AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 10.

Microbiology diagnosis of diseases, caused by Chlamydiae and Mycoplasma

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. 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.

2. Pathogenic mycoplasmas, morpho-biological characteristics.

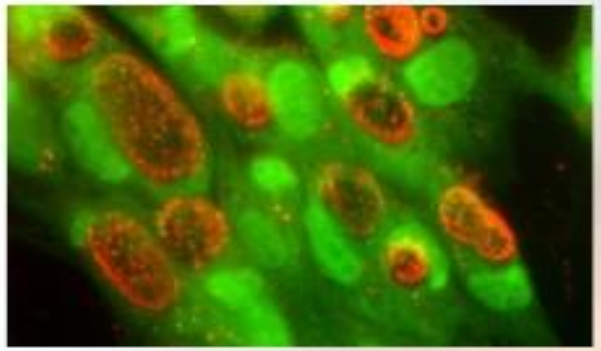
- *Mycoplasma* genus, morpho-biological characteristics, classification. Pathogenicity factors. Human diseases. Microbiological diagnostics.

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

Purpose of the lesson:

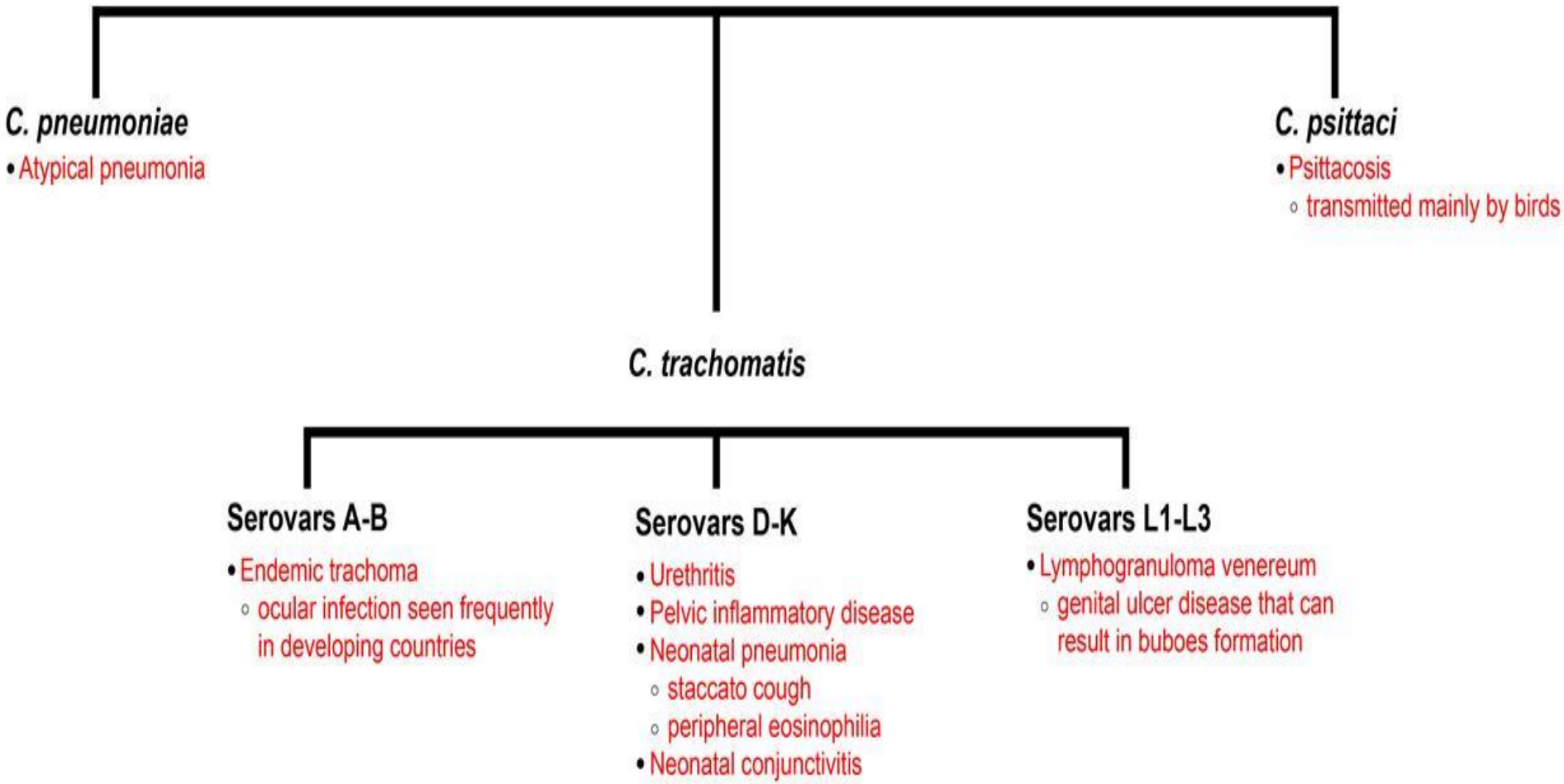
- To acquaint students with the morpho-biological features of pathogenic chlamydia (Chlamydia trachomatis, Chlamydia psittaci, Chlamydia pneumoniae) and mycoplasmas (Mycoplasma and Ureaplasma genera), pathogenesis of diseases, microbiological diagnosis, treatment and prevention principles.

Chlamydia



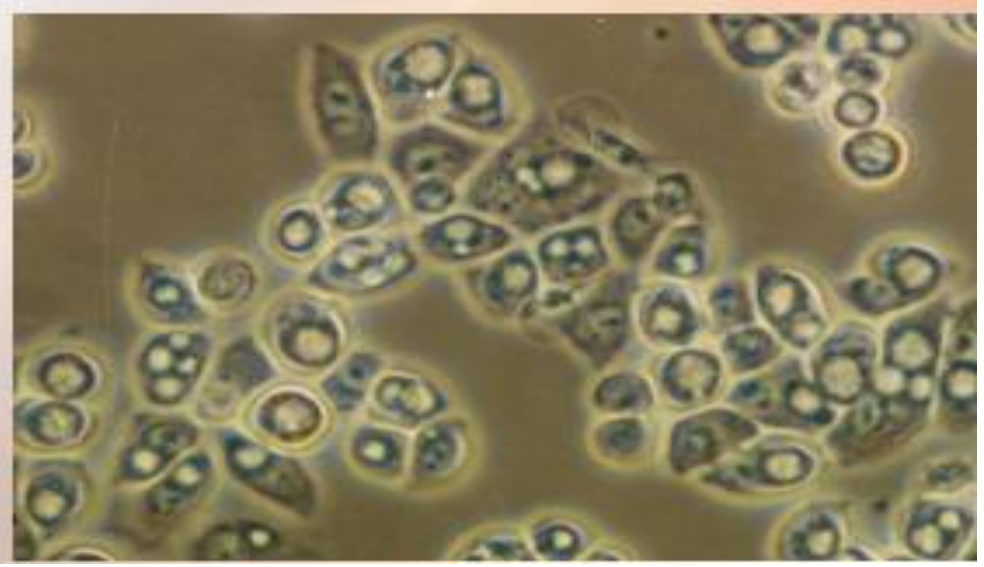
Chlamydia

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

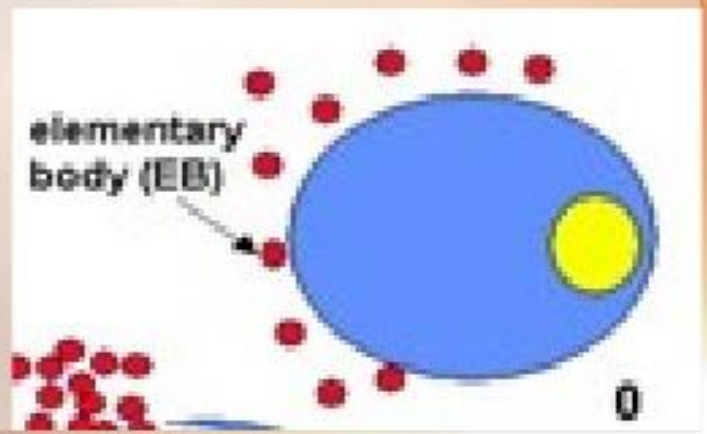


Developmental cycle

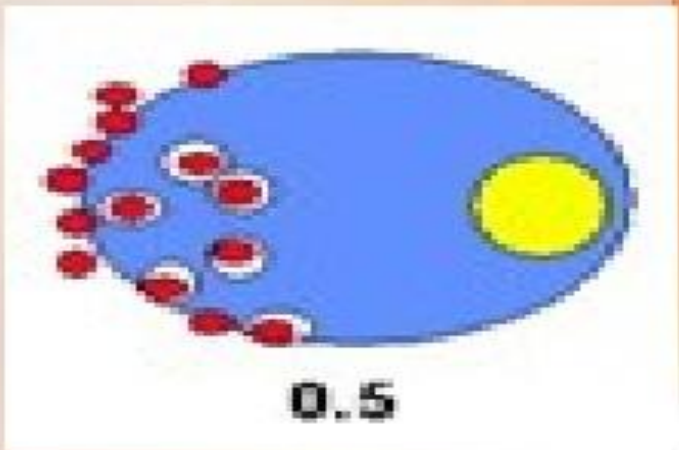
- Morphologically distinct infectious and replicative forms
- Stage 1. Attachment of elementary bodies (EBs)

  Infectious form
  - found in secretions
  - relatively resistant to environment

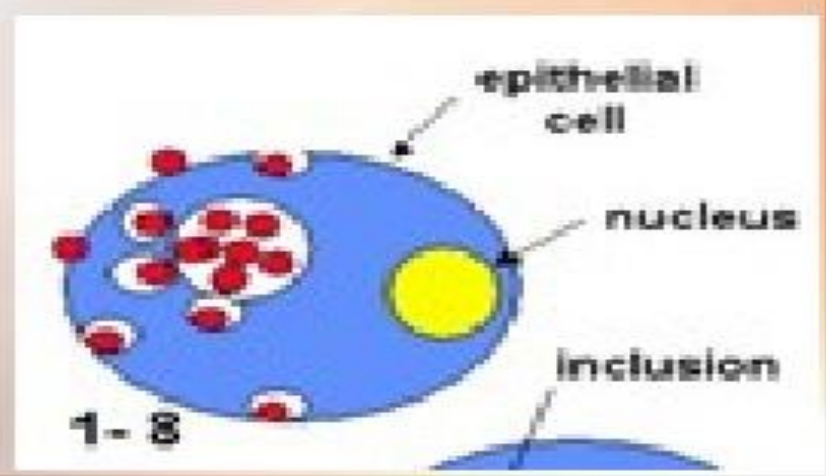
  Usual target cell
  - columnar epithelial cells of mucous membranes
  - not normally phagocytic



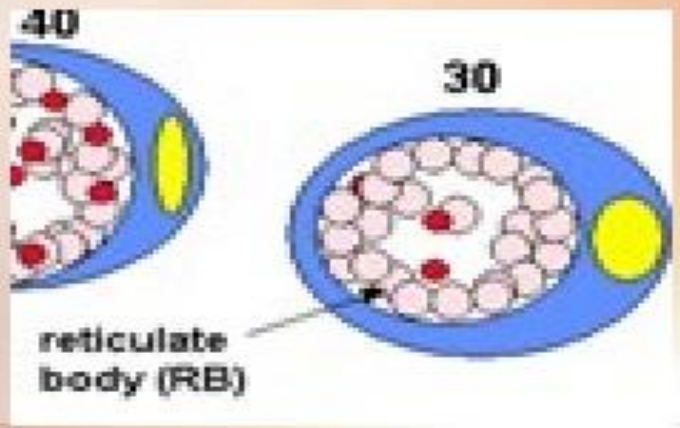
- **Stage 2. Entry of EBs**
- Chlamydia-specific receptor mediated **endocytosis**
- EBs enter cell within a phagosome
  - All development occurs here until rupture
  - Called an inclusion when visible



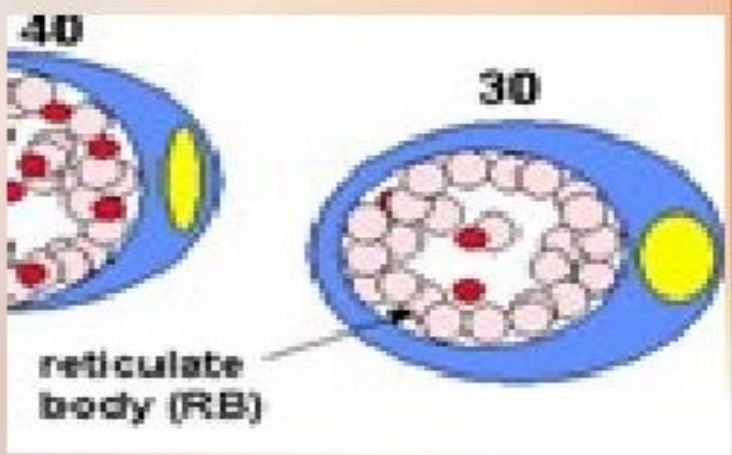
- **Stage 3. EBs change morphologically to reticulate bodies (RBs)**
  - 8 hours after entry
  - RB more permeable
    - **metabolically active**
  - not infectious at this stage



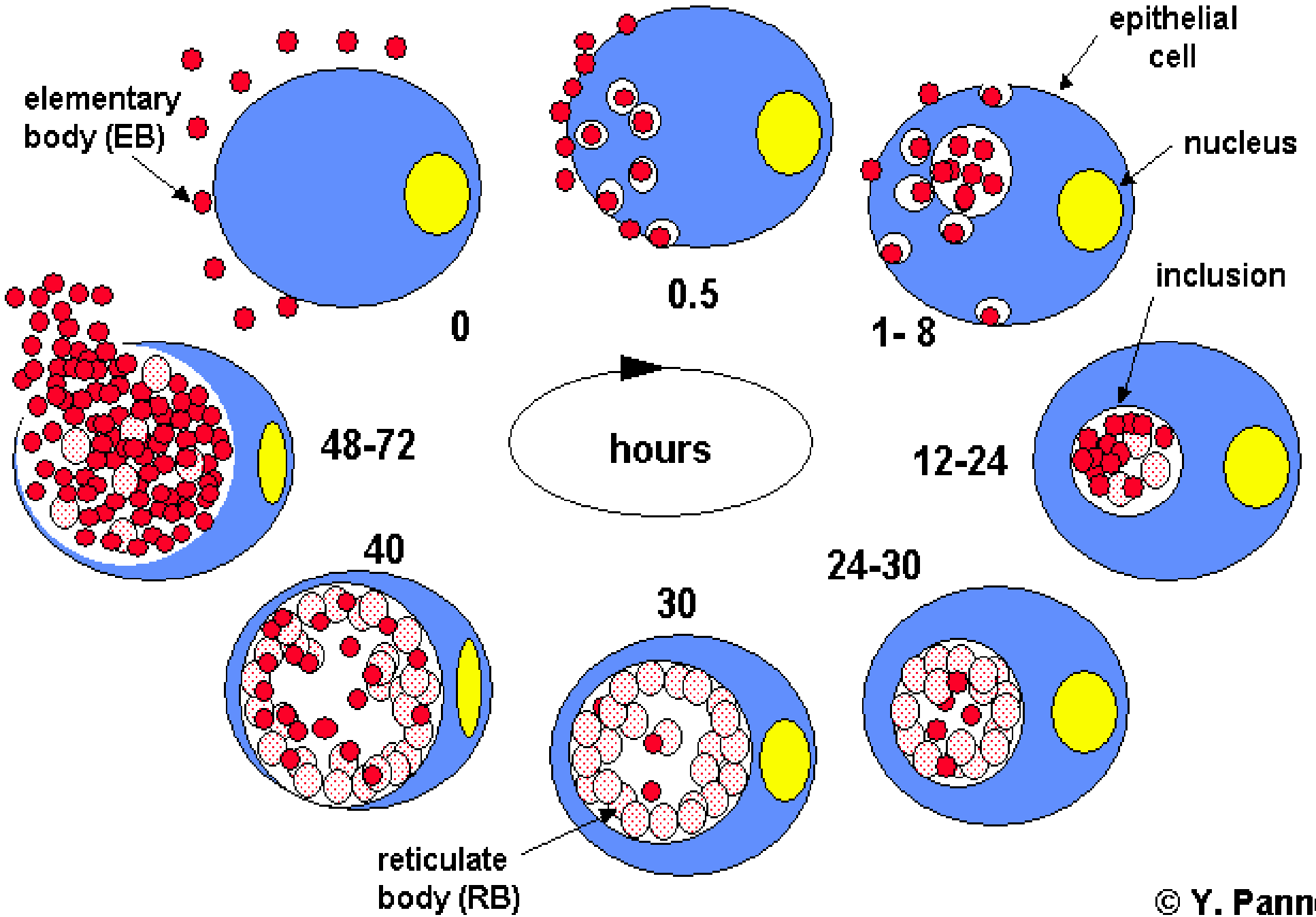
- **Stage 4. Replication**
  - RBs divide by binary fission for 20-24 hours



- **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


elementary
body (EB)
0
0.5
epithelial
cell
nucleus
1- 8
inclusion
12-24
24-30
30
40
48-72
hours
reticulate
body (RB)
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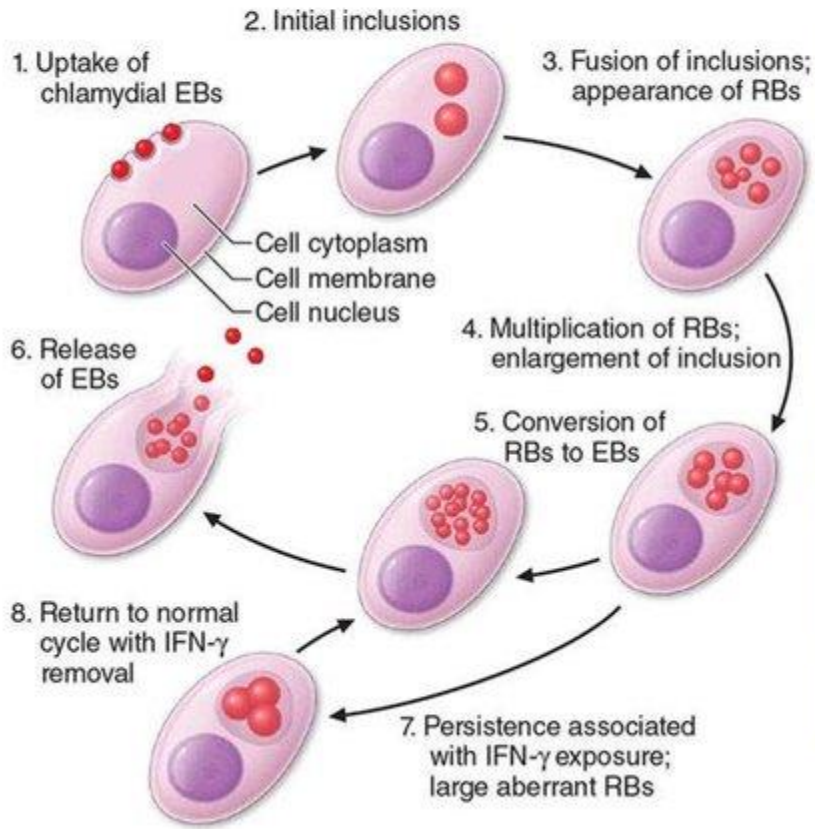
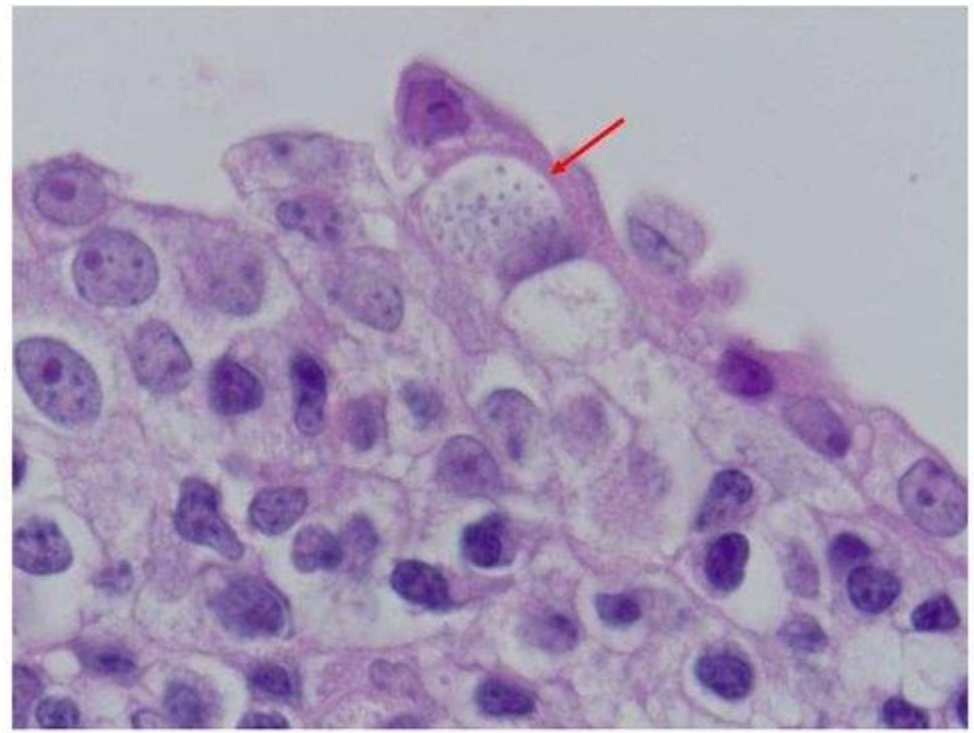
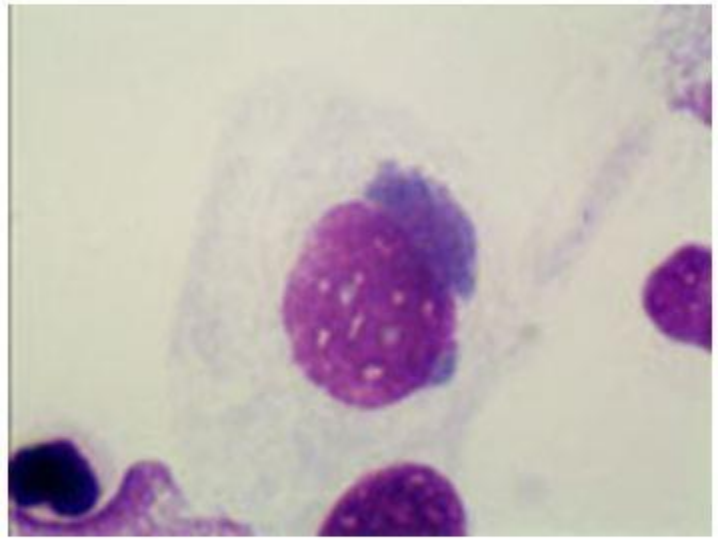
CHLAMYDIA TRACHOMATIS: MORPHOLOGY



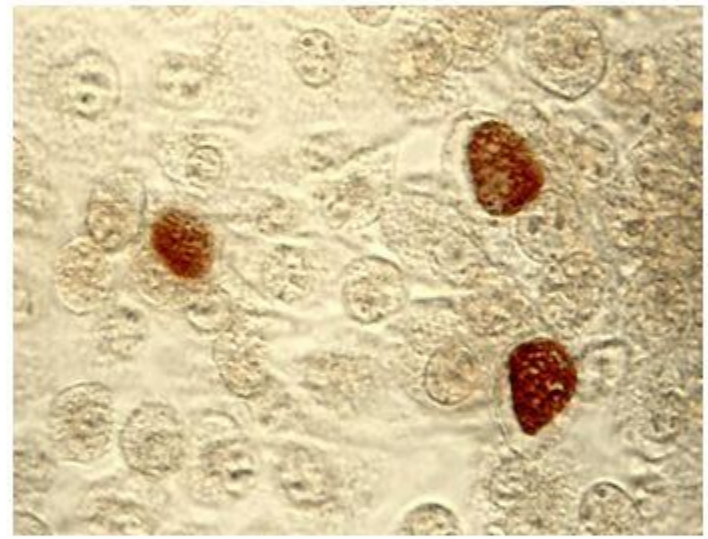


Chlamydia trachomatis

With Giema stain

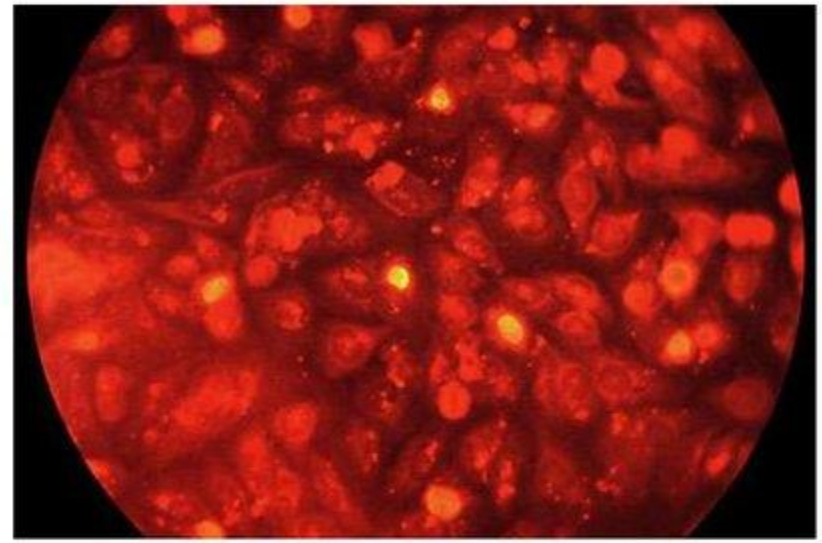


With Iodine stain



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

Trachoma: *Pathogenesis and ocular manifestations*

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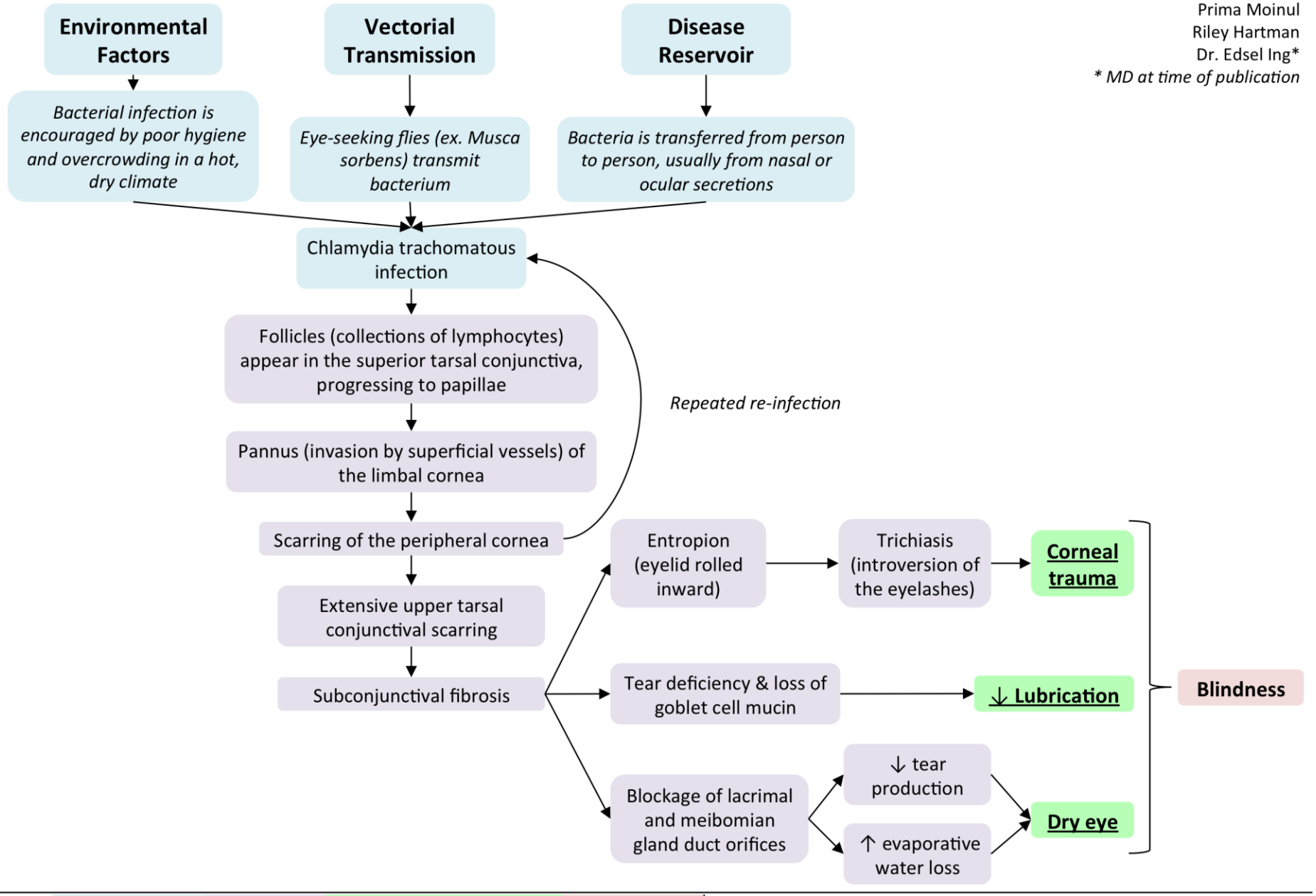

Legend: Pathophysiology | Mechanism | Sign/Symptom/Lab Finding | Complications

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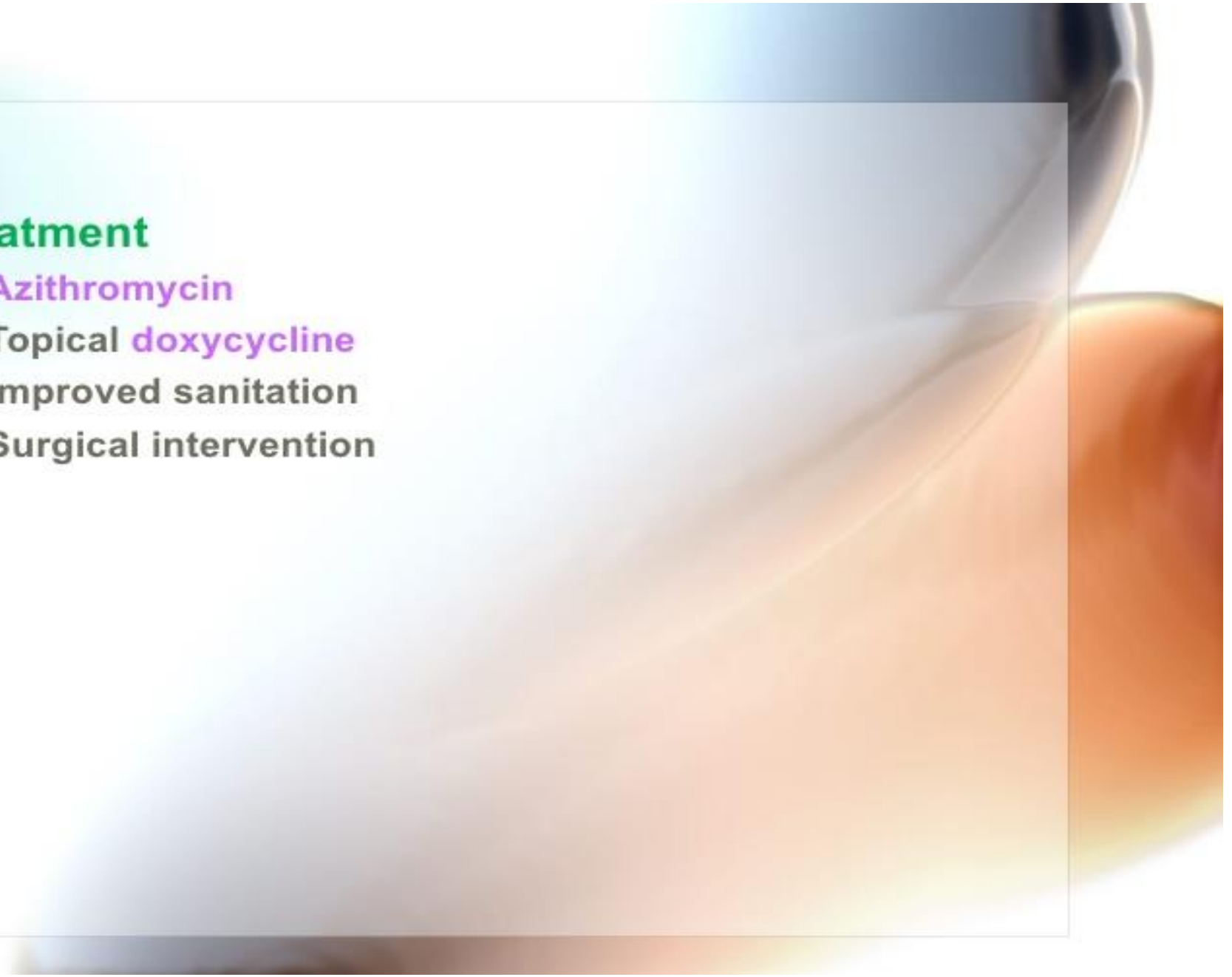
Trachoma

- **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**

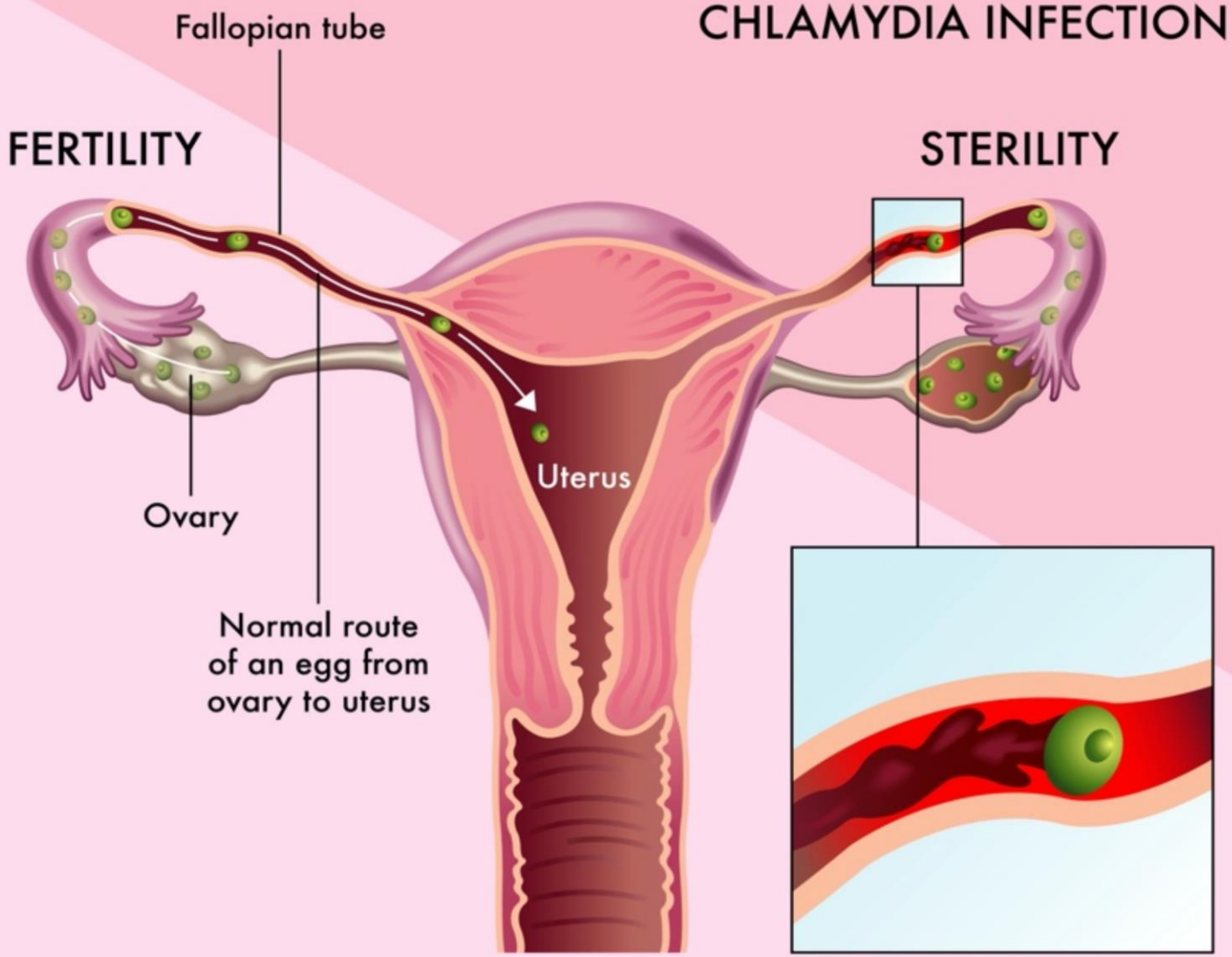
Male

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

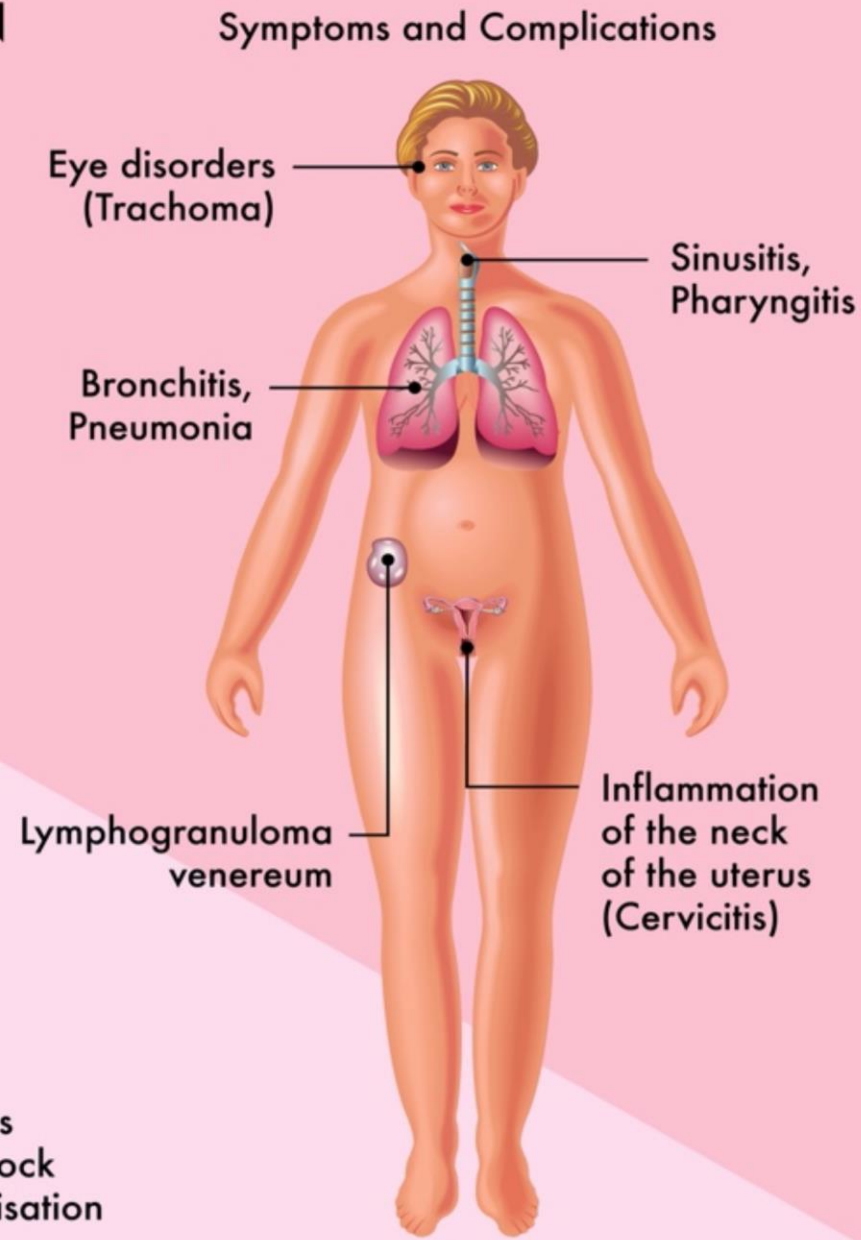
Infections in female

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

CHLAMYDIA INFECTION



The Chlamydia infection causes
a build-up of scarring that can block
the fallopian tube and prevent fertilisation



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

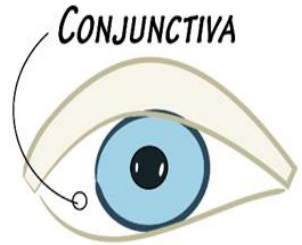
C.trachomatis

Complications

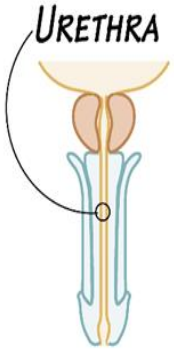
- **Reiter's syndrome**
  - A reactive arthritis secondary to an immune-mediated response
    - o It may present as asymmetric polyarthritis, urethritis, inflammatory eye disease, mouth ulcers
    - o 80% of affected patients are HLA-B27 positive
- **Deeper pelvic complications in the female**
  - o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

CLASSIC TRIAD

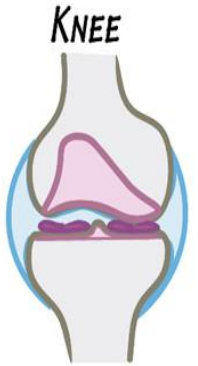
Conjunctivitis
(CAN'T SEE)



Urethritis
(CAN'T PEE)



Arthritis
(CAN'T BEND THE KNEE)



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

Lymphogranuloma venereum)

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

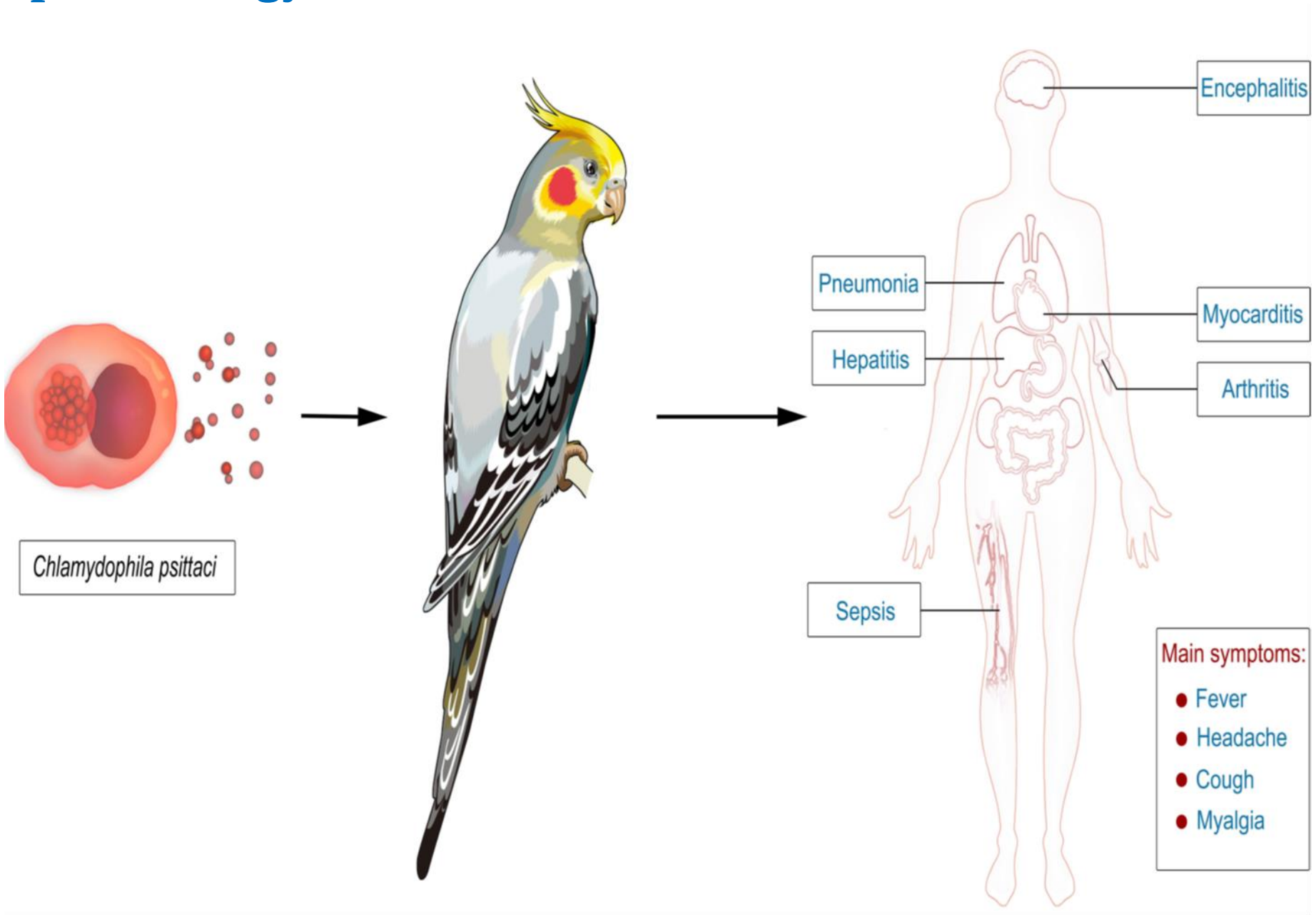
Transmission

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

Epidemiology and clinical manifestations of ornithosis:



- **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

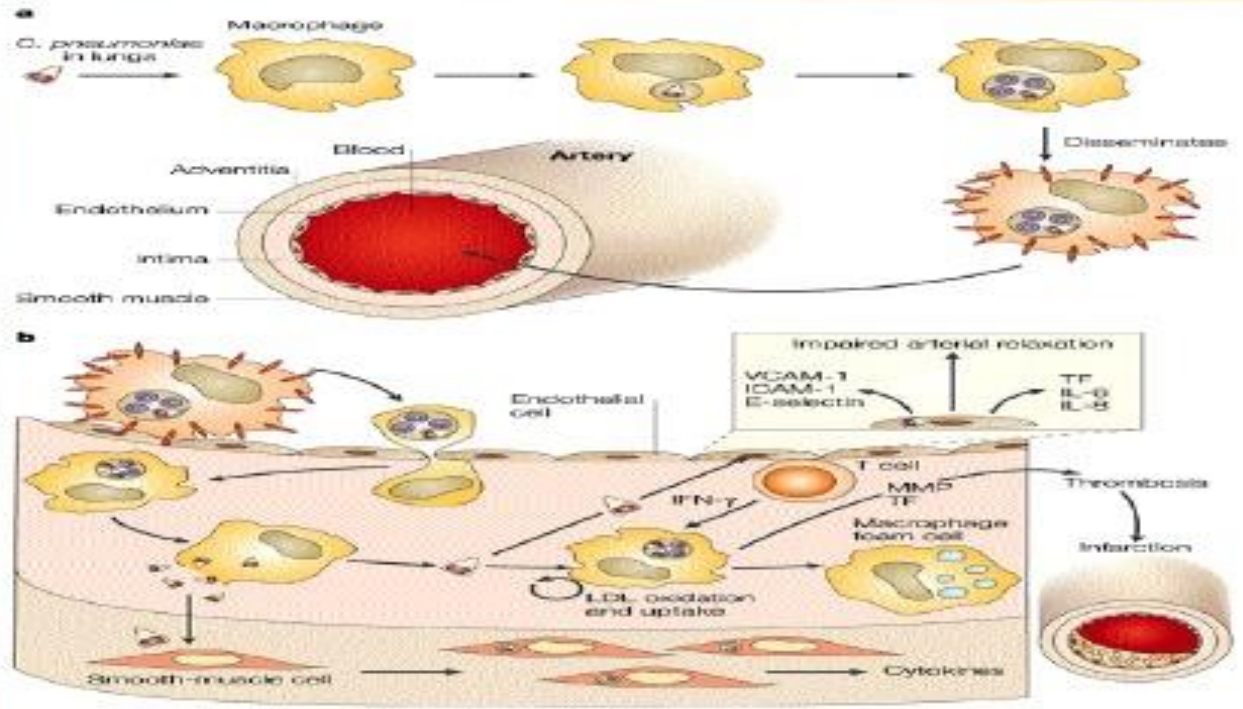
Chlamydia pneumoniae

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

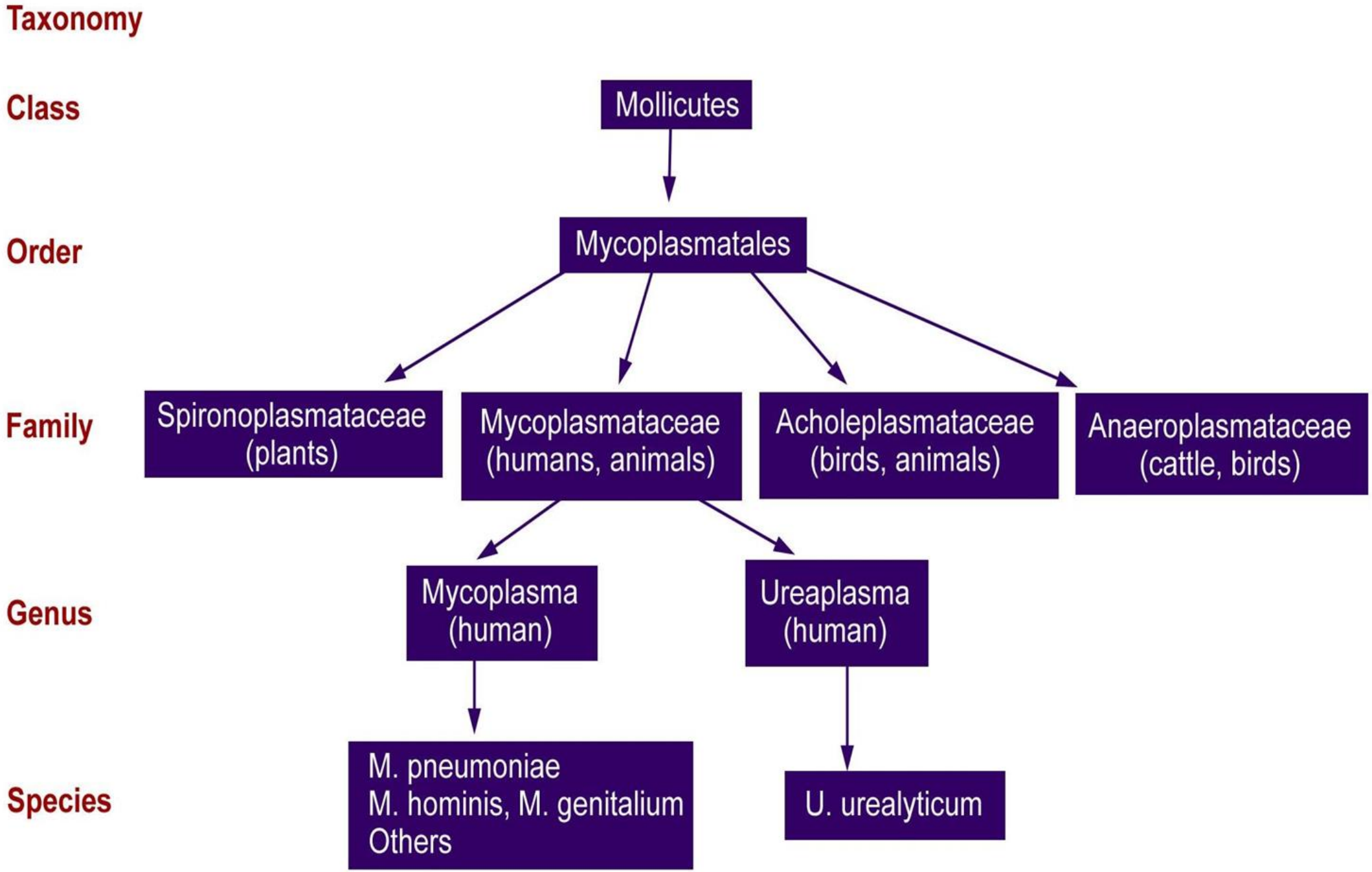
Epidemiology

- 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

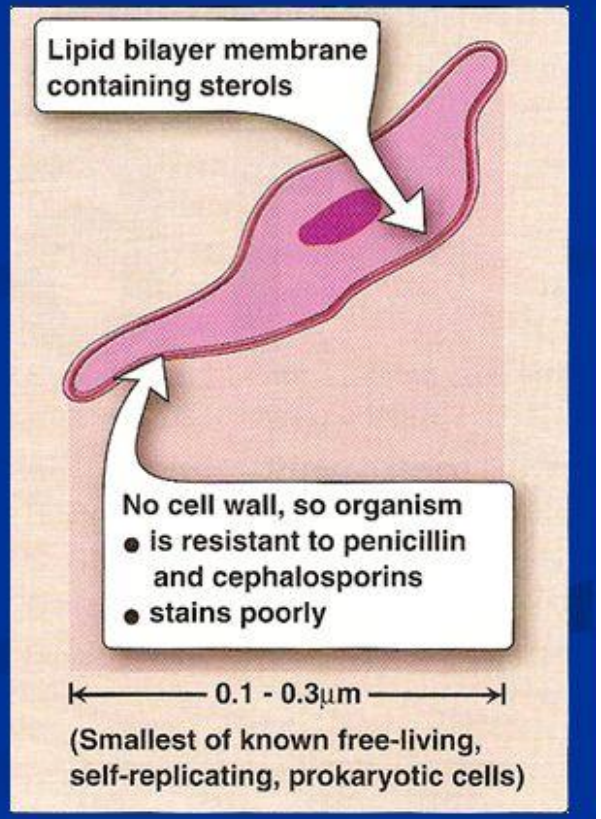


Taxonomy

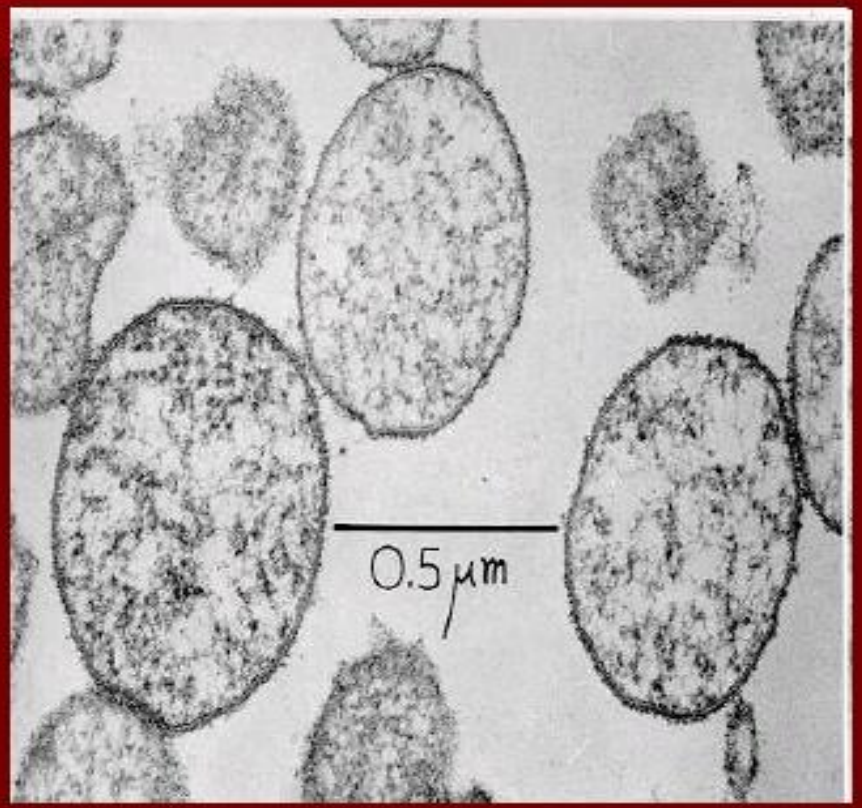

Class
Mollicutes
Order
Mycoplasmatales
Family
Spironoplasmataceae (plants)
Mycoplasmataceae (humans, animals)
Acholeplasmataceae (birds, animals)
Anaeroplasmataceae (cattle, birds)
Genus
Mycoplasma (human)
Ureaplasma (human)
Species
M. pneumoniae
M. hominis, M. genitalium
Others
U. urealyticum

MYCOPLASMAS
Characteristics

- Lacking cell wall
  - Resistant to antibacterials that inhibit cell wall synthesis
  - Gram's stain : Not useful
  - Pleomorphic
  - Cannot be classified as either cocci or bacilli
- Enclosed in a plasma membrane
  - Lipid bilayer membrane containing sterols
- Smallest free-living organisms
  - Pass through bacteriologic filters
- Can be cultured in vitro.

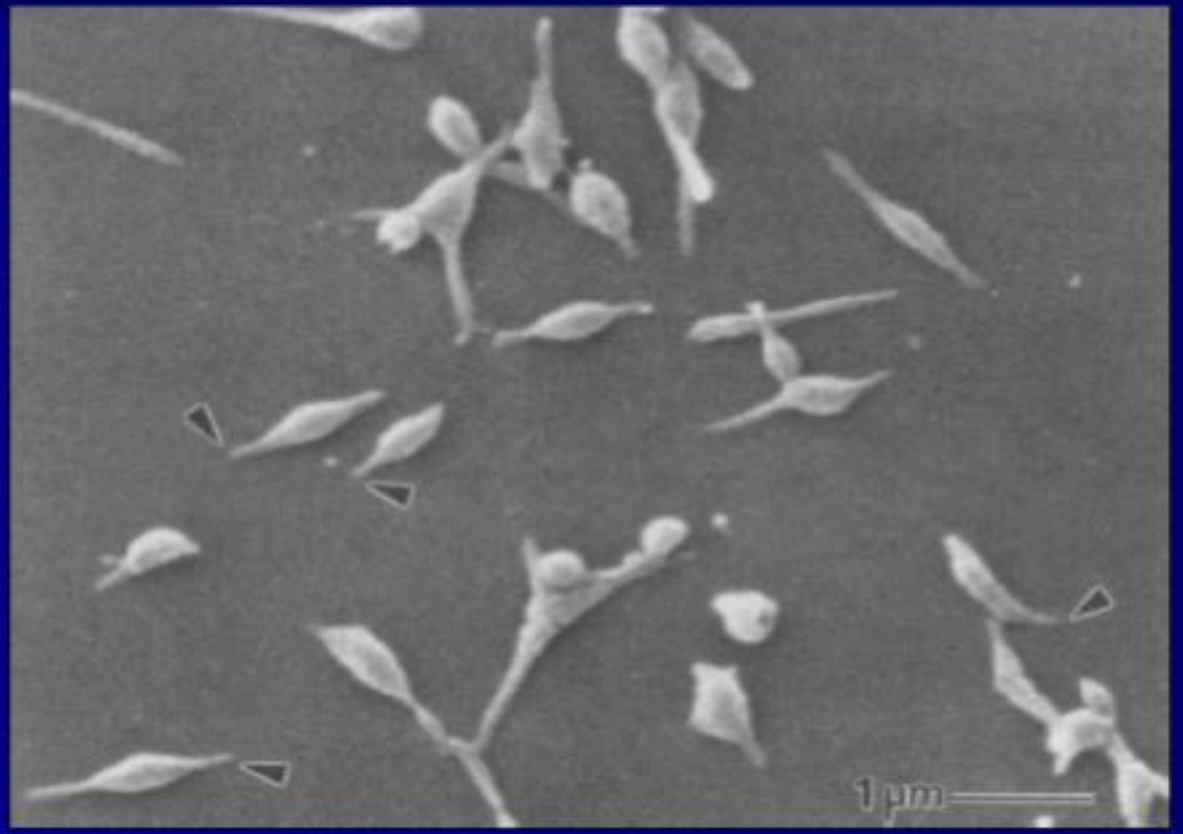


Mycoplasma are cell wall deficient microorganisms



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

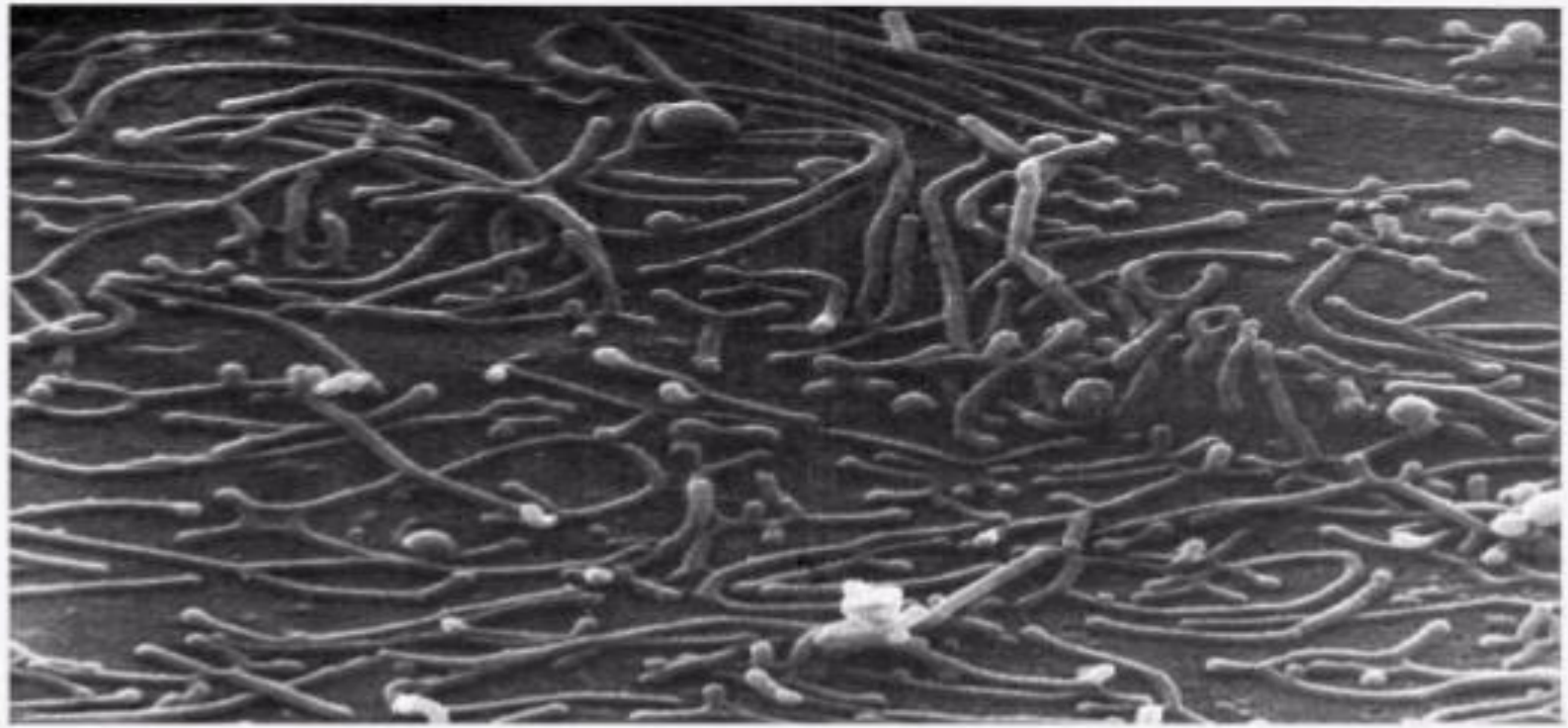
Mycoplasma (Scanning EM)



Morphology

Size :- 300nm to 0.2nm

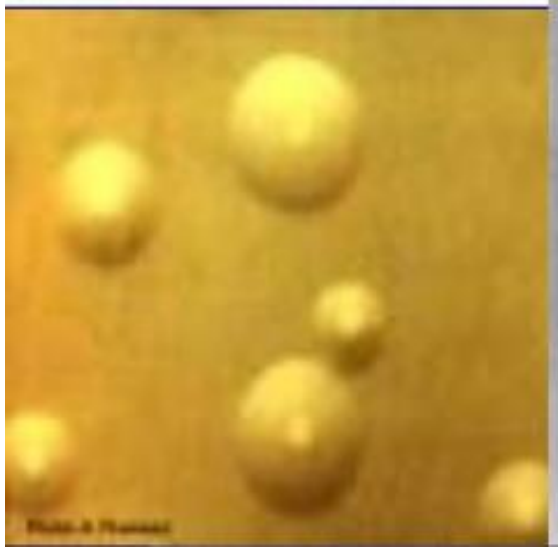
Shape :- Coccoidal,Diploform,Filamentus,Spiral shape &Teardrop shape.



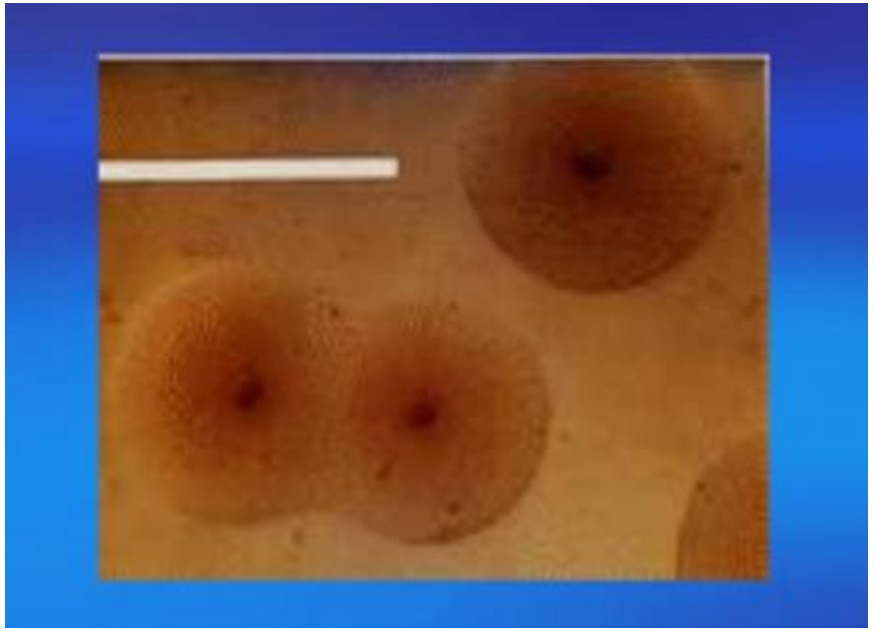
CULTURE CHARACTERISTICS

- **Most mycoplasmas require a rich medium(liquid or solid)containing a sterol and serum proteins for growth. Temperature 35-37 °C.**
- **On solid media, they form minute, transparent colonies. When viewed under low-power magnification, the colony looks like a "fried egg".**
- **The different strains vary in their growth rate and may take from two days to several weeks to form a colony.**

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

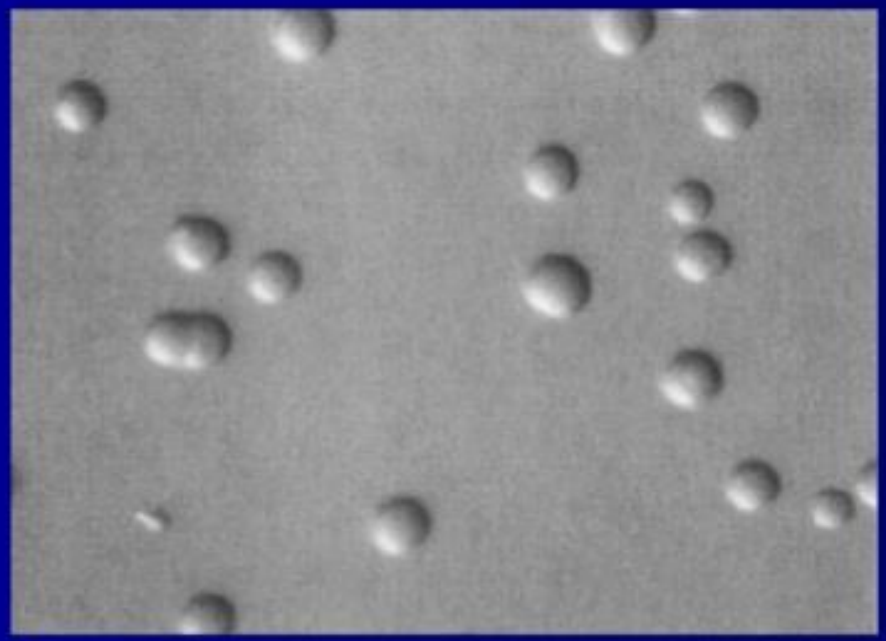
Laboratory Diagnosis - *M. pneumoniae*

- 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

Ureaplasma *urealyticum*

Characteristics

a bacterium that is found in the urogenital tracts of humans.

It stains gram negative, but that is because it lacks a cell wall.

Ureaplasma can spread during sex

Ureaplasma is a very small bacterium that both men and women can catch and transmit to each other.

Ureaplasma infection is a little known but common STI.

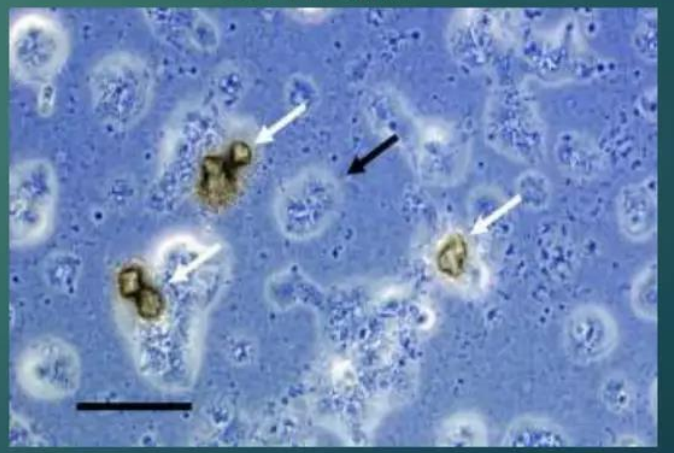
DISEASE

nongonococcal urethritis

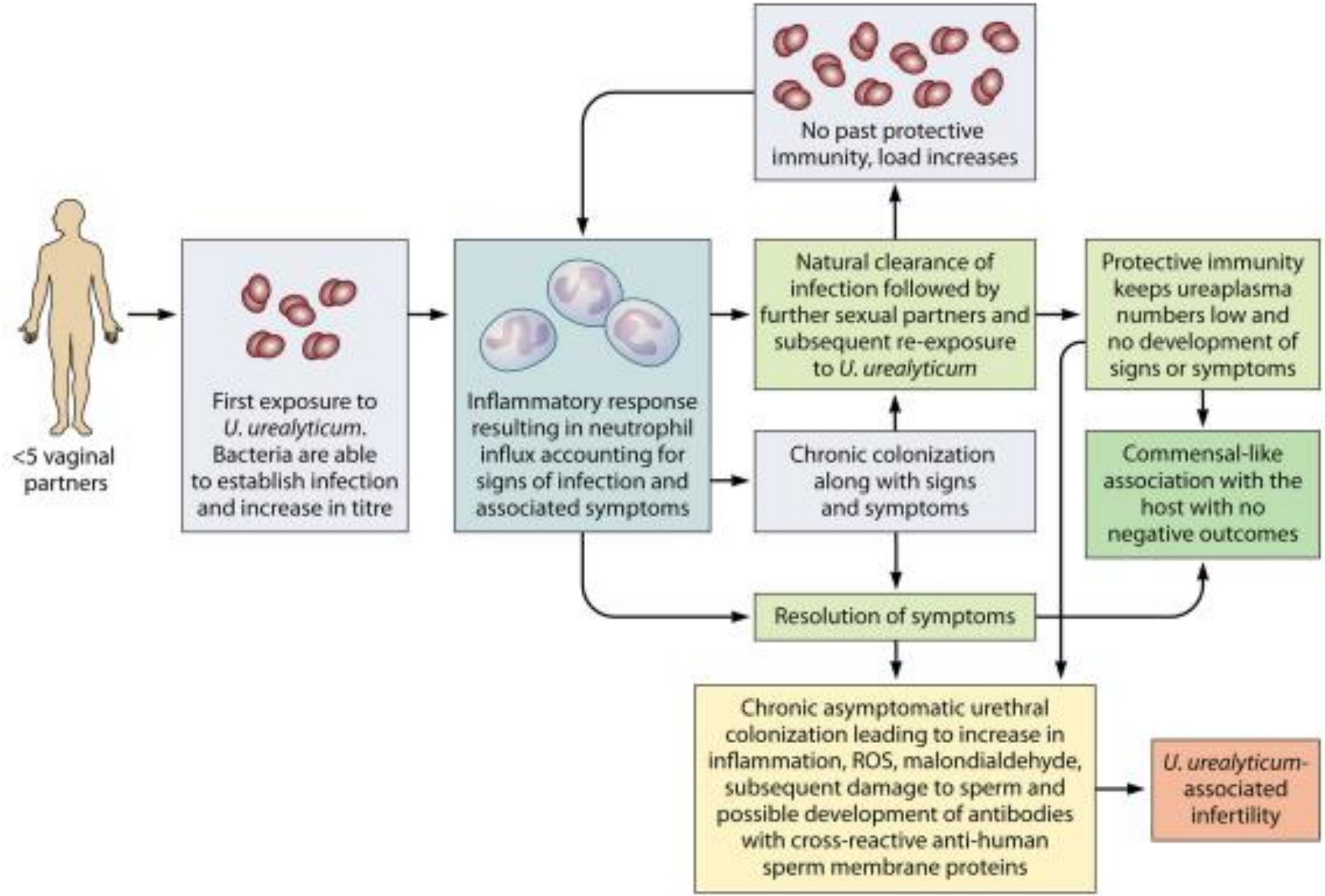
SYMPTOMS

• it hurts when you pee • You're belly pain • it is pain, odor or discharge from the vagina.

On media



Under microscope


<5 vaginal partners
First exposure to U. urealyticum. Bacteria are able to establish infection and increase in titre
Inflammatory response resulting in neutrophil influx accounting for signs of infection and associated symptoms
No past protective immunity, load increases
Natural clearance of infection followed by further sexual partners and subsequent re-exposure to U. urealyticum
Chronic colonization along with signs and symptoms
Protective immunity keeps ureaplasma numbers low and no development of signs or symptoms
Commensal-like association with the host with no negative outcomes
Resolution of symptoms
Chronic asymptomatic urethral colonization leading to increase in inflammation, ROS, malondialdehyde, subsequent damage to sperm and possible development of antibodies with cross-reactive anti-human sperm membrane proteins
U. urealyticum-associated infertility

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

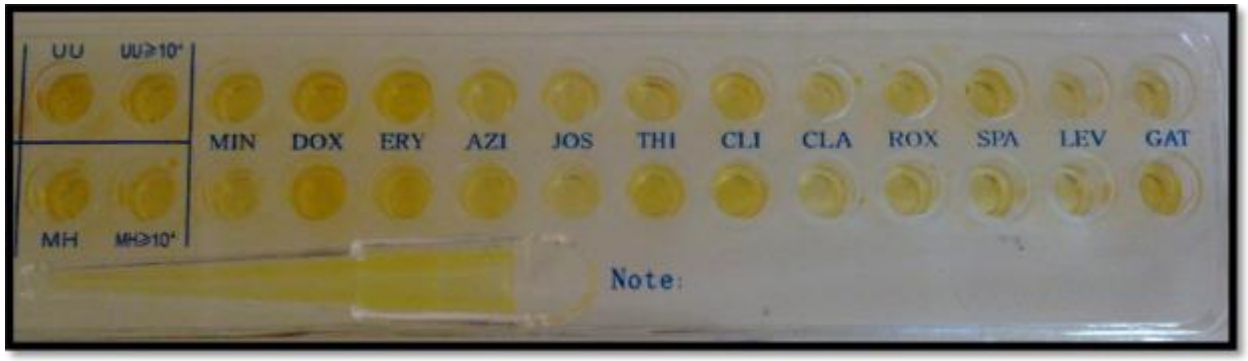
- Laboratory diagnosis
  - Culture (except *M. genitalium*)
  - Fried egg appearence of colonies on PPLO agar

Determination of mycoplasma and ureaplasma by express method (microcultivation):

- **Material -** urethral or vaginal swab
- **Principle of the test**
- The Mycoplasma kit is based on the reaction of cultivation and biochemistry.
- Urea can be decomposed by Urease for *U.urealyti*cum and release NH3 and arginine can be decomposed by Arginase for *M.hominis* and release NH3. Then NH3 causes an increased pH of the liquid medium. The corresponding color change of the indicator is used to judge the result.
- The susceptibility strip contains 12-antibiotics, each one in two concentrations. If Mycoplasma is sensitive to antibiotic, the activity of the enzyme is inhibited causing no change in color.

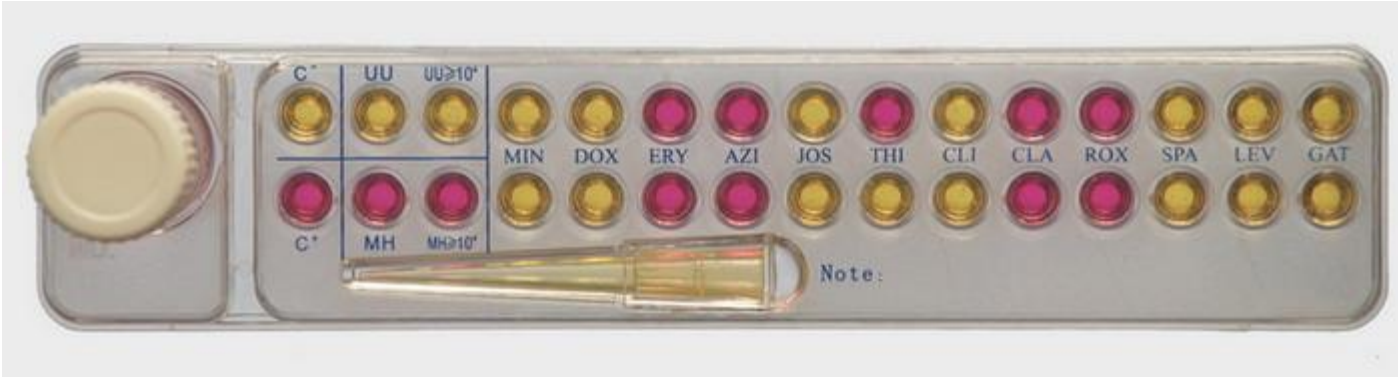
Determination of mycoplasma and ureaplasma by express method (microcultivation):

before



Cultivation

after



Tetracyclines - Minocycline (MIN), Doxycycline (DOX)
Macrolides - Roxithromycin (ROX), Erythromycin (ERY), Azithromycin (AZI), Clarithromycin (CLA), Josamycin (JOS)
Chloramphenicols - Thiamphenicol (THI)
Lincosamides - Clindamycin (CLI)
Quinolones - Sparfloxacin (SPA), Levofloxacin (LEV), Gatifloxacin (GAT)

Genital Mycoplasmosis -Treatment

- Tetracycline or
- Erythromycin