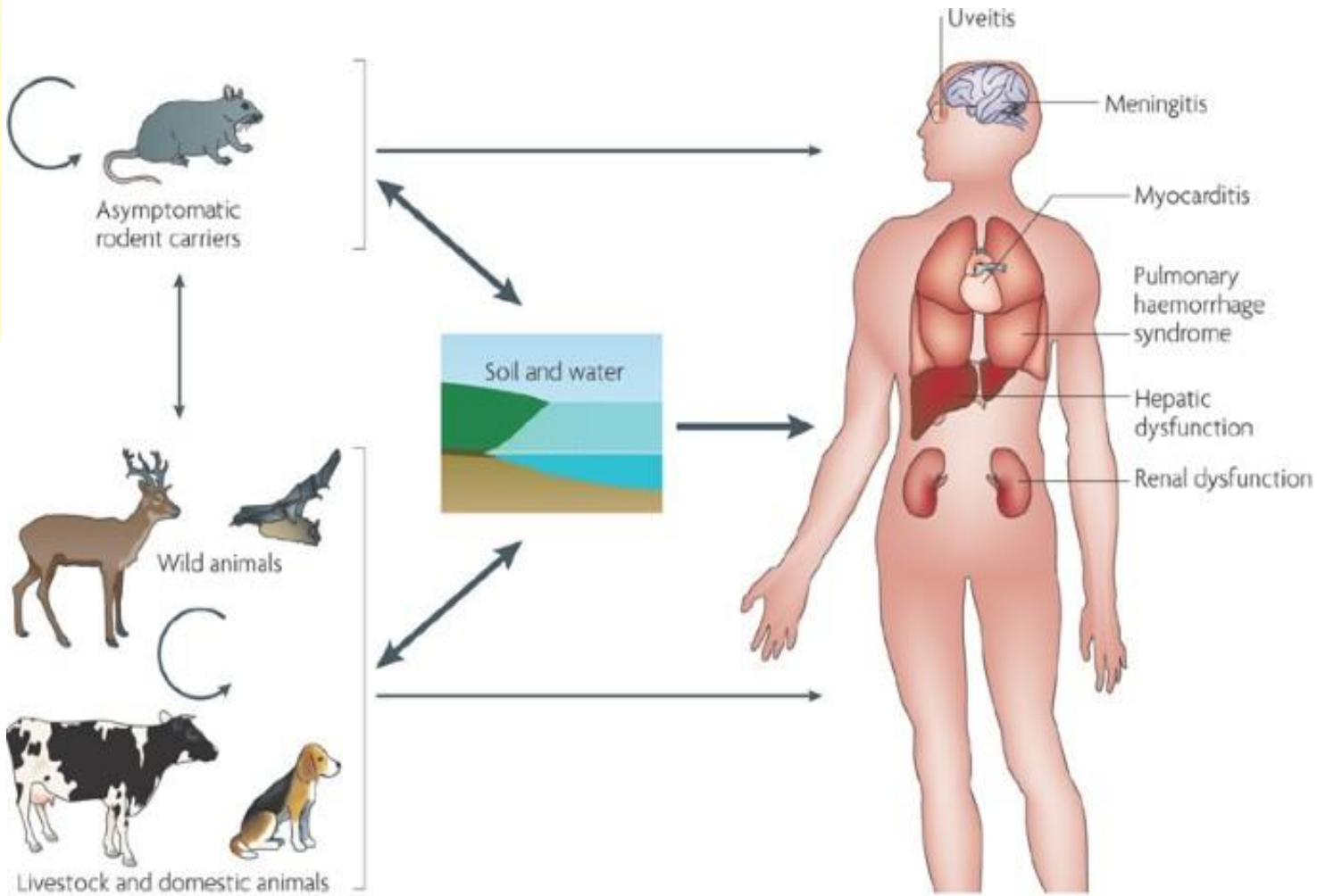
- Mainly a zoonotic disease
  - Transmitted to humans from a variety of wild and domesticated animal hosts
  - Most common reservoirs rodents (rats), dogs, farm animals and wild animals
- Organism can penetrate the skin through minor breaks in the epidermis or with intact mucus membranes
- Indirect contact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

# Transmission



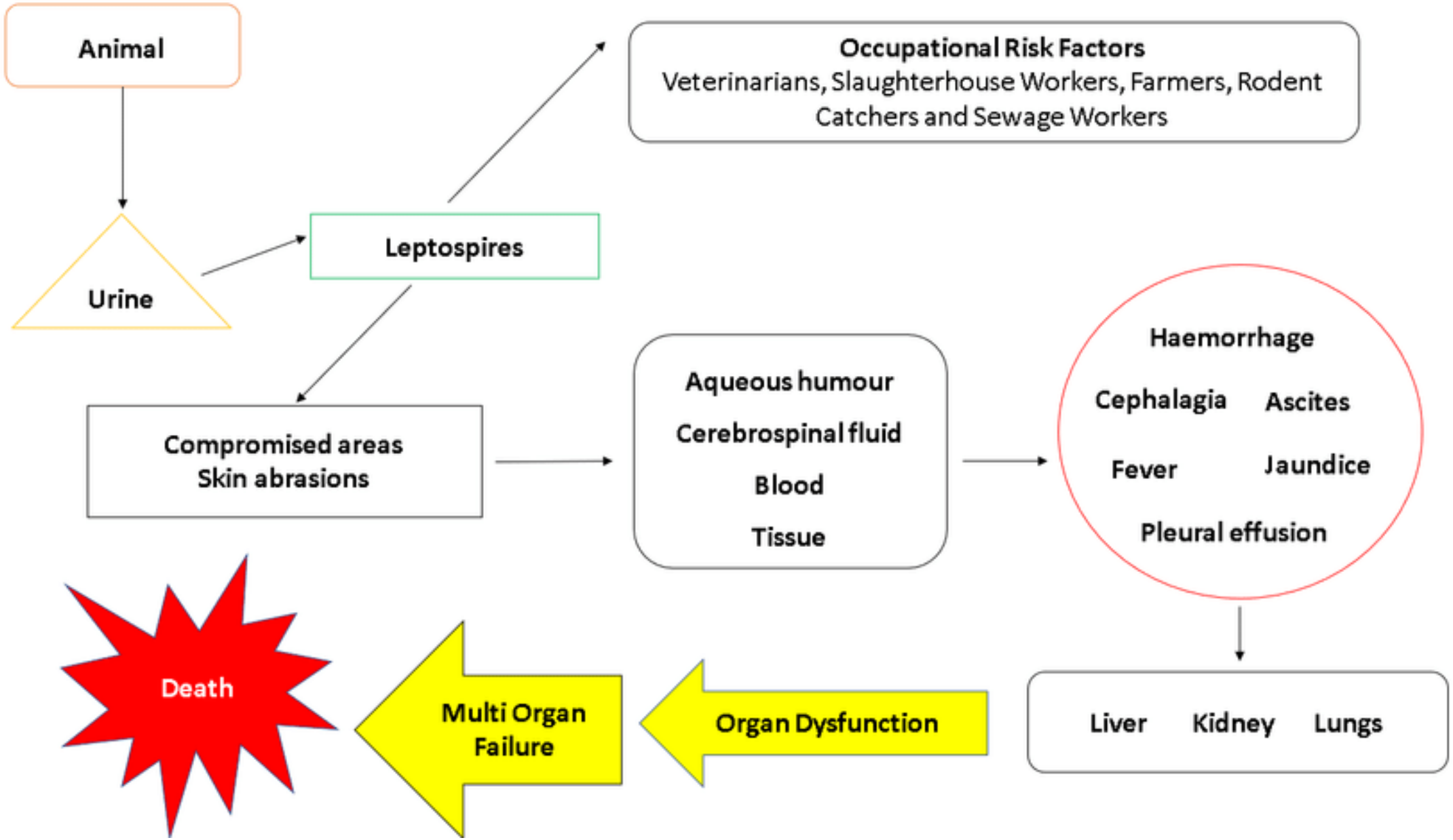

**Human infection is accidental**

**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

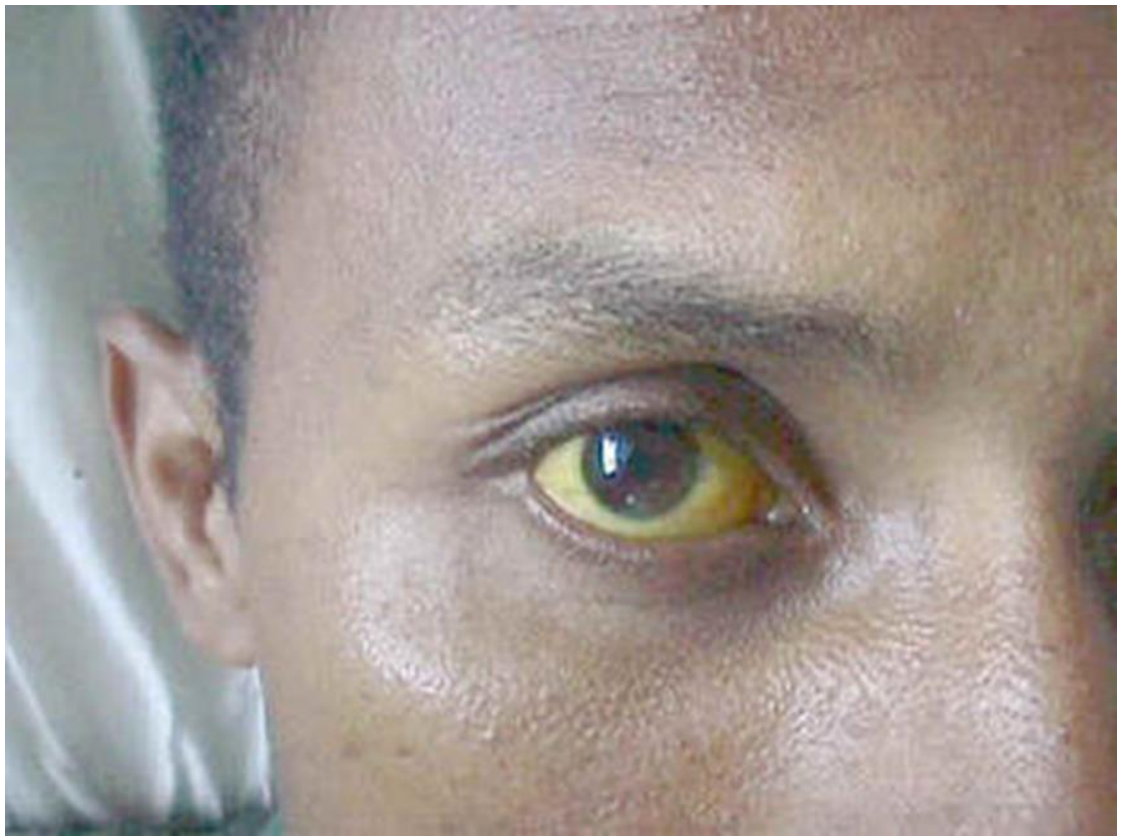

Animal
Urine
Leptospires
Occupational Risk Factors
Veterinarians, Slaughterhouse Workers, Farmers, Rodent Catchers and Sewage Workers
Compromised areas
Skin abrasions
Aqueous humour
Cerebrospinal fluid
Blood
Tissue
Haemorrhage
Cephalagia
Ascites
Fever
Jaundice
Pleural effusion
Liver
Kidney
Lungs
Organ Dysfunction
Multi Organ Failure
Death

# *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

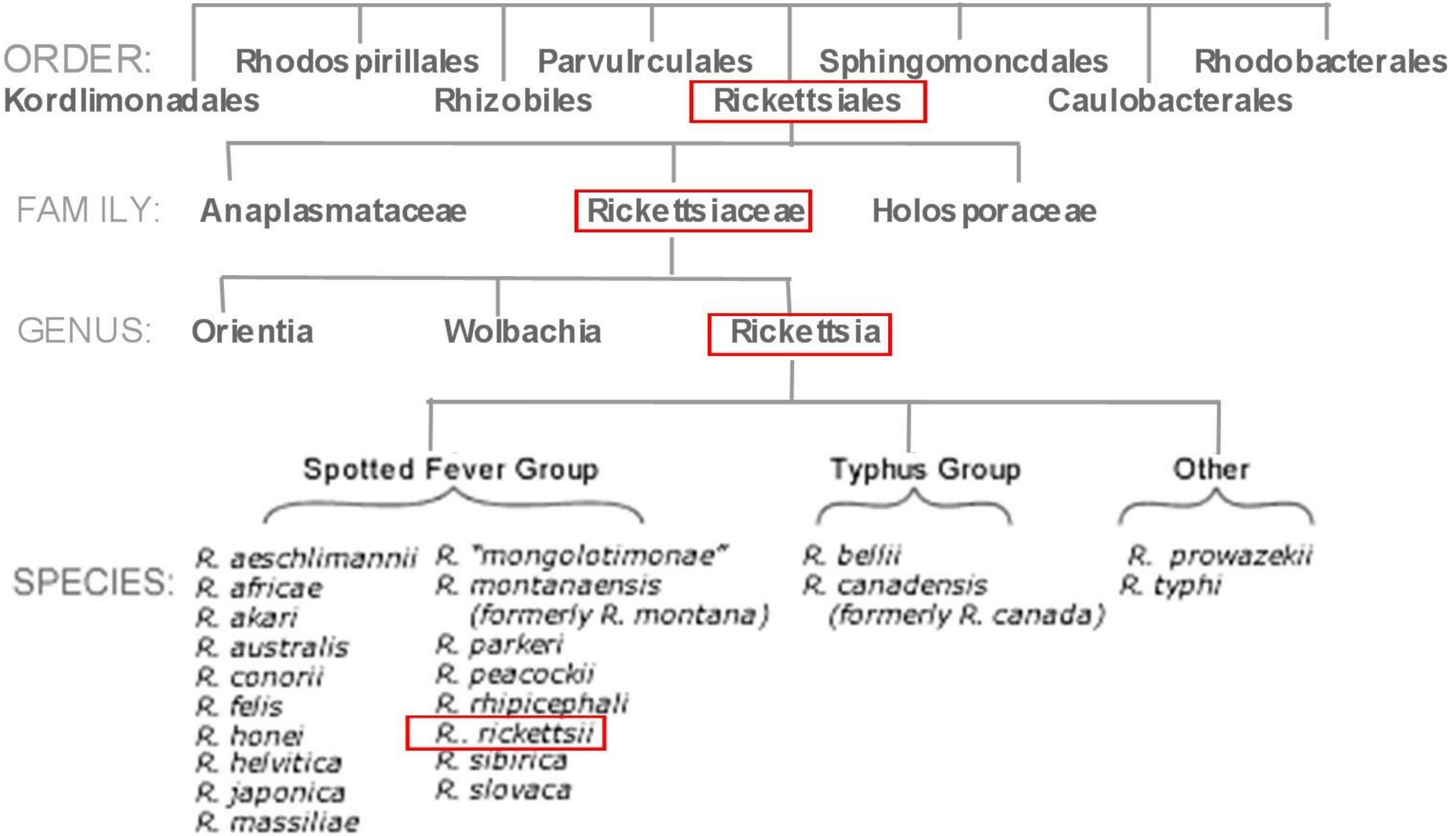
- △ **Direct microscopy**
  - ◎ **Dark-ground microscopy**
  - ◎ **Silver stain**
  - ◎ **Fluoresent antibody staining**
- △ **Culture isolation and identification : Korthof liquid medium**
- △ **Animal test**
- △ **Molecular diagnostics**

# Serological examinations

**Paired serum**

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

# RICKETTSIA


ORDER:
Rhodospirillales
Parvularculales
Sphingomonadales
Rhodobacterales
Kordlimonadales
Rhizobiles
Rickettsiales
Caulobacterales
FAMILY:
Anaplasmataceae
Rickettsiaceae
Holosporaceae
GENUS:
Orientia
Wolbachia
Rickettsia
Spotted Fever Group
Typhus Group
Other
SPECIES:
R. aeschlimannii
R. africae
R. akari
R. australis
R. conorii
R. felis
R. honei
R. helvitica
R. japonica
R. massiliae
R. "mongolotimonae"
R. montanaensis
(formerly R. montana)
R. parkeri
R. peacockii
R. rhipicephali
R. rickettsii
R. sibirica
R. slovaca
R. bellii
R. canadensis
(formerly R. canada)
R. prowazekii
R. typhi

# *Rickettsiaceae* - Taxonomy

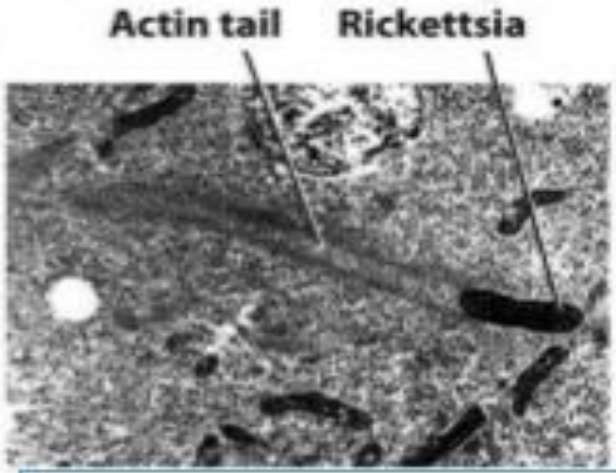
- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Alphaproteobacteria
- (Order): Rickettsiales
- (Family): Rickettsiaceae
- (Genus): Rickettsieae, Orientia
- **Növ** (Species):

# *Ehrlichiaceae* - Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Alphaproteobacteria
- (Order): Rickettsiales
- (Family): Ehrlichiaceae
- (Genus): Erlichia
- (Species):

# 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

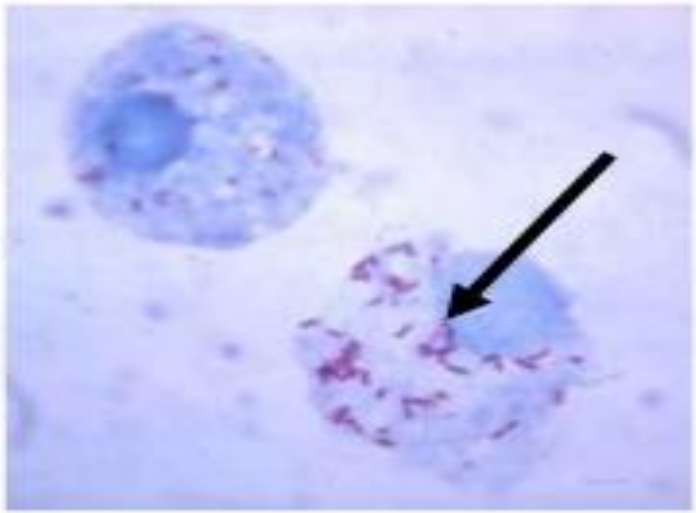



*Rickettsia rickettsii*

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.

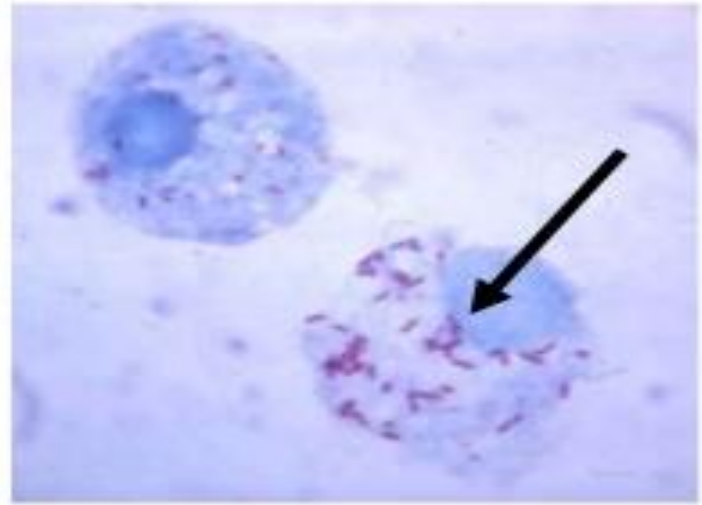


Rickettsia inside the host cell




# 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.



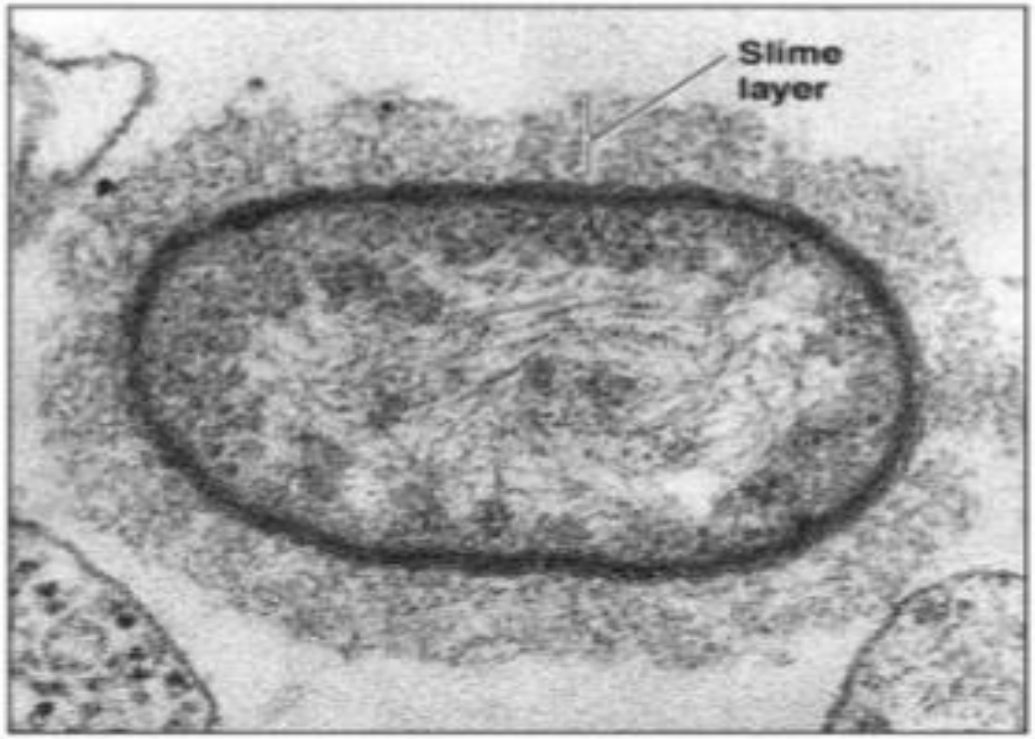
**Rickettsia inside the host cell**

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

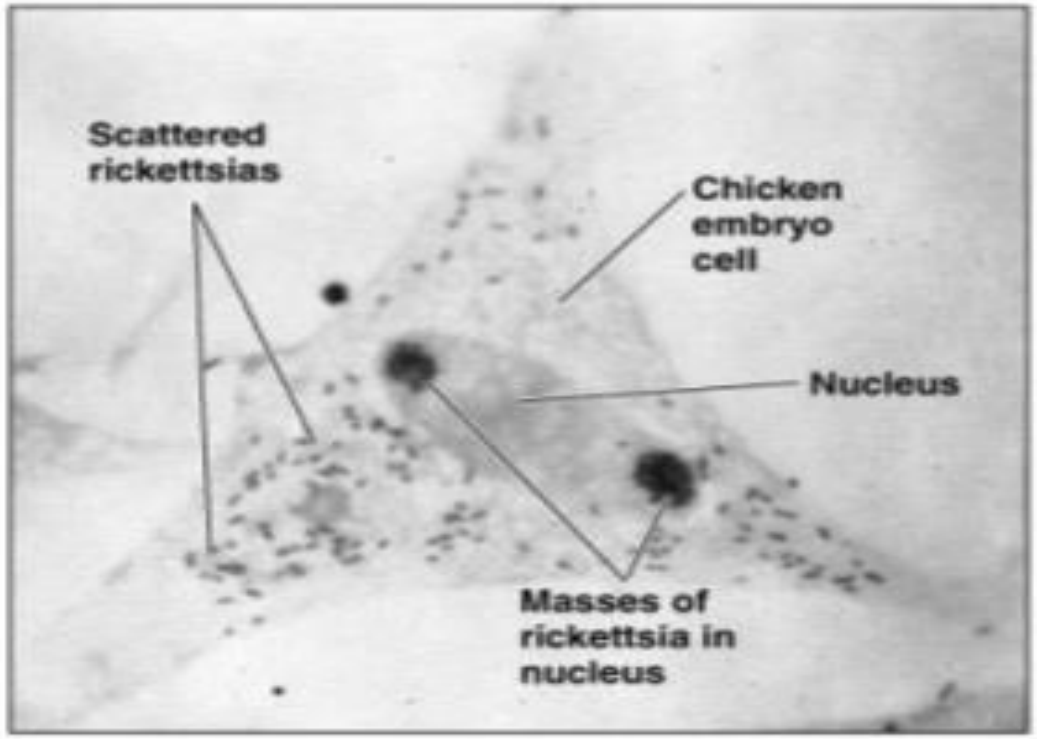
# General characteristics

- 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

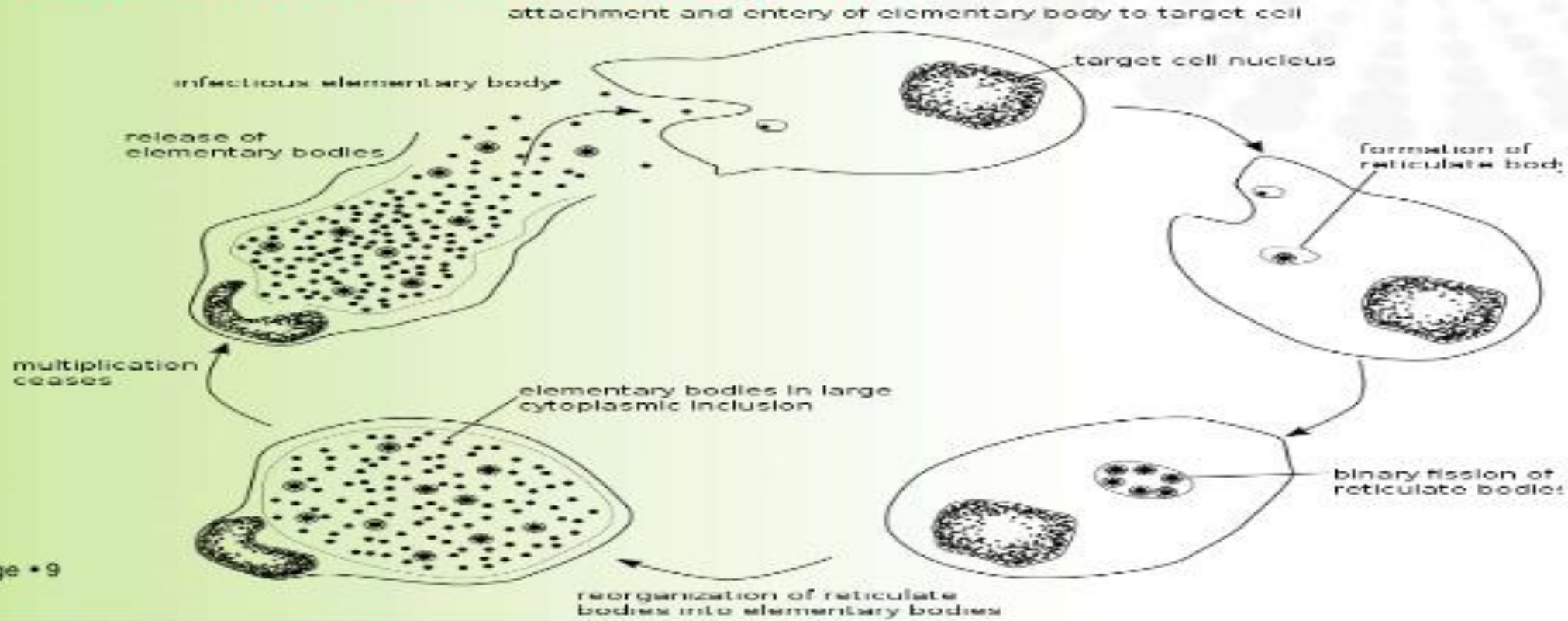
| Species | Disease | Reservoir |
|---|---|---|
| *R. prowazekii* | Epidemic typhus, Brill-Zinsser disease | Human body louse |
| *R. typhi* | Endemic typhus | Rat flea |
| *R. rickettsii* | Rocky-Mountain spotted fever | Ticks |
| *R. conori* | Boutonneuse fever | Ticks |
| *R. australis* | Australian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

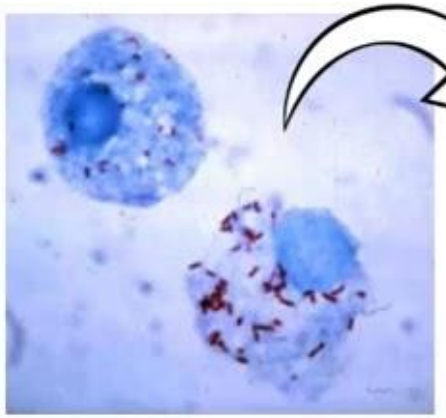
- Blood is inoculated in guinea pigs/mice.
- Observed on 3rd – 4th week.
- Animal responds to different rickettsial species can vary.
- Difficult & dangerous because of the highly infectious nature of rickettsiae.
- Symptoms:
  - ✓ Rise in temperature – all species.
  - ✓ Scrotal inflammation,swelling,necrosis – *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.

# Rickettsial species and its disease

| Species | Disease | Reservoir |
|---|---|---|
| *R. prowazekil* | Epidemic typhus, Brill-Zinsser disease | Human body louse |
| *R. typhi* | Endemic typhus | Rat flea |
| *R. rickettsil* | 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 |



Bacteria
Rickettsiae

Male

Nymph

Feeds on small and medium sized mammals

Infects Tick, Mite, Flea

Infects Human

Spotted fever group rickettsioses (spotted fevers).

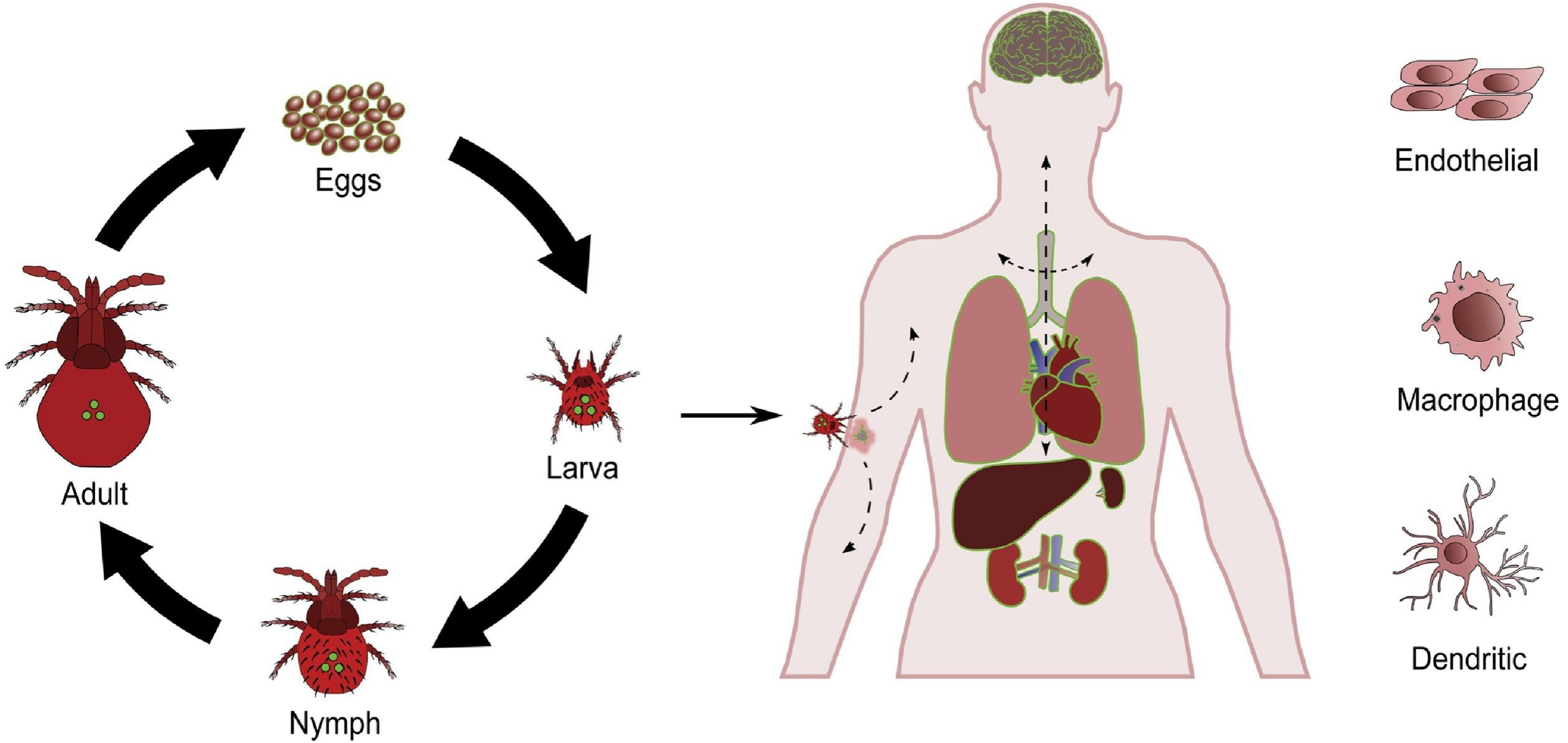
Rocky Mountain spotted fever (RMSF)
(Most Important) Can be Fatal

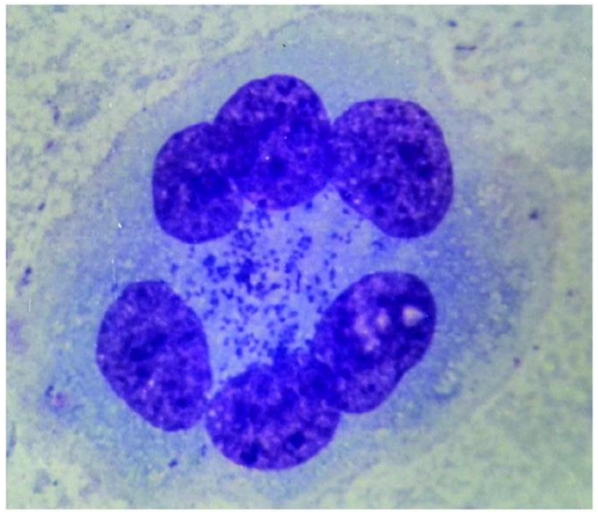
Symptoms: Fever, Headache, Rash

Rash – 2-4 days after fever begins

Diagnosis: Clinical Symptoms

# *Orientia tsutsugamushi*

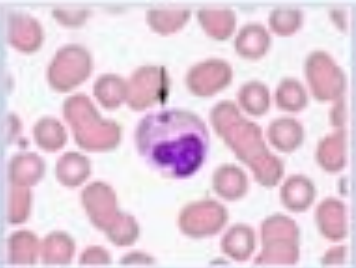
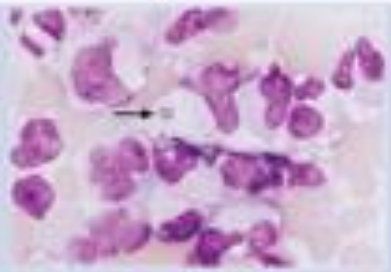
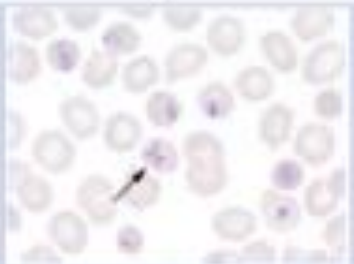




***Orientia tsutsugamushi***

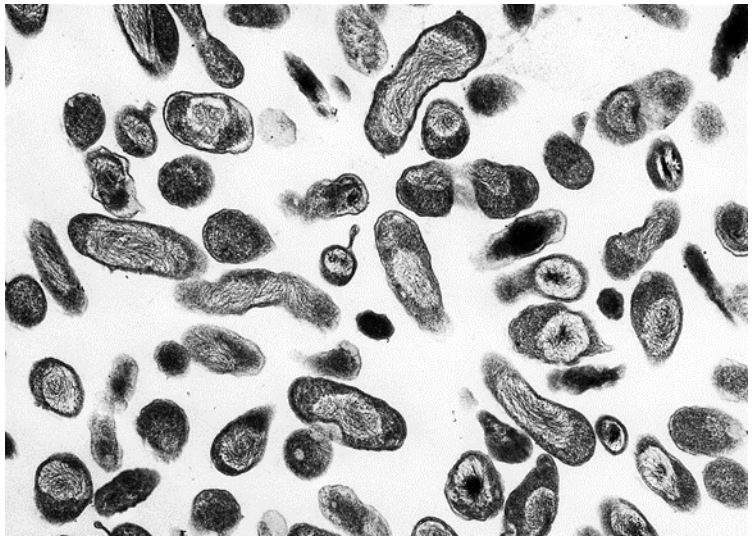
Giemsa stain

# *ERLICHIOSIS*

| | Human granulocytic anaplasmosis | Erlichiosis | Babesiosis |
|---|---|---|---|
| Most common agent | *Anaplasma phagocythopilum* | *Ehrlichia chaffeensis* | *Babesia microti* |
| Tick | *Ioxedes tick* | *Lone Star tick* | *Ioxedes tick* |
| Clinic | Nonspecific febrile illness with severe headache and myalgia | | |
| Labs | Leukopenia, thrombocytopenia and increased LFTs | | *Hemolytic anemia, jaundice, hemoglobinuria |
| Generalized rash | + | +++ | + |
| CNS involvement | + | +++ | + |
| Smear |  |  |  |
| Diagnosis | NAAT<br>Blood smear (S 25-75%)<br>Baseline serology | NAAT<br>Blood smear (Only 3% cells affected)<br>Baseline serology | Blood smear<br>NAAT<br>Baseline serology |
| | Serology (>1:256 or 4-fold) - NAAT | | |
| Treatment | Doxycycline | Doxycycline | Atovaquone PLUS azithromycin |
| Fatality | 0.3%<br>Response in 48h! | 42% admission<br>17% severe disease<br>3% mortality | Severe cases in immunosuppressed |

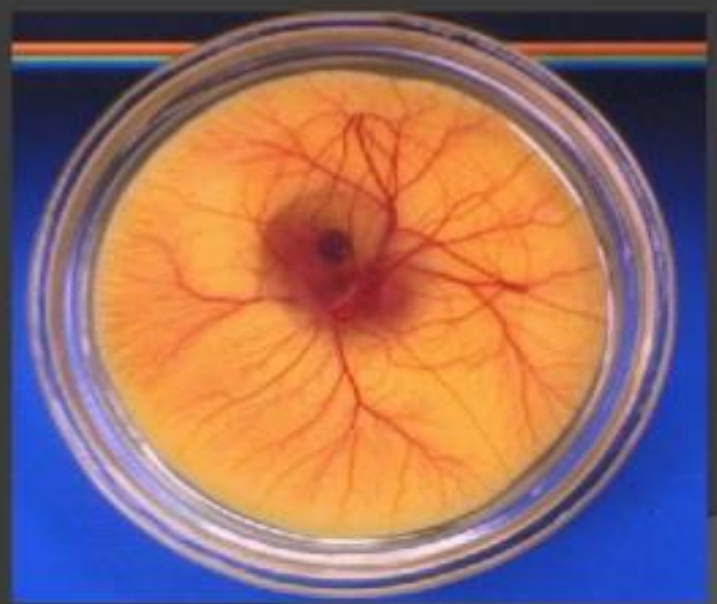
# Introduction

- ***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

# 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

# Primary Reservoir

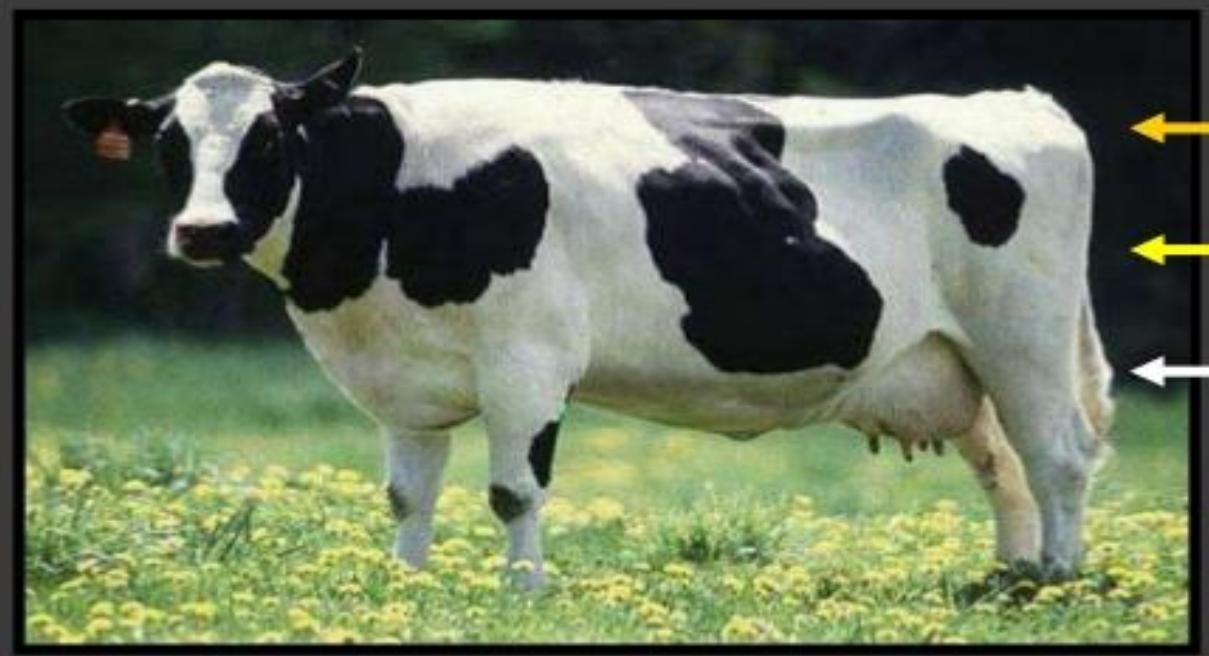
Goats

Cattle

Sheep

* All eukaryotes can be infected

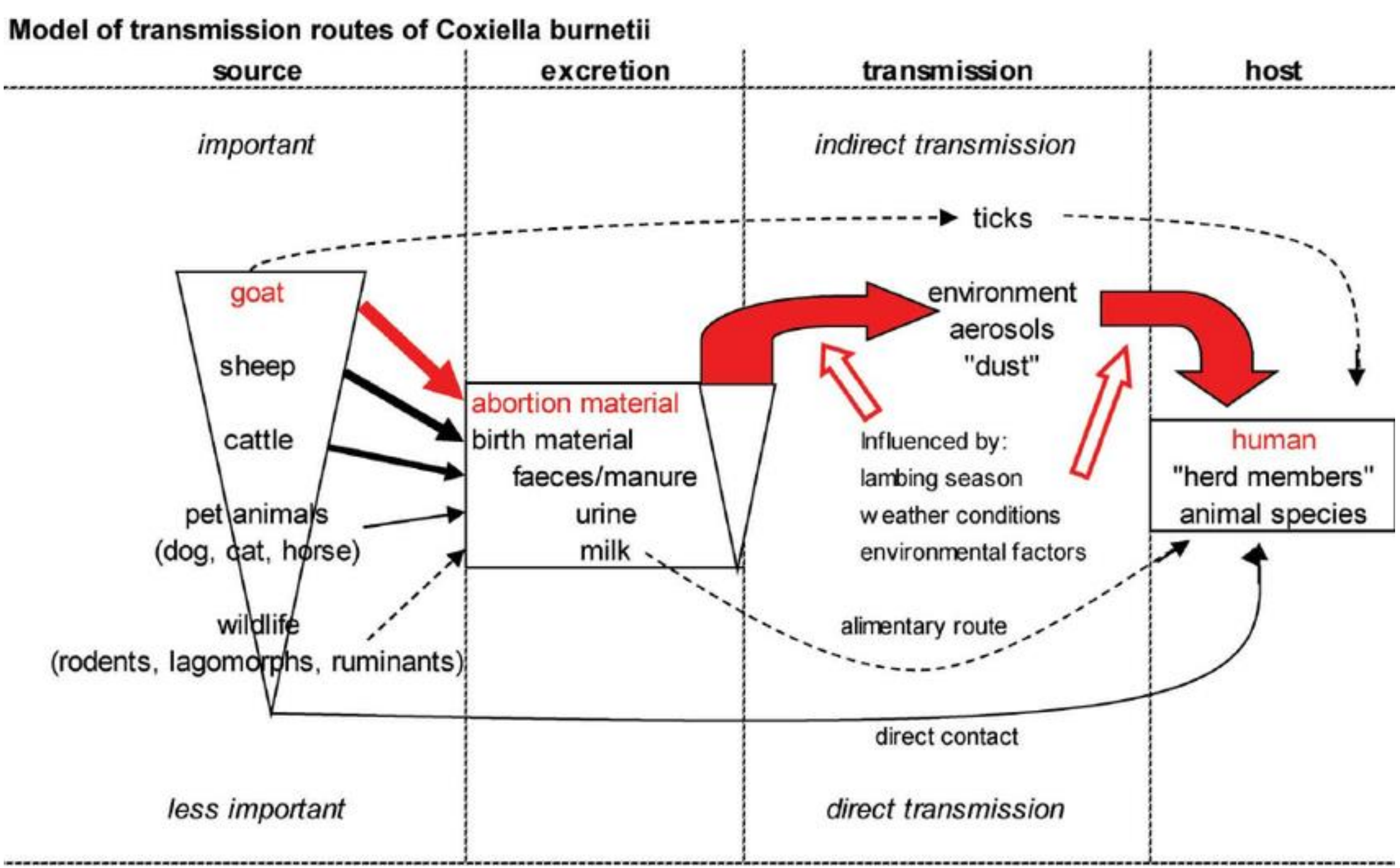
# Bacteria is excreted in:

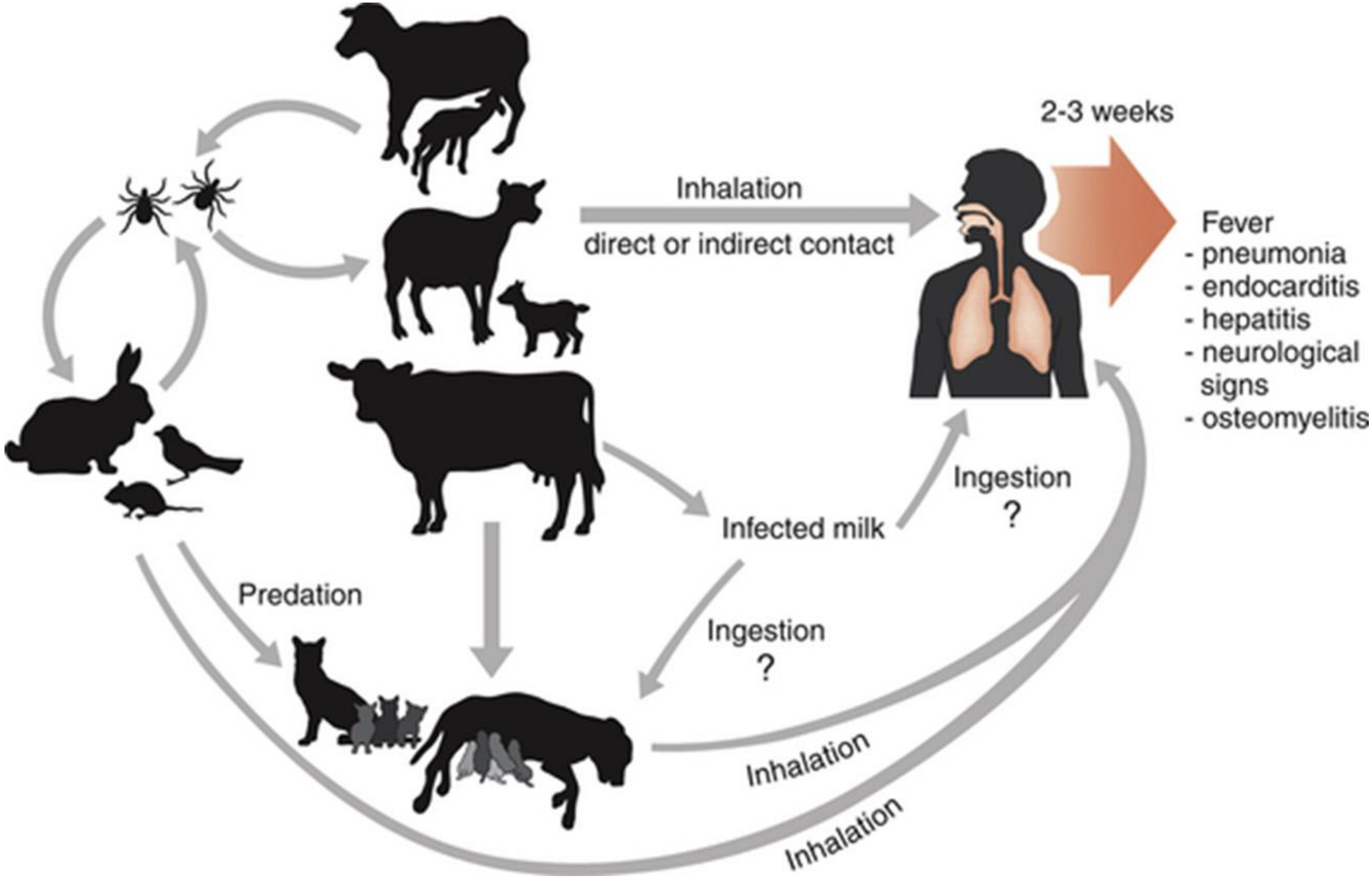



of infected animals

# 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


Model of transmission routes of Coxiella burnetii
source
excretion
transmission
host
important
indirect transmission
ticks
goat
sheep
cattle
pet animals
(dog, cat, horse)
wildlife
(rodents, lagomorphs, ruminants)
abortion material
birth material
faeces/manure
urine
milk
environment
aerosols
"dust"
Influenced by:
lambing season
weather conditions
environmental factors
human
"herd members"
animal species
alimentary route
direct contact
less important
direct transmission


2-3 weeks
Inhalation
direct or indirect contact
Fever
- pneumonia
- endocarditis
- hepatitis
- neurological signs
- osteomyelitis
Infected milk
Ingestion
?
Predation
Ingestion
?
Inhalation
Inhalation

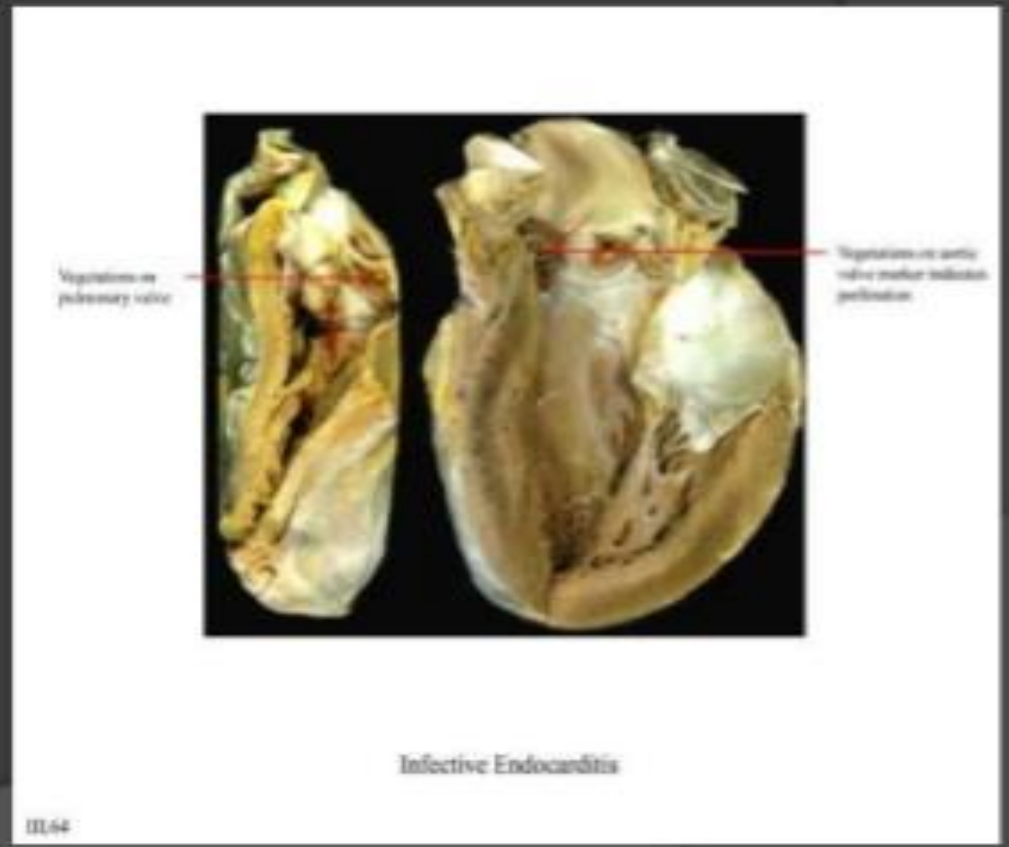
# Symptoms

## 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