



Semmelweis University
Institute of Medical Microbiology

Epi- and obligate intracellular
bacteria:

Mycoplasma,
Chlamydia, Rickettsia,
Anaplasma, Coxiella

PROF. KÁROLY NAGY

Comparison of the smallest bacteria

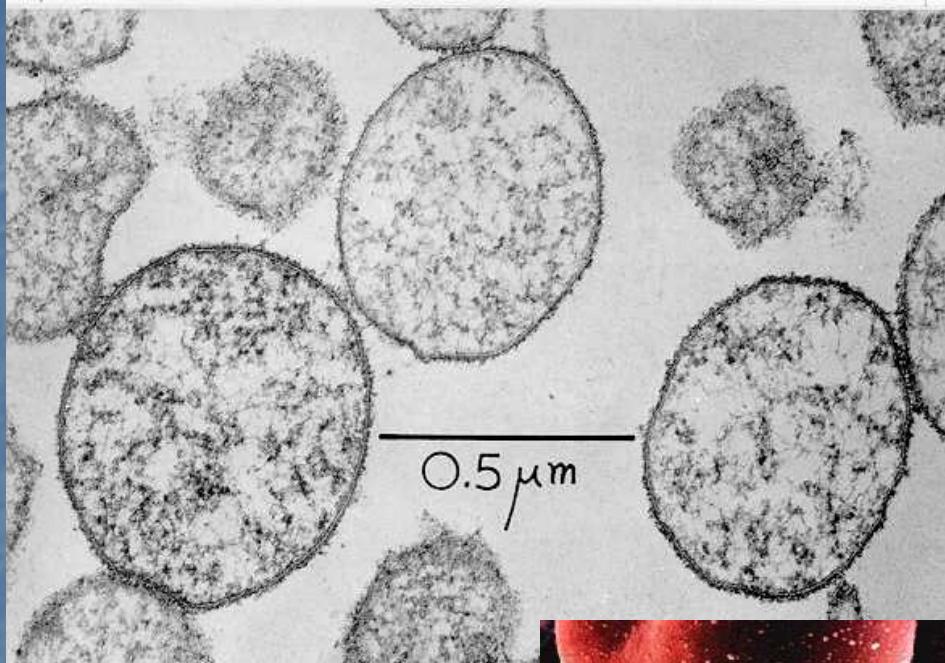
	<i>Mycoplasma</i>	<i>Chlamydia</i>	<i>Rickettsia</i> <i>Anaplasma</i> <i>Coxiella</i>
Size (μm)	0.1-0.3	0.3	0.25-0.4
Cell wall	-	+?	+?
Growth in cell free medium	+	-	-
Resist cell wall active antibiotics	+	+?	+

Mycoplasmataceae

General characteristics

- The smallest free-living bacteria and genome 580 kbp,
- triple, sterol containing cell membrane
- *no cell wall*, pleomorph
- slow growth (weeks) on special synthetic media (sterol, amino acid and nucleic acid requirement, antibiotics)
- strong adhesion to epithelial cells: P1 adhesin (epicellular surface parazites)

Mycoplasma EM picture

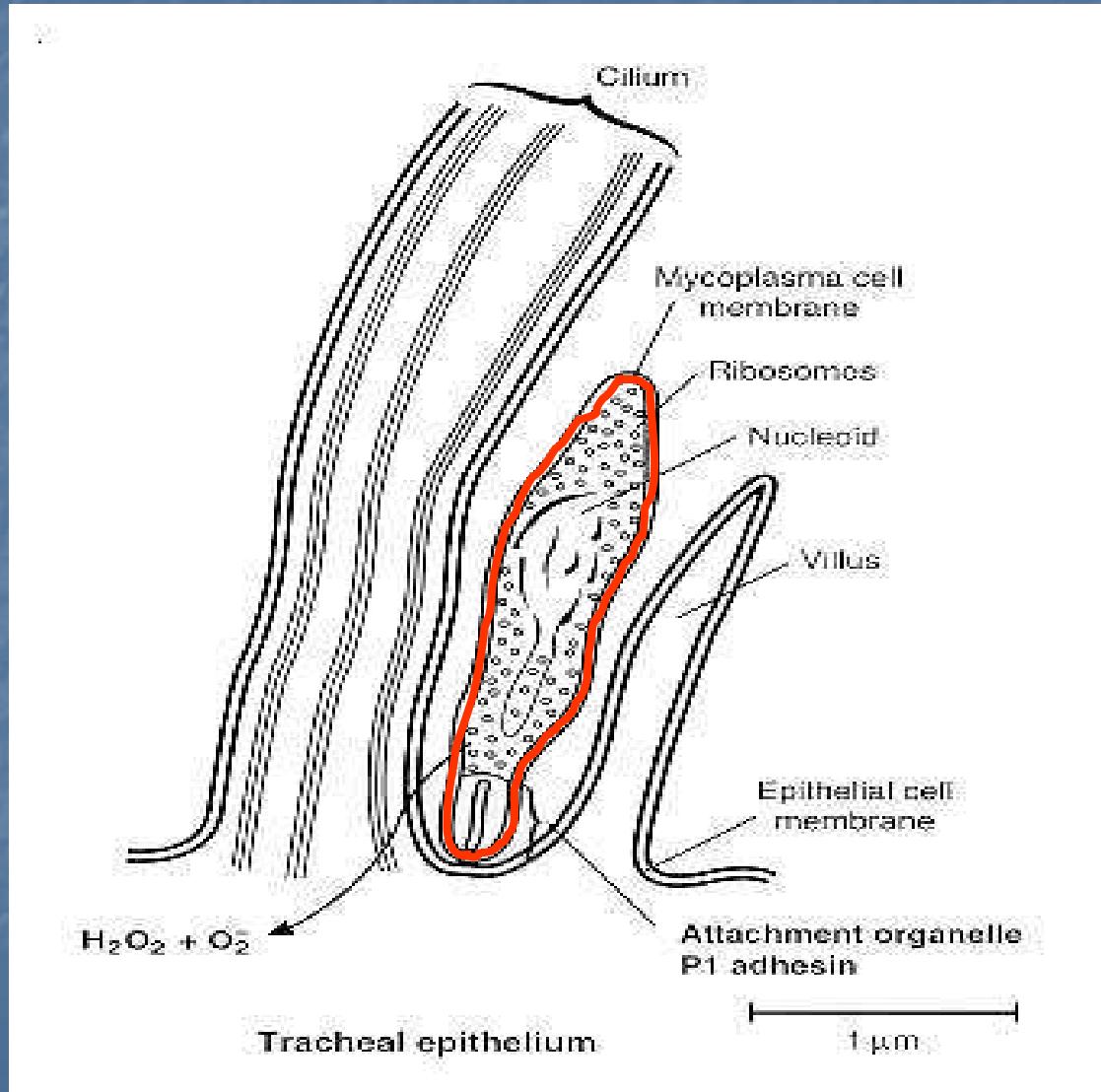


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www.venereology.ru

Pathogenesis: attachment, ciliostasis, destruction superantigen! (respiratory and urogenital epithel)



Taxonomy: more than 200 species

TABLE 37-1 Taxonomy and Properties of Mycoplasmas Capable of Infecting Humans^a

Genus	No of Established Species	Genome Size (kbp)	G+C Content (mol%)	Cholesterol Requirement	Distinctive Properties	Hosts
<i>Mycoplasma</i>	98	580-1300	23-40	+	None	Humans, other animals
<i>Ureaplasma</i>	6	760-1140	27-29	+	Urease positive	Humans, other animals

Human adapted: *Mycoplasma* and *Ureaplasma* genus 16 species Pathogen: 6 species

- *Mycoplasma pneumoniae*: upper respiratory tract infections, atypical pneumonia, meningoencephalitis
- *Mycoplasma hominis*: pyelonephritis, stillbirth, postpartum fever, PID, epididymitis
- *M. fermentans*: rheumatoid arthritis
- *M. genitalium*: NGU (non Go), arthritis (SARA)
- *M. penetrans*: in immunosuppressed patients
- *Ureaplasma urealyticum*: newborn pneumonia urinary stones, NGU

Mycoplasmas: respiratory and urogenital diseases, erythema multiforme



Cultivation: 4 days-3 weeks

CULTURAL CHARACTERISTICS OF HUMAN MYCOPLASMAS AND UREAPLASMAS

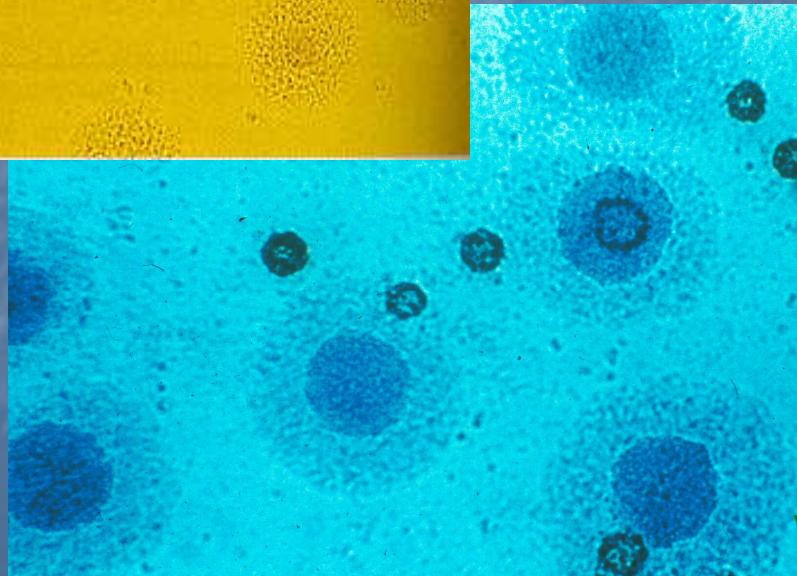
SPECIES	SUBSTRATE METABOLIZED			pH OF MEDIUM	INCUBATION ATMOSPHERE (AGAR CULTURES) ^a		
	GLUCOSE	ARGININE	UREA		AEROBIC	ANAEROBIC ^b	CANDLE JAR
RESPIRATORY							
<i>M. PNEUMONIAE</i>	+	-	-	7.8	++	±	++
<i>M. SALIVARIUM</i>	-	+	-	7.0	+	++	?
<i>M. ORALE</i>	-	+	-	7.0	+	++	?
<i>M. BUCCALE</i>	-	+	-	7.0	+	++	?
<i>M. FAUCIUM</i>	-	+	-	7.0	+	++	?
<i>M. LIPOPHILUM</i>	-	+	-	7.0	+	++	?
GENITAL							
<i>M. HOMINIS</i>	-	+	-	7.0 - 7.2	++	++	++
<i>U. UREALYTICUM</i>	-	-	+	6.0	++	++	++
<i>M. FERMENTANS</i>	+	+	-	7.6 - 7.8	+	++	?
<i>M. GENITALIUM</i>	+	-	-	7.4 - 7.6	+	±	++

^a GROWTH ON AGAR CULTURES : ++, STRONG GROWTH; +, MODERATE GROWTH; ±, MINIMAL GROWTH; ?, NOT DETERMINED

^b GASPAK, CARBON DIOXID, AND HYDROGEN

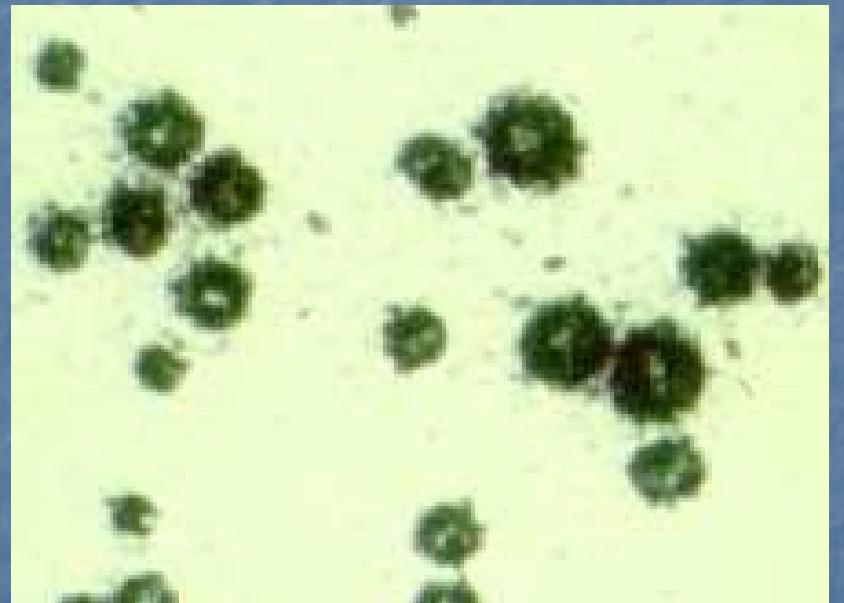
Colony morphology: on heart infusion agar (HIA) the smallest colonies 0.1-0.2 mm

Mycoplasma hominis fried-egg appearance



Ureaplasma

dark, small, dense



www-instruct.nmu.edu, www.gravidanzaonline.it

Other diagnostic methods, Therapy

- Ag detection, PCR? for *M. pneumoniae*
M. genitalium, Ureaplasma
- CF, IgM, IgA, IgG ELISA, Western blot
(*M. pneumoniae*)

Treatment: erythromycin, doxycyline, fluoroquinolons
Note! *M. hominis* resistant to macrolides, susceptible to clindamycin

CHLAMYDIA

Chlamydiae definition:

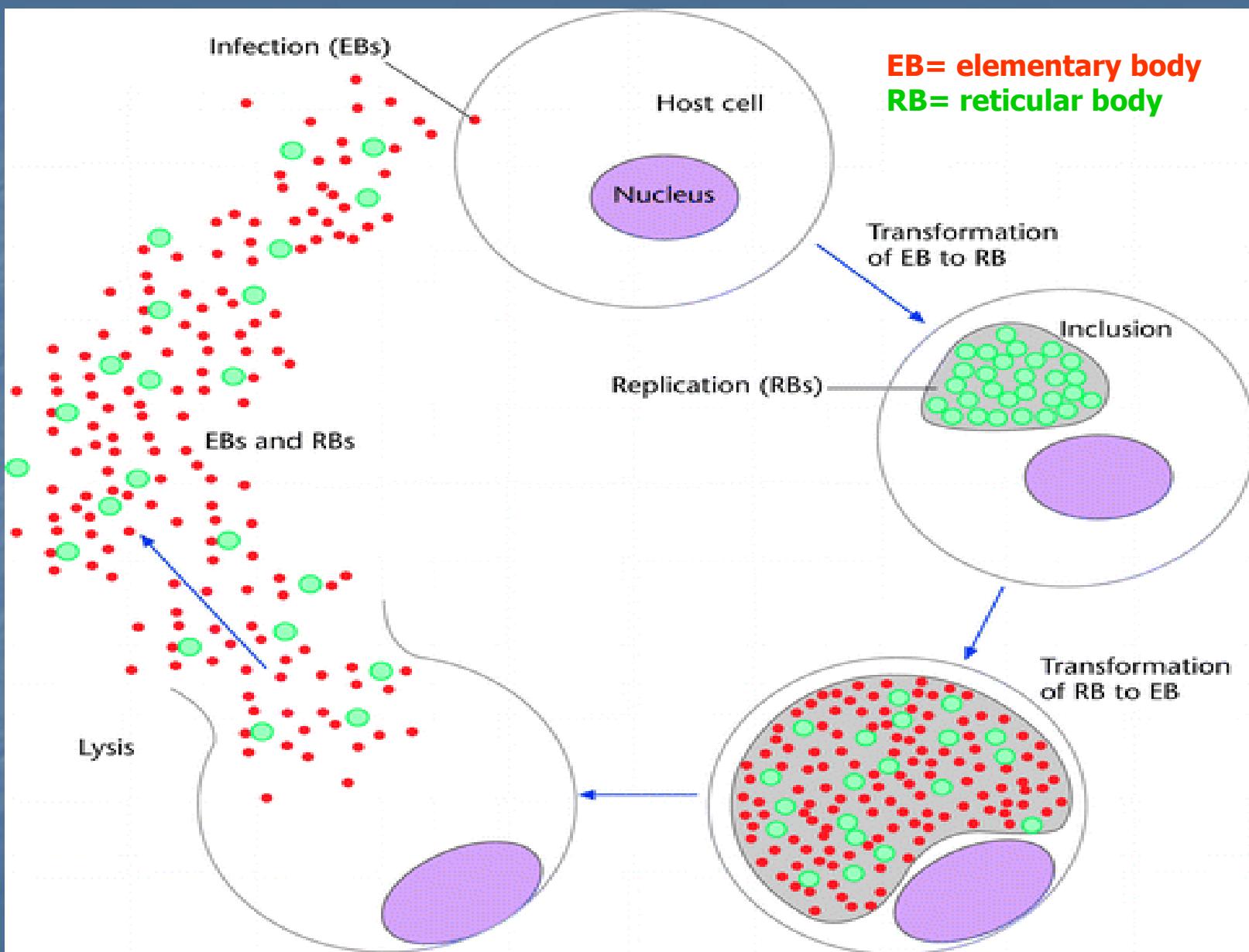
Gram-negative (LPS+ muramic acid-),

obligate intracellular,

energy (ATP) parasite bacteria with unique

biphasic life cycle

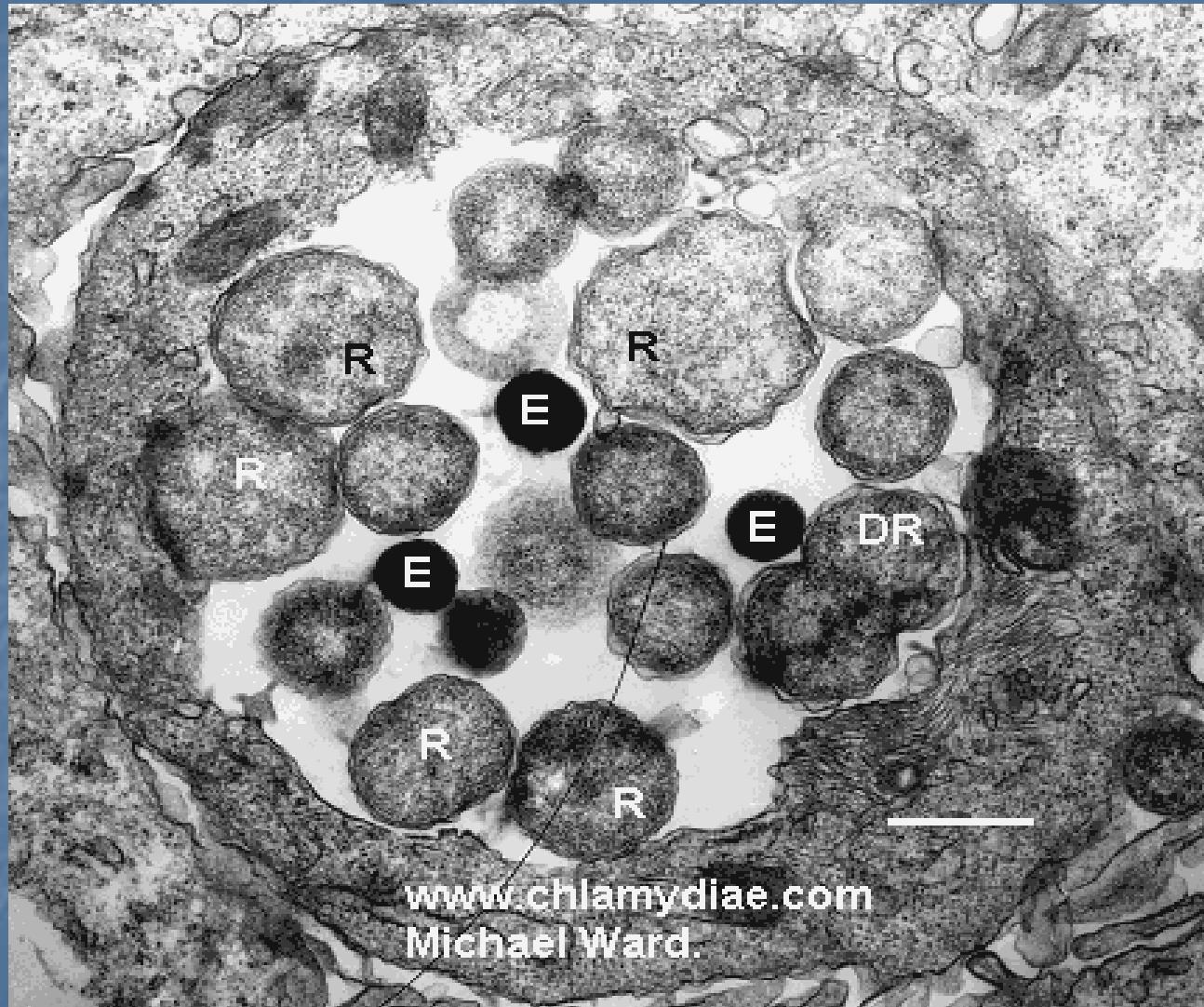
Growth cycle of Chlamydiae: 48-50 hours



Comparison of EB and RB

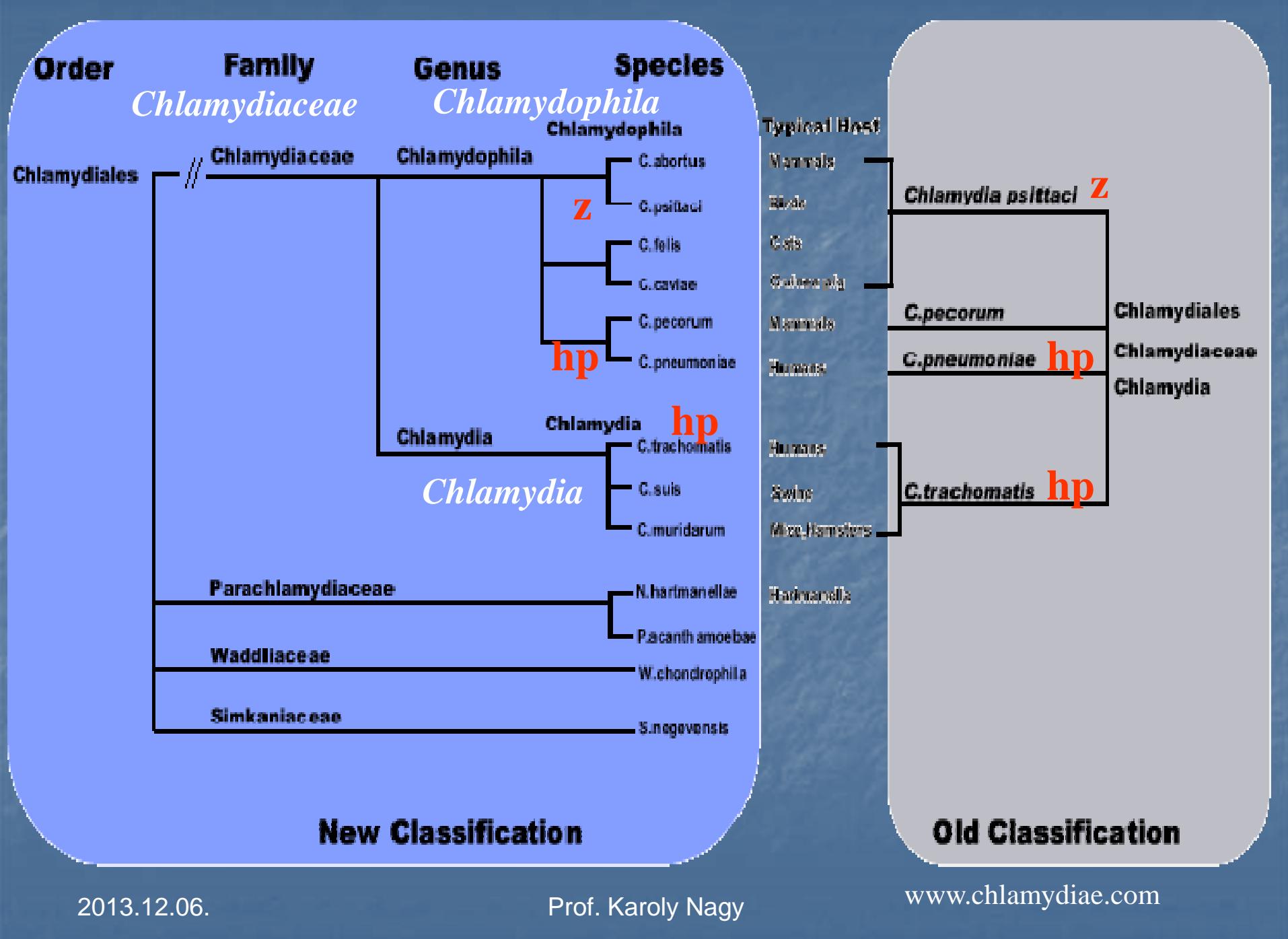
- Elementary body size
0,3µm
- RNA:DNA content 1:1
- *Infectious*
- Toxic for mice
- Extracellular survival
- Induces endocytosis
- Metabolically inactive
- Resistant to trypsin
- Reticulate body size
0,5 -1.0 µm
- 3:1
- Not infectious
- Not toxic for mice
- Intracellular growth
- No endocytosis
- Active
- Susceptible

Electron micrograph of EB-s, RB-s



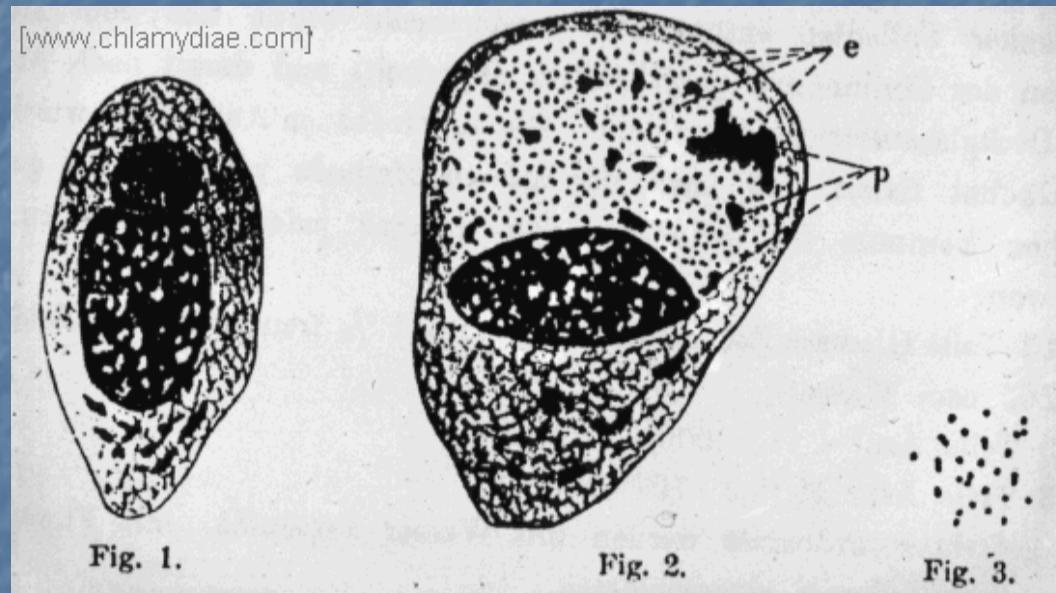
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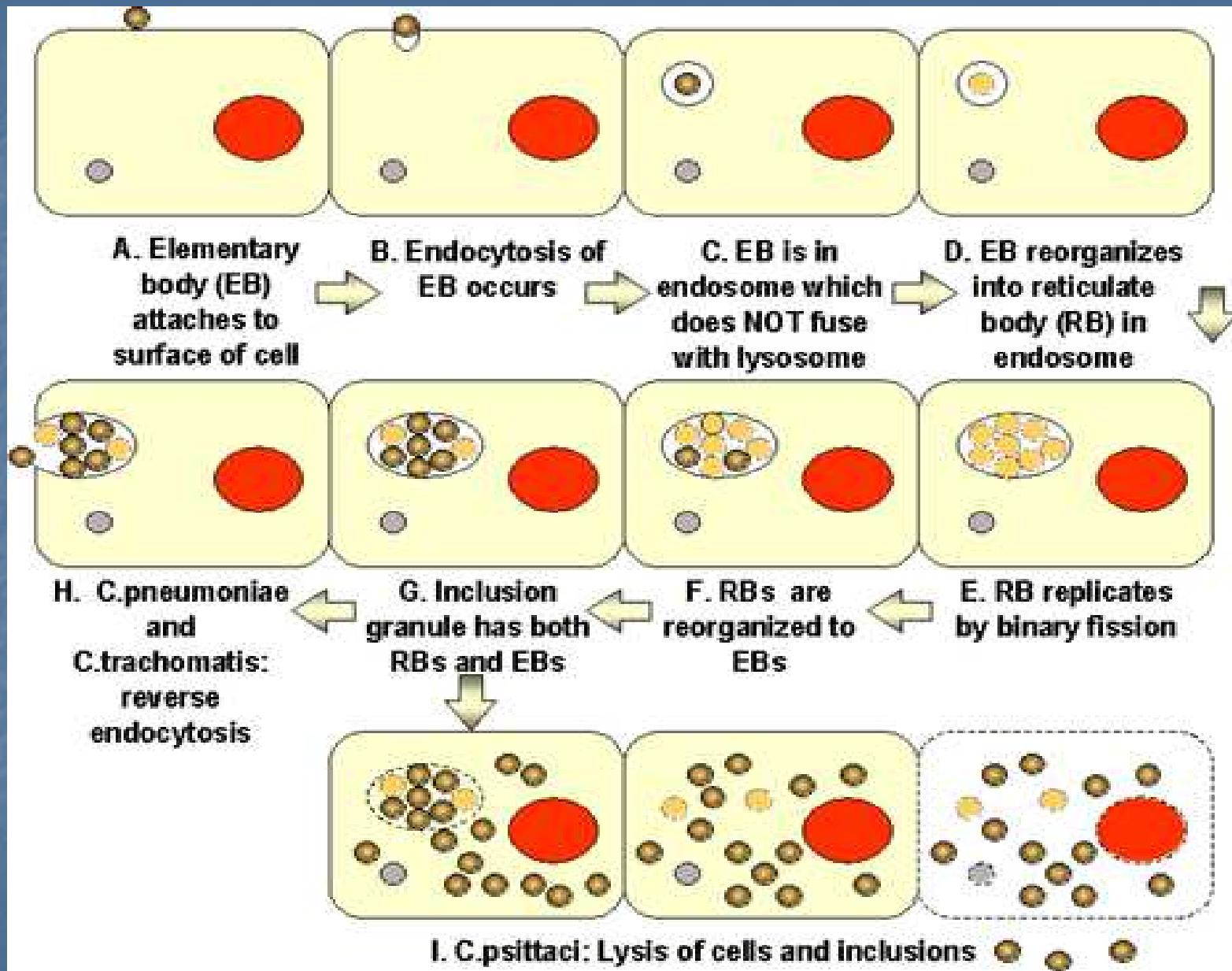
History

- *C. trachomatis* 1907 Prowazek, Halberstaedter



- *C. psittaci* 1930 Bedson
- 1957 embryonated eggs,
- 1965 tissue culture, 1985 ELISA, 1989 PCR
- *C. pneumoniae* 1989 Grayston

Chlamydial infections: pathogenesis



Human pathogens, Diseases 1

- *C. trachomatis*: 2 biotypes, 20 serotypes

- A, B, Ba, C serotypes: **trachoma**

- D-K: **oculogenital infections, pneumonia of newborns**

- L1-L3: **lymphogranuloma venereum (LGV)**

- Target cell: **epithel, lymphoid**

Human pathogens, Diseases 2

- ***Chlamydophila pneumoniae***: upper and lower respiratory tract infections, **atypical pneumonia**, ?
- Target cells: **macrophages, lung parenchyma**

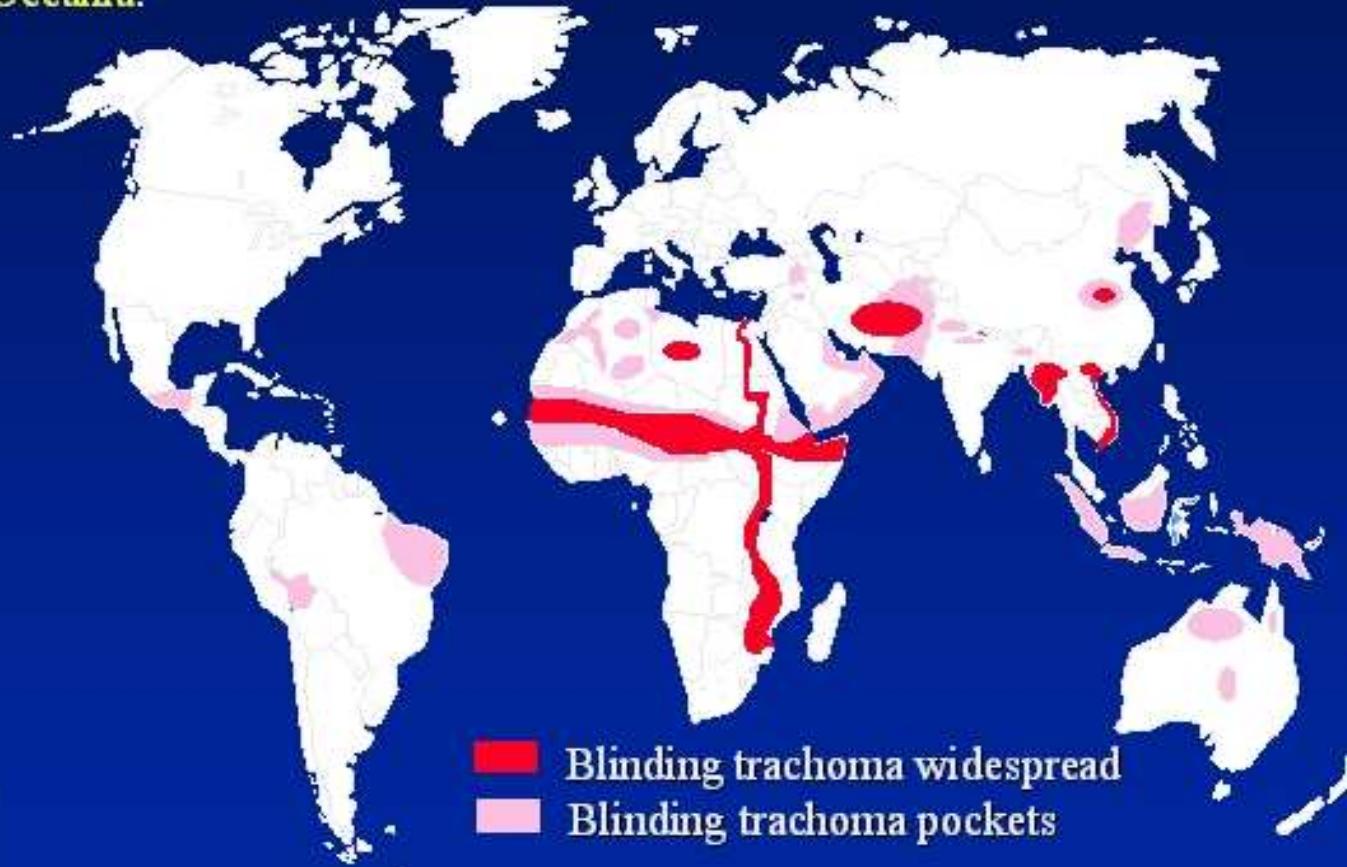
- ***Chlamydophila psittaci***: pneumonia
(psittacosis)
- Target cell: **macrophages, lung parenchyma**

Trachoma (*C. trachomatis* A-C serotypes):
well known, ancient (China, Egypt)
chronic inflammatory eye disease,
but today still is one of the leading
causes of blindness!

160 million acute new infections,
500 million trachoma patients,
6-8 million blind people
WHO project: eliminate blinding trachoma to 2020

Trachoma endemic areas WHO

It represents an important public health problem in a number of countries in Africa, eastern Mediterranean, dry areas of the Indian sub-continent, south-east Asia, western Pacific and some areas of Oceania.



Trachoma spread: contact or fly

Disease clinical stages



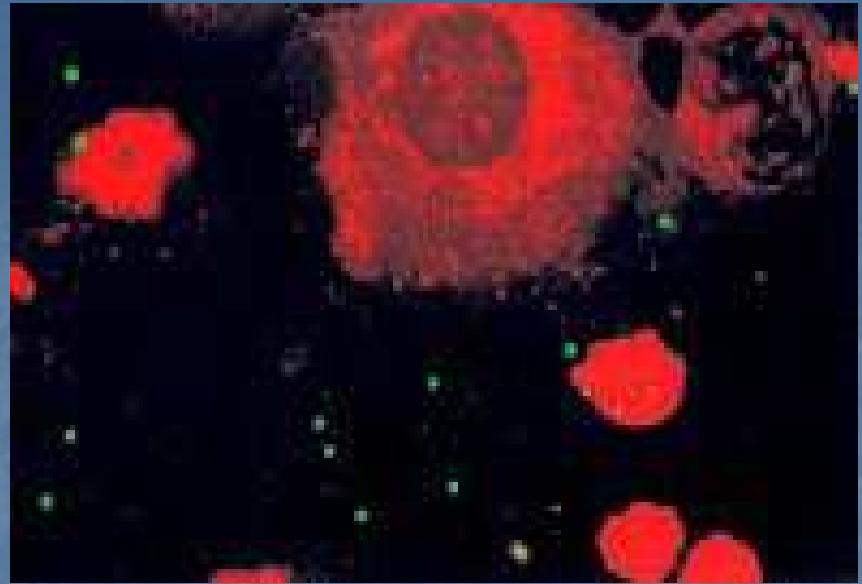
www.chlamydiae.com, www.eyeatlas.com

www.med.cmu.ac.th

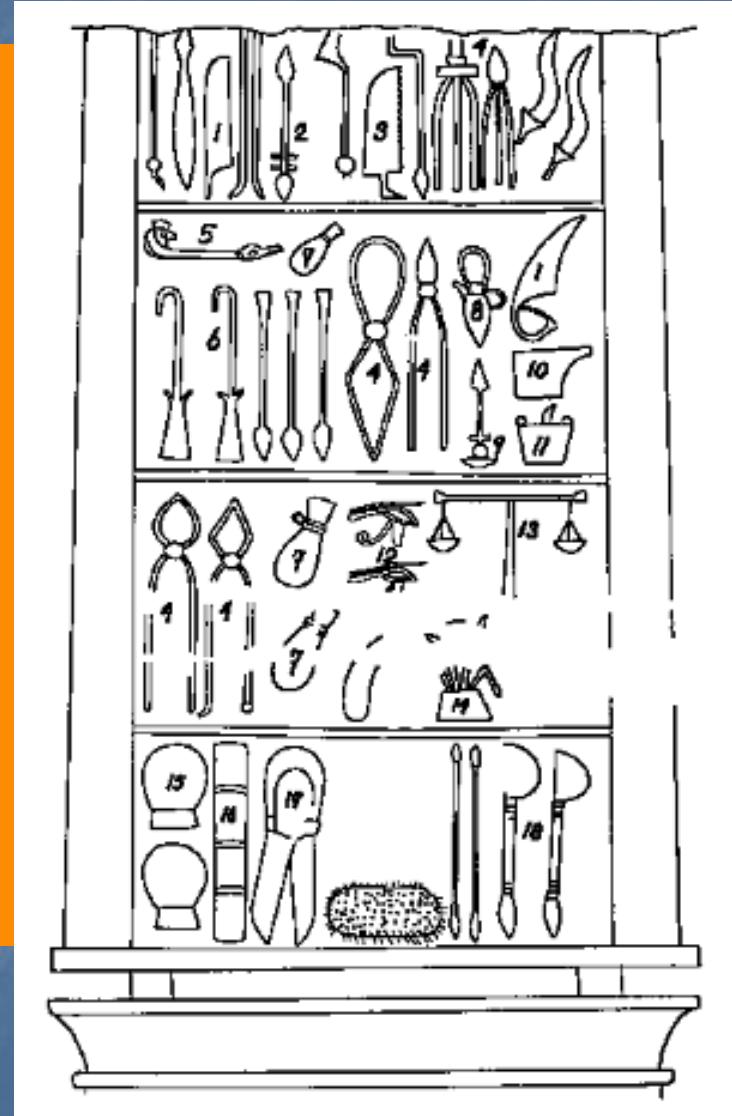
- Follicular inflammation (conjunctiva)
- After reinfections: follicular scarring and fibrosis
- Trichiasis: entropion of the lid, inturned eye-lashes
- Corneal opacity, visual loss

Trachoma diagnosis, Therapy

- Clinical
- Laboratory: DIF, Ag detection ELISA, Nucleic acid detection (PCR)
- Th: azithromycin per os! + surgery
- **Control, prevention:** face hygiene, sanitation, fly control!
in Europe was endemic from the Napoleon's war until 1960



Trachoma treatment and prevention in Egypt



Oculogenital *C. trachomatis* ac and chr infections: D-K serotypes

Spread: sexual, contact, vertical

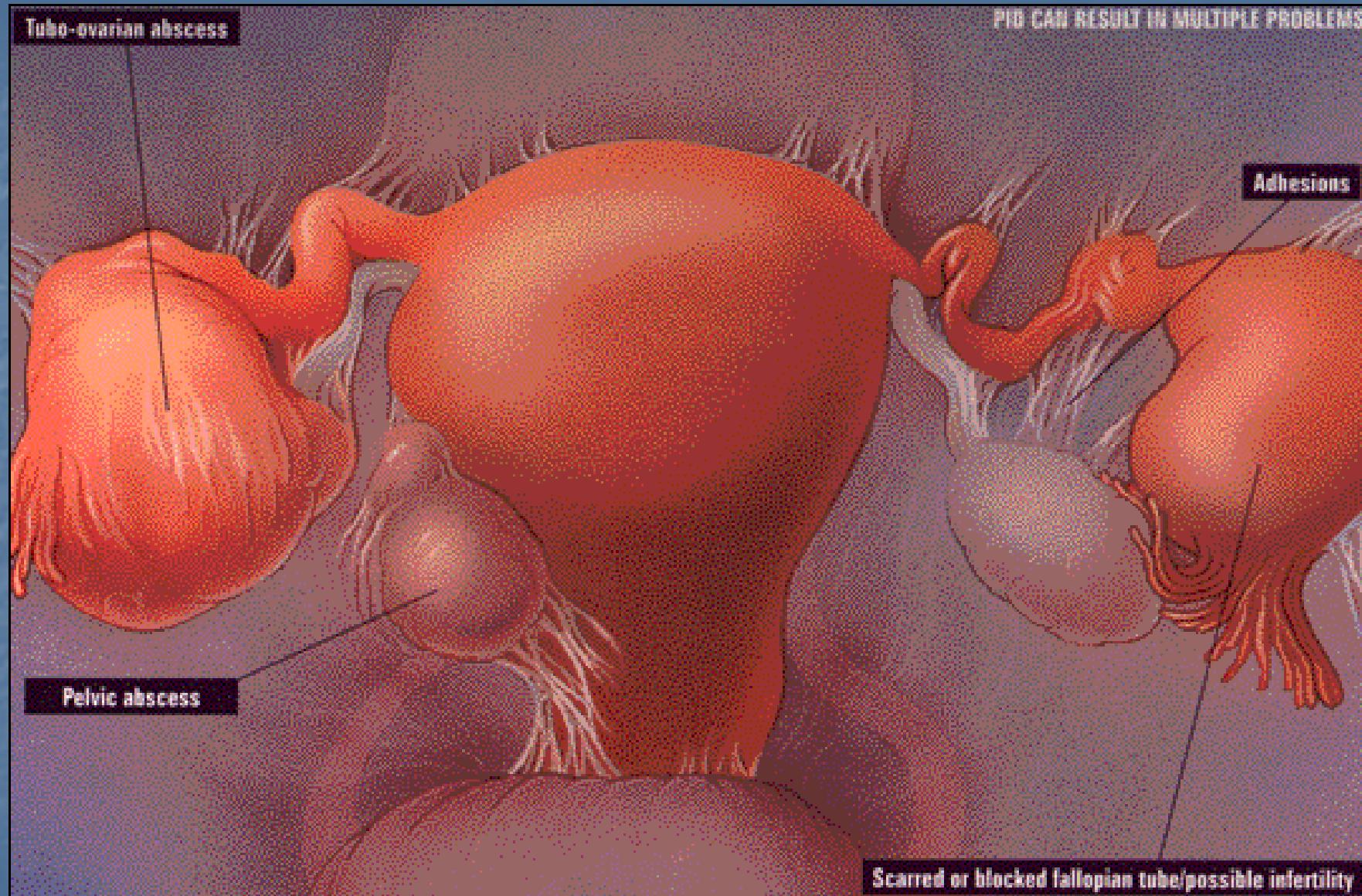
Target cell: columnar epithel



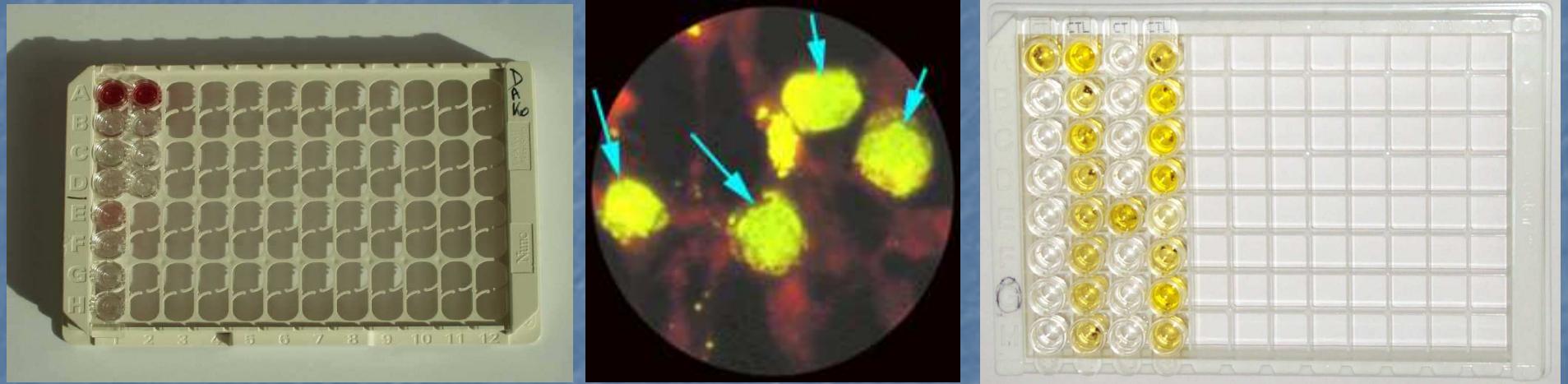
- urethritis, cervicitis, inclusion conjunctivitis
- epididymitis, Reiter-trias , perihepatitis, pelvic inflammatory disease (PID), infertility
- newborn pneumonia

www.worldsbest-sexual-health-site.com

PID



Diagnosis of oculogenital D-K infections



- Clinical+laboratory in acute forms :
DIF, Ag ELISA, cell culture (McCoy), NAAT (PCR)
- In systemic cases as above + antibody detection:
IgM, IgA and IgG ELISA or indirect MIF

New genetic variant of *Chlamydia trachomatis* Sweden 2006

- ★ First description: 13% , then 20-65% of all detected chlamydia cases in County Halland (Southwest Sweden)
- ★ 377bp deletion in the cryptic plasmid
- ★ Escape of chlamydia detection for those commercial tests with this target
- ★ False negative results

Oculogenital (D-K) *C. trachomatis* diseases: Therapy, Prevention

- Macrolides or doxycycline for 2 weeks
- Single dose azithromycin in acute adult infections, repeat 2x!
- Newborn infections: systemic+ local macrolides

- Safe sex, screening of risk groups, asymptomatic partners and treat them

C. trachomatis L1-L3 serotypes: Lymphogranuloma venereum (LGV)



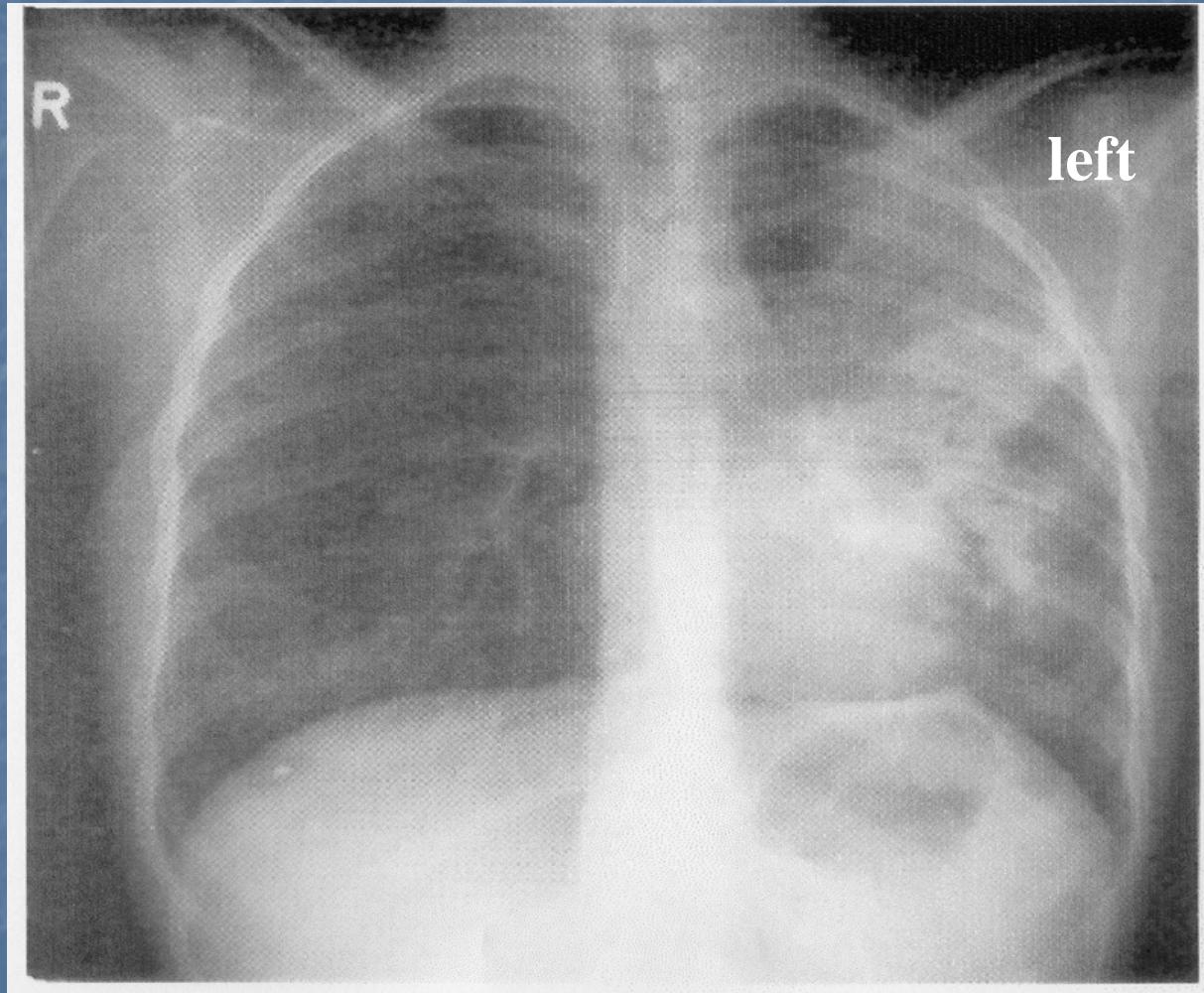
- Systemic STD disease with local ulcer, proctitis, lymphadenitis, high fever, meningoencephalitis
- Laboratory: DIF , PCR and indirect MIF or ELISA antibody detection IgM, IgA, IgG
- Therapy: doxycycline, (macrolides) 3weeks
- Prevention: safe sex

www.phac-aspc.gc.ca, www.med.cmu.ac.th, www.moondragon.org

Chlamydophila (Chlamydia) pneumoniae atypical interstitial pneumonia

- Air-born spread, human pathogen, common from 3 years, family and community outbreaks, no strong seasonality,
- 7-20 days incubation time
- mild to severe form (ARDS), low grade fever, dry cough for weeks, leukocytes often normal,
- X-ray?

C. pneumoniae interstitial pneumonia X-ray



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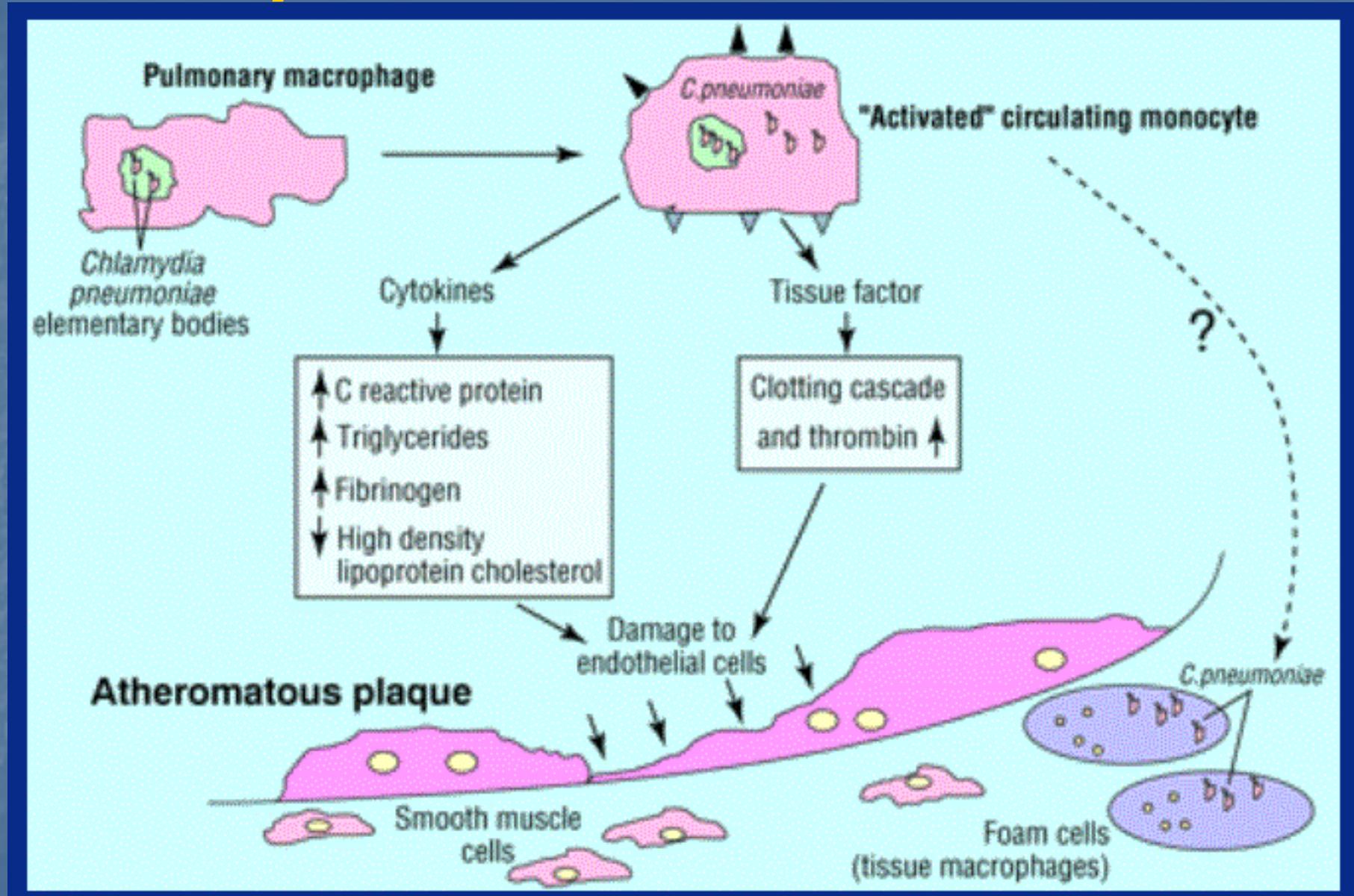
C. pneumoniae atypical pneumonia laboratory diagnosis and therapy

- **Laboratory:** bronchial aspirate or lung tissue PCR, DIF or cell culture (HeLa, Hep2) (not routine), more common serology: IgM, IgA and IgG species specific ELISA or MIF
- **Therapy:** macrolides, doxycycline or new fluoroquinolons for 2-3 weeks or longer
- **Vaccine?:** DNA subunit animal trial

C. pneumoniae disease courses

- Acute
- Re-infections common (short term immunity)
- Chronic or persistent (intracellular surviving!) among adult population 50-70% seropositivity!
- sequelae: arthritis, Kawasaki syndrome, atherosclerosis?, Alzheimer?, sclerosis multiplex ?
- pulmonary carcinoma?

C. pneumoniae and atherosclerosis



Chlamydophila psittaci psittacosis, ornithosis, parrots' disease

- Zoonotic
- Air-born spread (birds faeces)
(BSL3 !)
- Target cells: macrophages,
lung parenchyma
- Disease course: severe
atypical pneumonia
- Laboratory: CF or species
specific MIF serology
- Therapy: macrolides,
doxycycline for 3 weeks
- Prevention: veterinary control,
animal vaccine, respirators in
animal quarantine



Chlamydophila psittaci: psittacosis, ornithosis

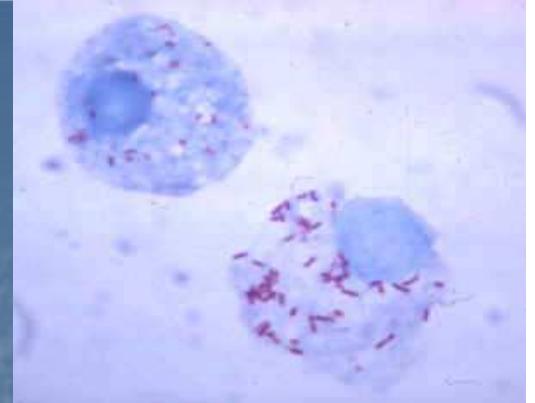


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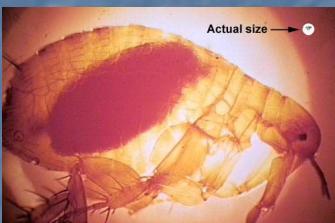
Rickettsia, Orientia



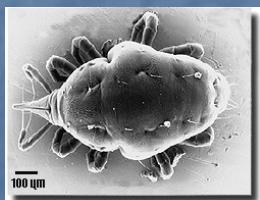
- The smallest obligate intracellular Gram-negative bacteria with permeable cell wall (NAD, coenzyme A, aminoacid glucose dependent)
- Adaptation to human **endothelial** cells
- Responsible for **arthropode born** human diseases
- Distribution: depends on arthropods (tick, flea, mite, lice) and rodents
- BSL3! cell culture or animal inoculation

Taxonomy: 2 genera, many species

■ *Rickettsia*



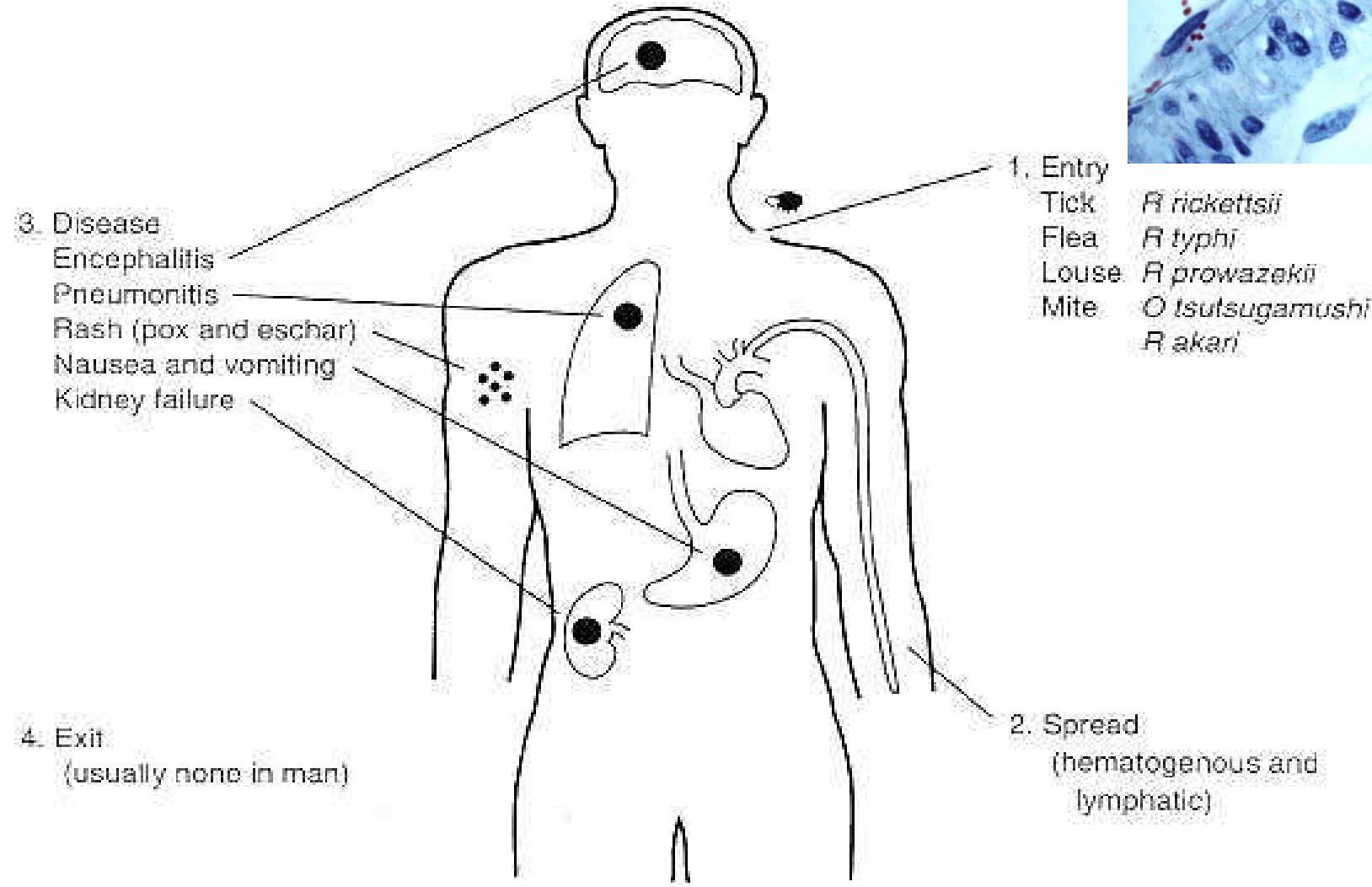
■ *Orientia* (Rickettsia)



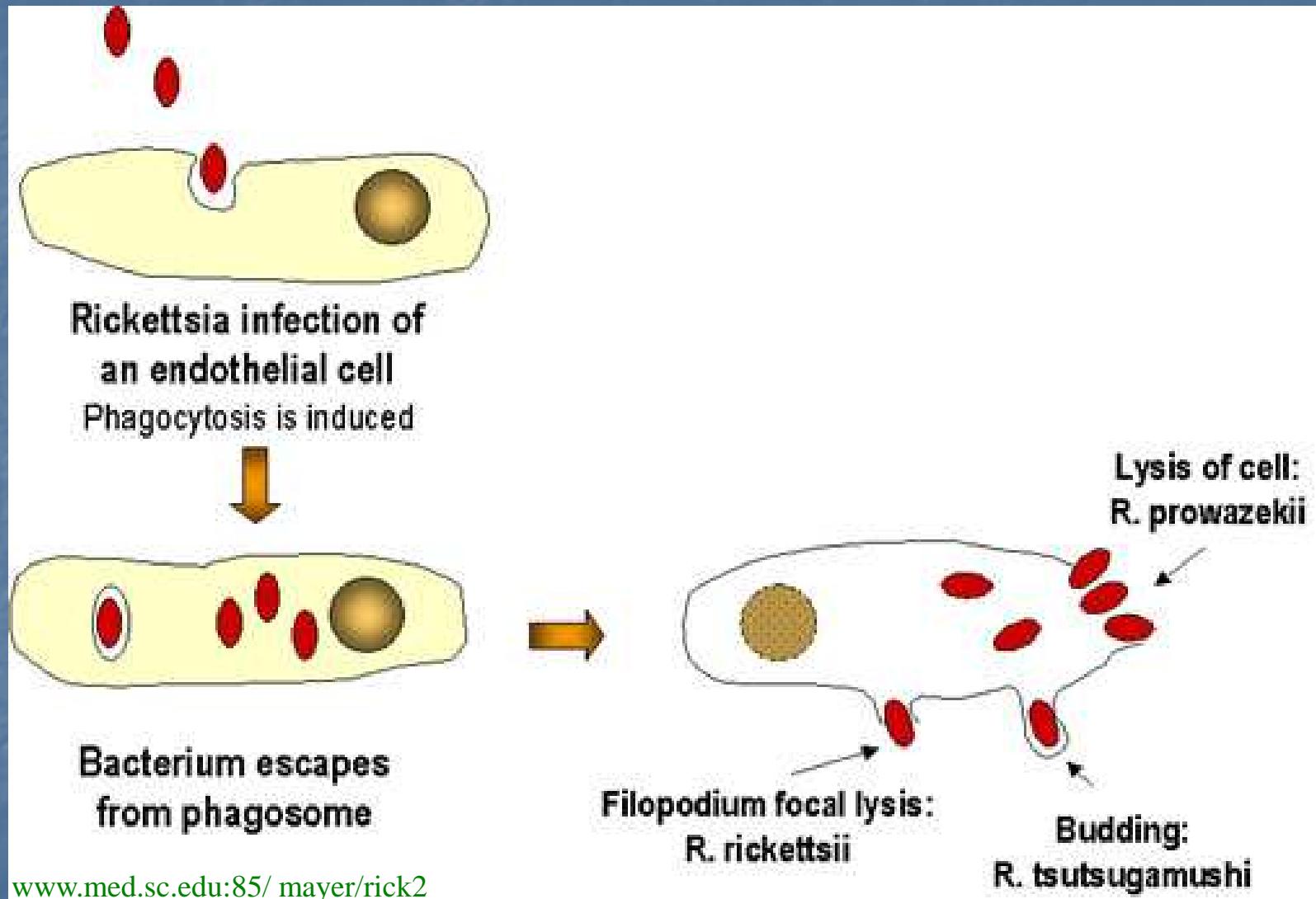
- **Spotted fever group:** Rocky Mountain (RMSF)- *R. rickettsii*
Mediterranean (MSF)- *R. conorii*,
+ others *R. helvetica*, *R. slovaca*,
Rickettsial-pox: *R. akari*
- **Typhus:** Epidemic *R. prowazekii*
Endemic (murine) *R. typhi*

- **Scrub typhus:** *O. tsutsugamushi*
no LPS and PG in cell wall!

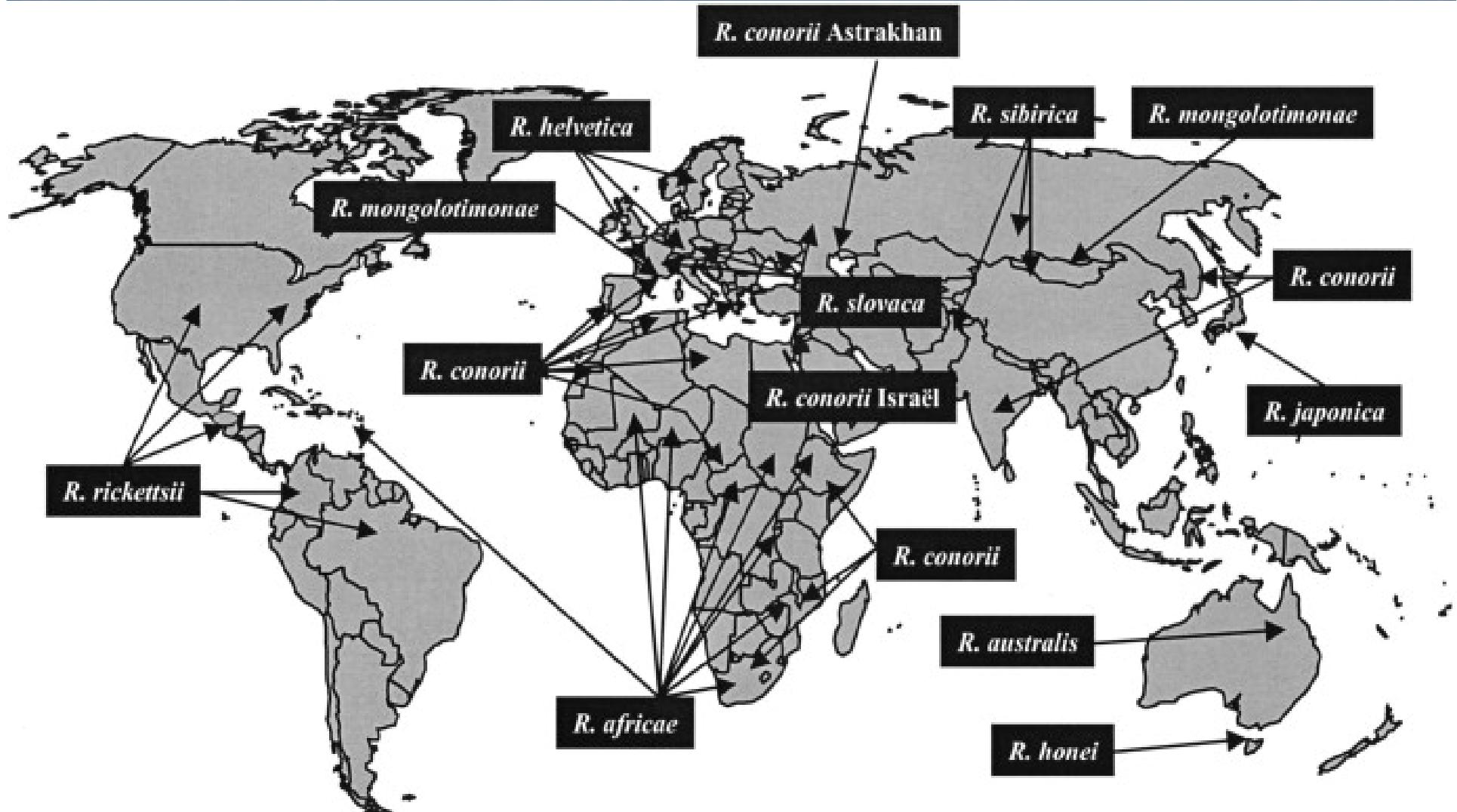
Rickettsial diseases-pathogenesis: vasculitis of small blood vessels



Rickettsial diseases: pathogenesis



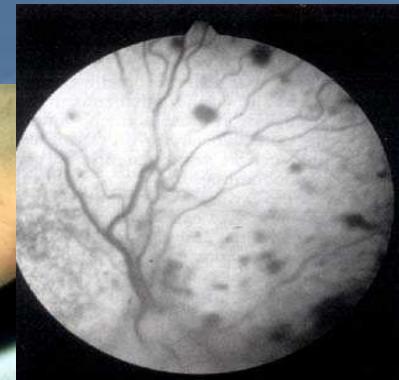
WORLDWIDE DISTRIBUTION OF RICETTSIA



Symptoms of rickettsial infections

- Fever, chills, headache, myalgias, thrombocytopenia
- Maculopapular rash, petechiae first on extremities , later on trunk in spotted fever,
- pneumonia, renal insuff, myo-pericarditis, encephalitis
- mortality 1-30 %

Rashes in spotted fever: early maculopapular and late petechial



CDC

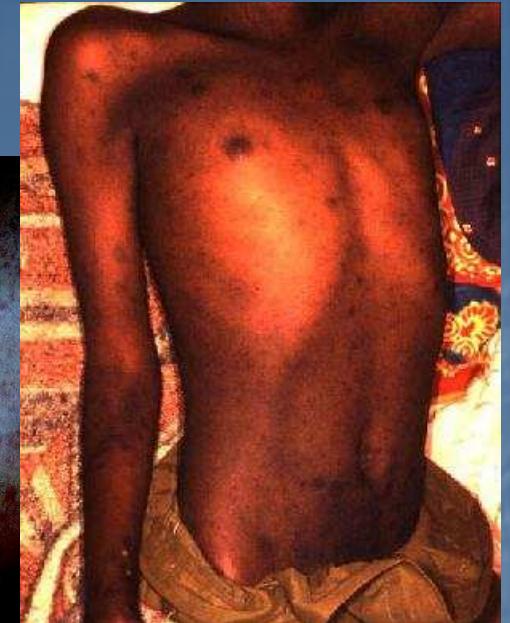
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www.med.sc.edu:85/mayer/rick2

Typhus exanthematicus

Epidemic typhus



- *R. prowazekii*
- Human to human spread by
- Vector: *Pediculus humanus corporis* (*louse*)

- Sudden onset, fever, chills, myalgia, rash on trunk, coma, multi-organ failure
- Rescrudescence typhus: Brill-Zinsser disease
- Therapy: doxycycline, killed vaccine for high risk

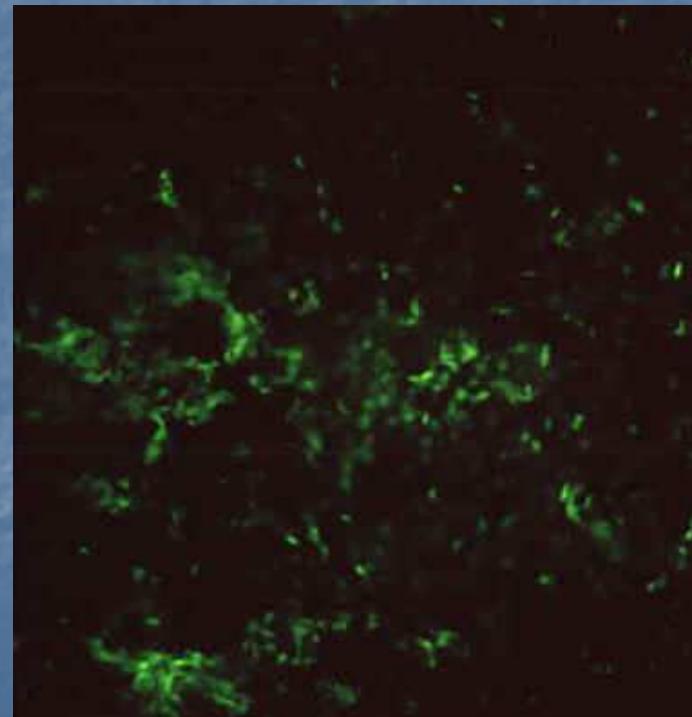
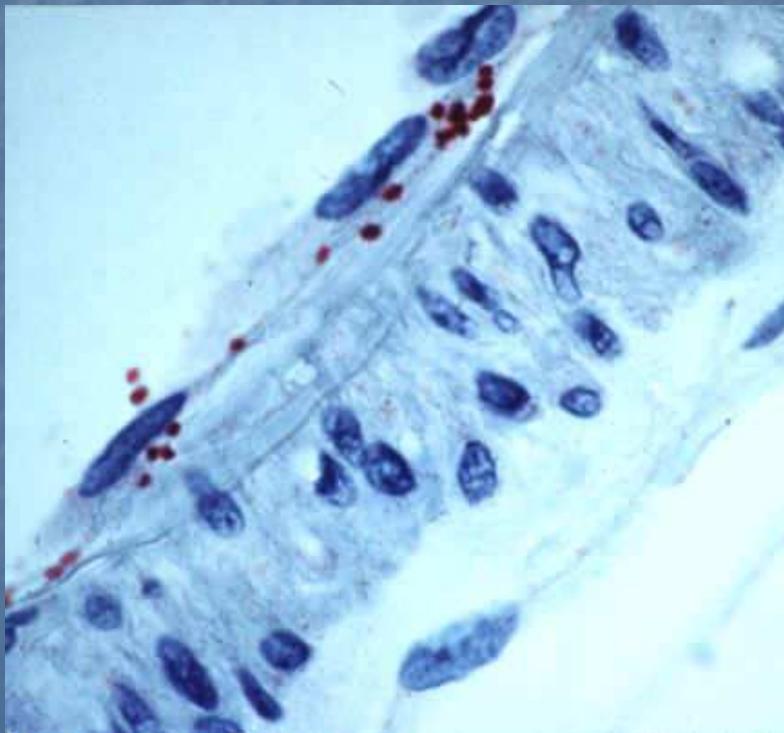


Laboratory diagnosis:
serology methods

Weil-Felix tube agglutination
(non specific Proteus OX-19, OX-2 antigen), CF,
specific indirect IF IgM, IgG

BSL3!

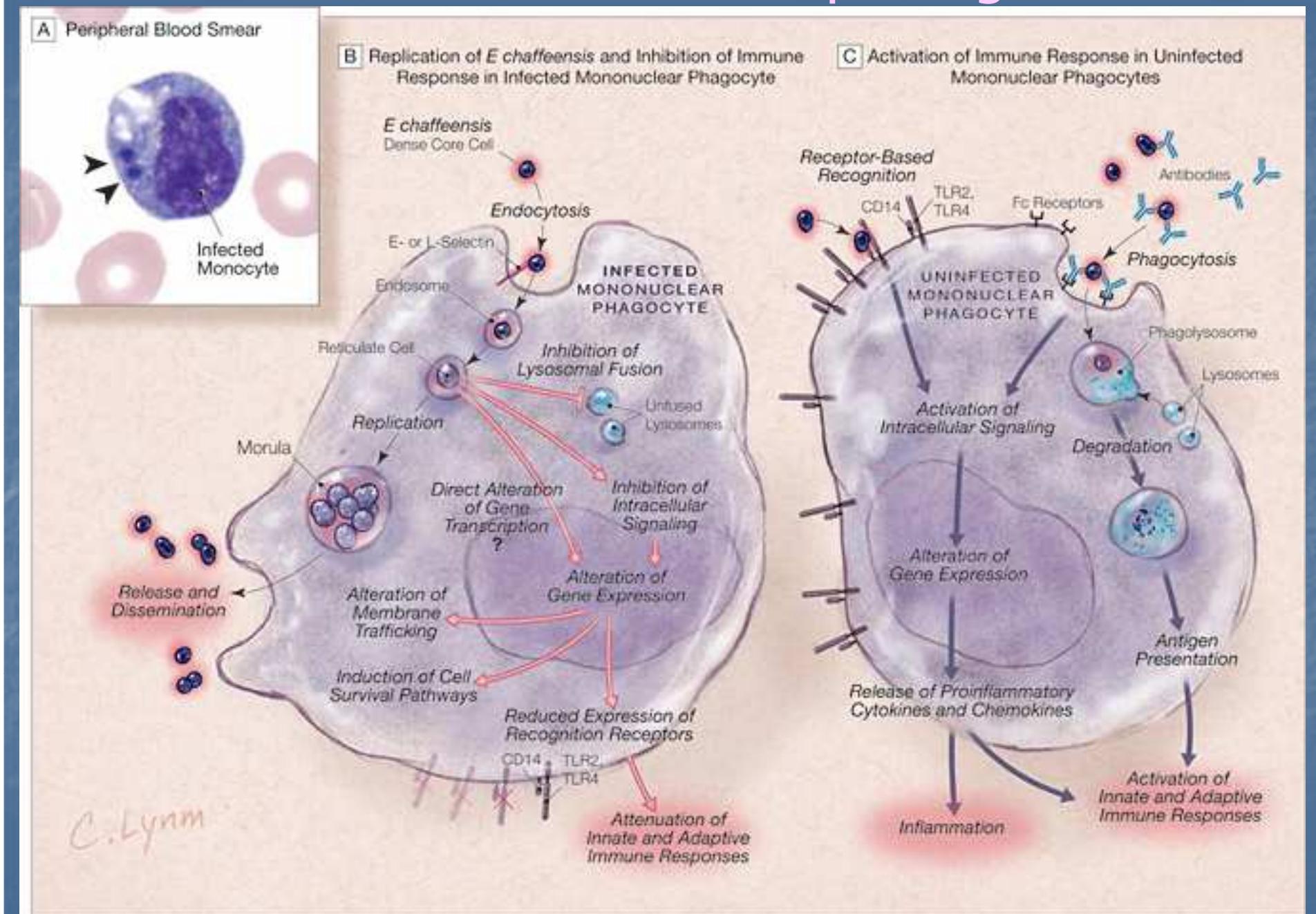
histochemistry from skin lesion, direct IF, PCR



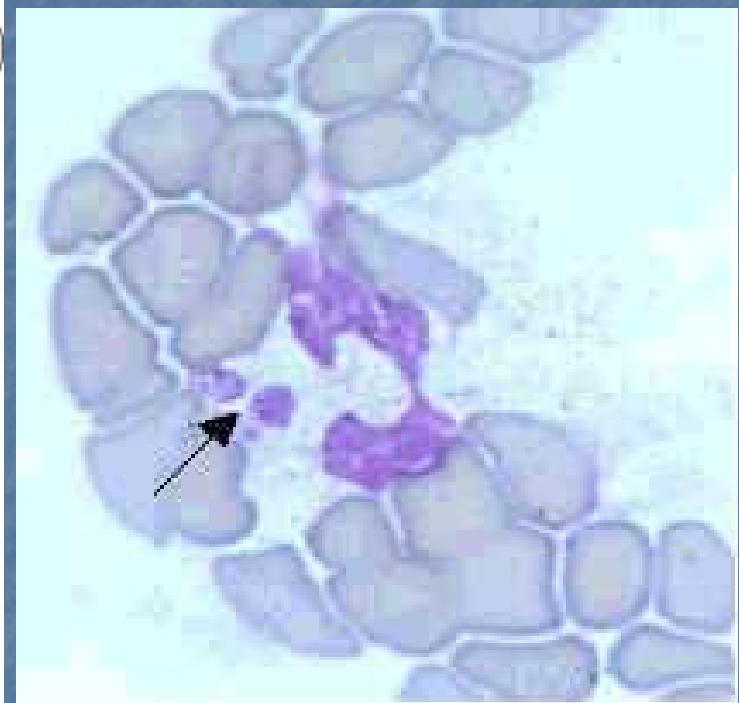
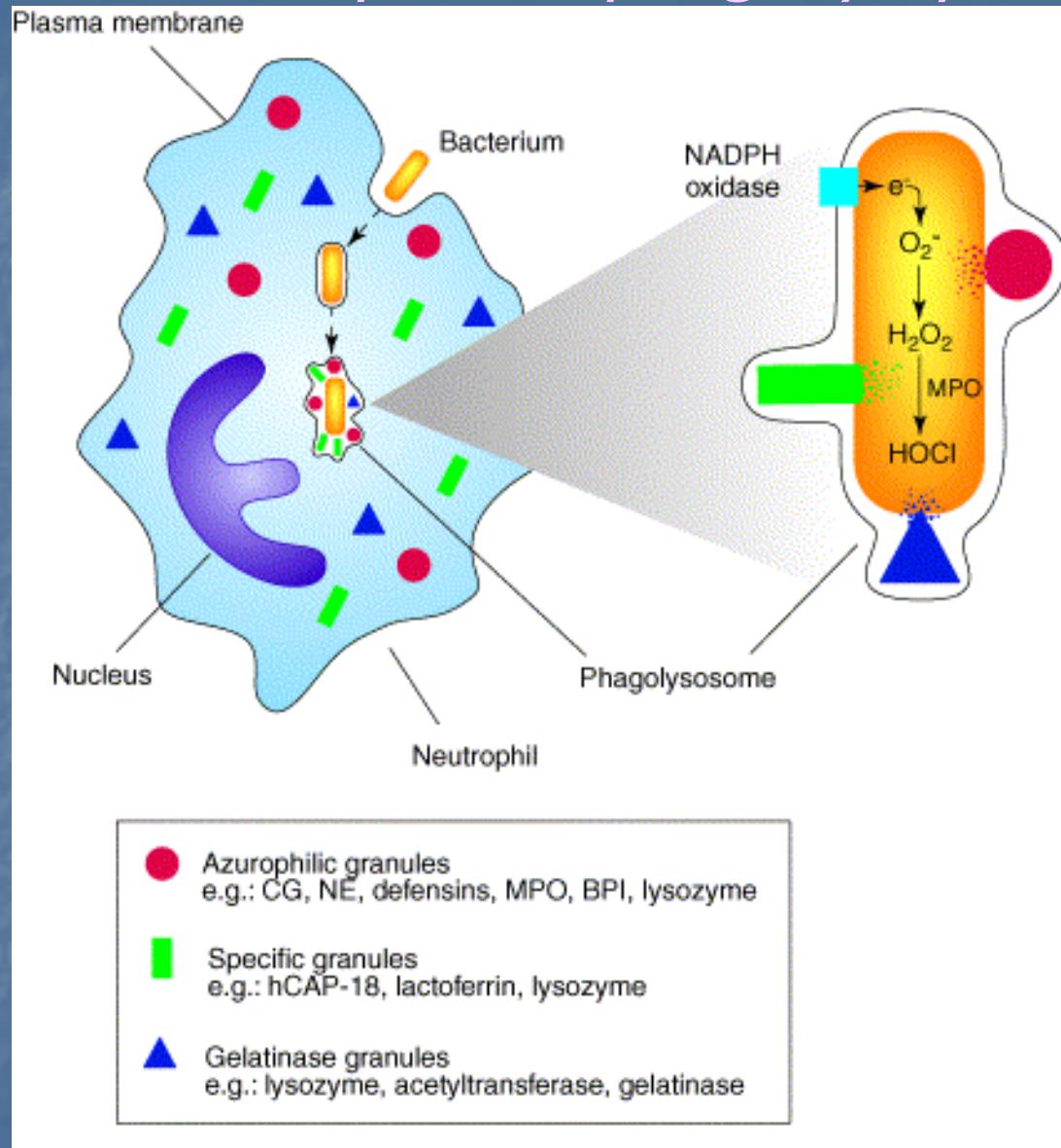
Ehrlichia, Anaplasma (Ehrlichia)

- parasiting in mononuclear cells or granulocytes
- no LPS and peptidoglycan
- prevent fusion of phago-lysosome
- form membrane enclosed **morulae** in cytoplasma of infected cells

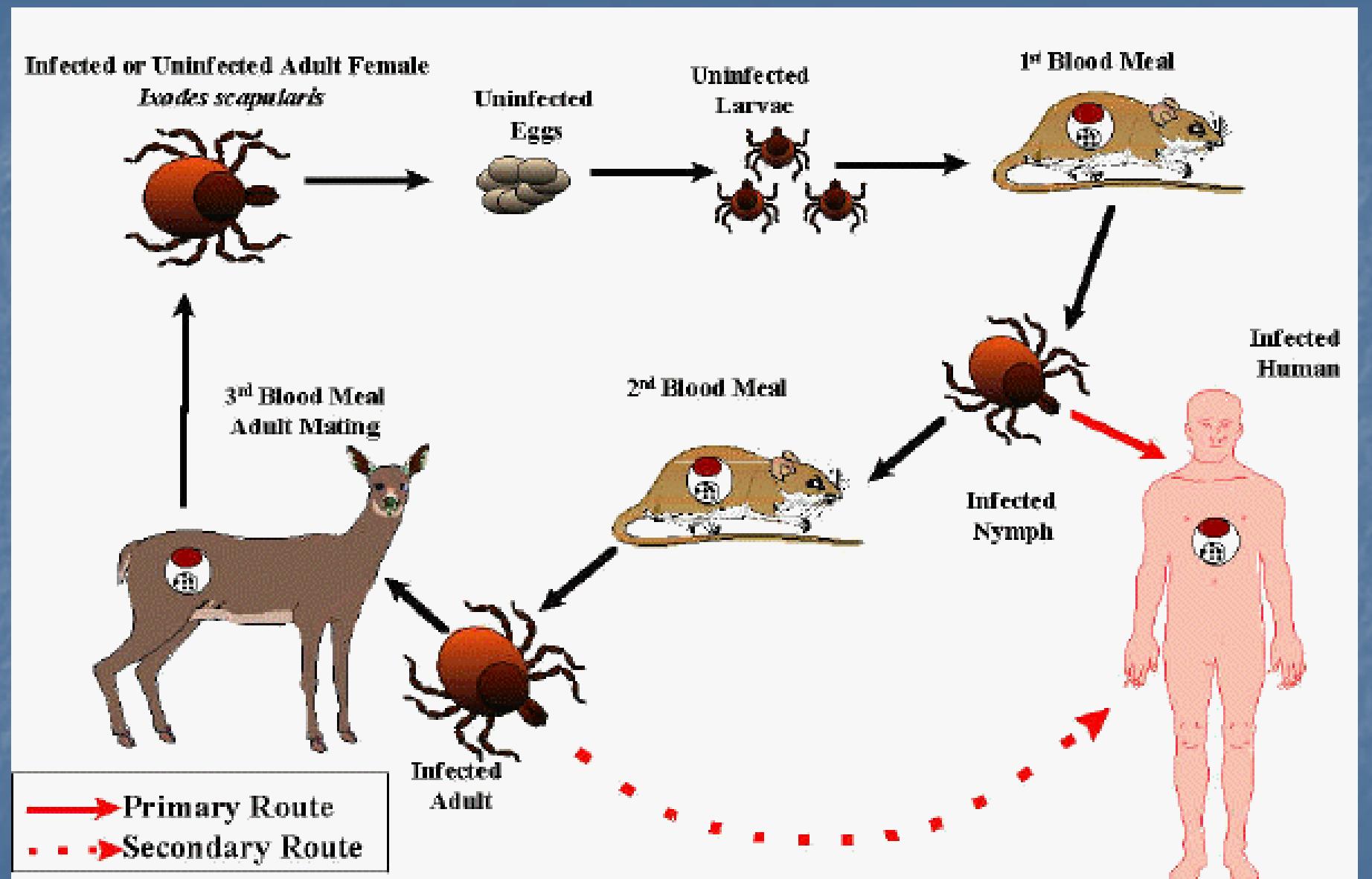
Ehrlichia chaffeensis HME pathogenesis



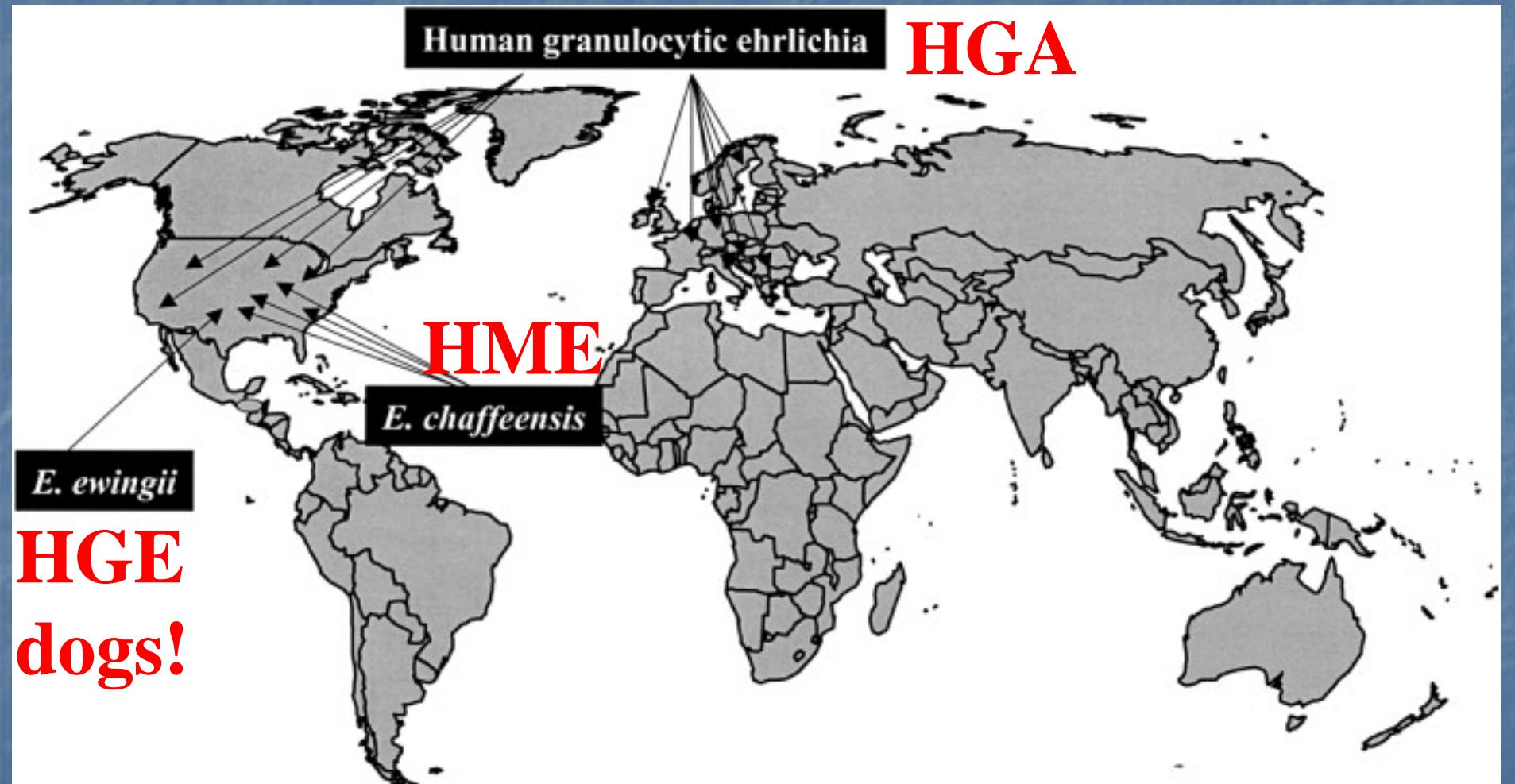
Anaplasma phagocytophilum pathogenesis



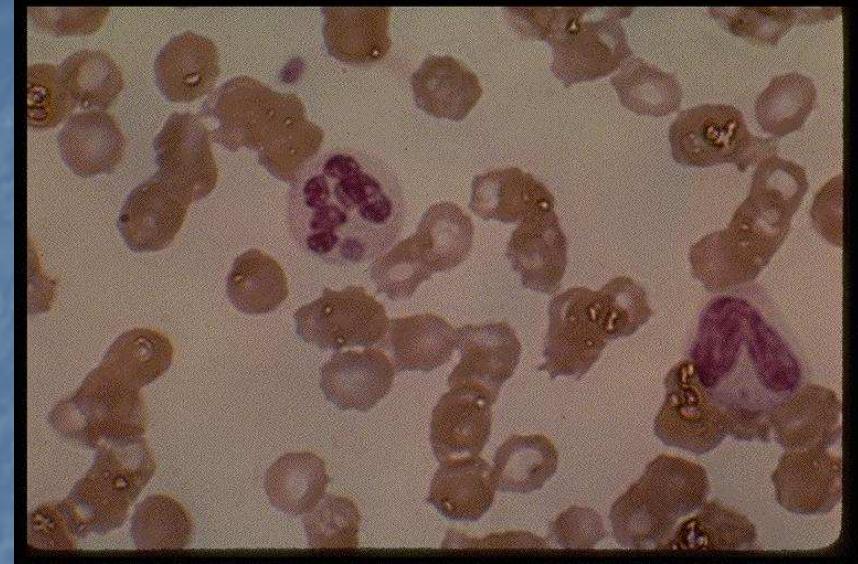
Ehrlichia (HME), Anaplasma (HGA) pathomechanism



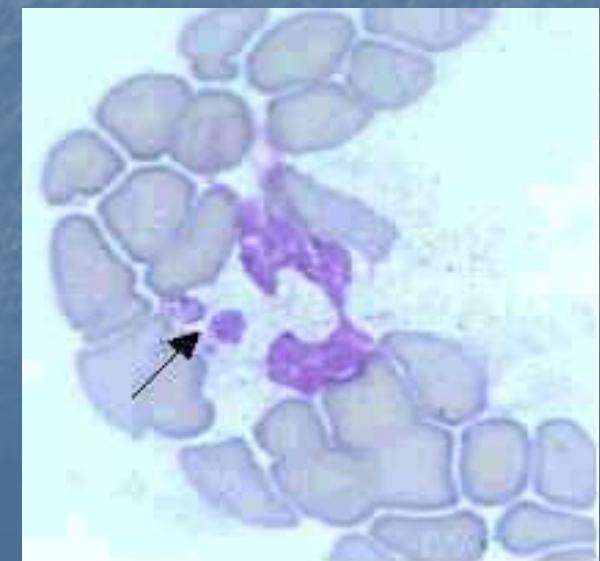
Ehrlichia HME, *Anaplasma* HGA host and vector distribution map



Anaplasma phagocytophilum HGA



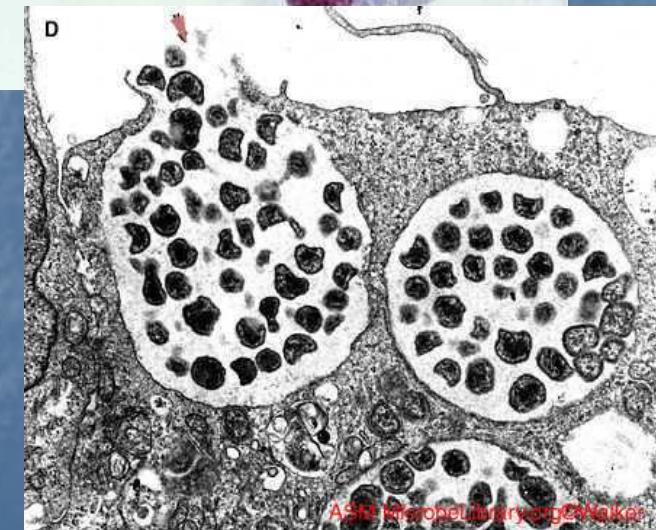
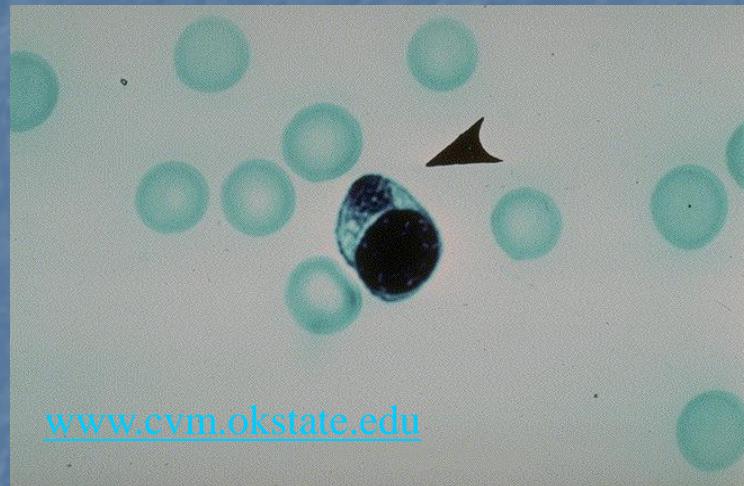
- Vector: *Ixodes* spp. ticks
- Symptoms: fever, headache, rash in 20%, low WBC and thrombocyte count, SGOT, SGPT elevation
- Giemsa-stain: peripheral blood or bone marrow, PCR
- indirect IF IgM, IgG
- Mortality : 1 %



Human monocytic ehrlichiosis (HME) :

Ehrlichia chaffeensis

Morulae in monocytes! Mortality: 3%



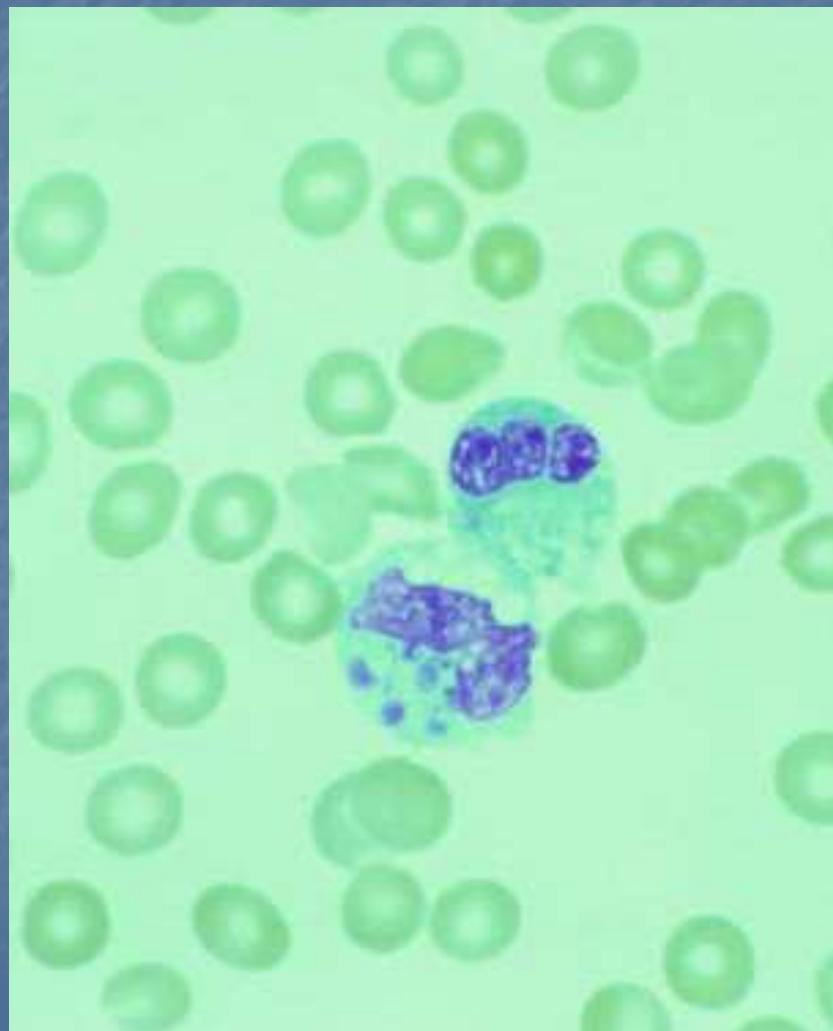
Dense-core cells of *E. chaffeensis* are seen here exiting the host cell following rupture of the morula and the host cell cytoplasmic membrane. These ehrlichiae will now go on to infect additional host cells or they may be ingested by a feeding tick, thus spreading the infection.

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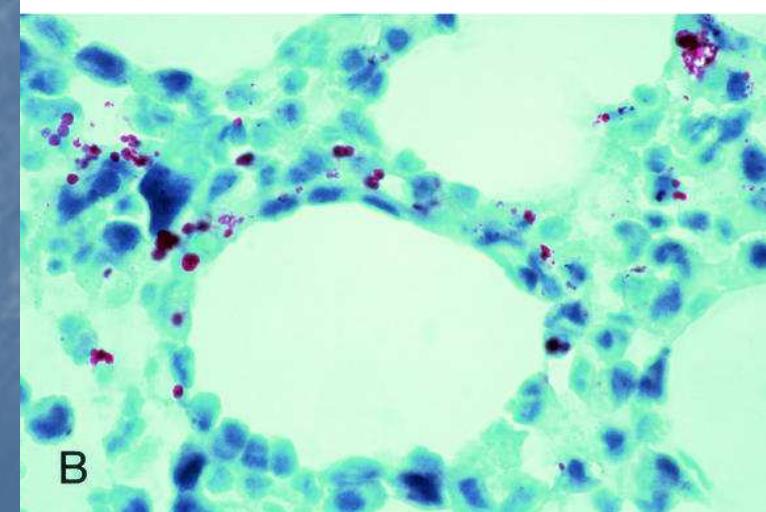
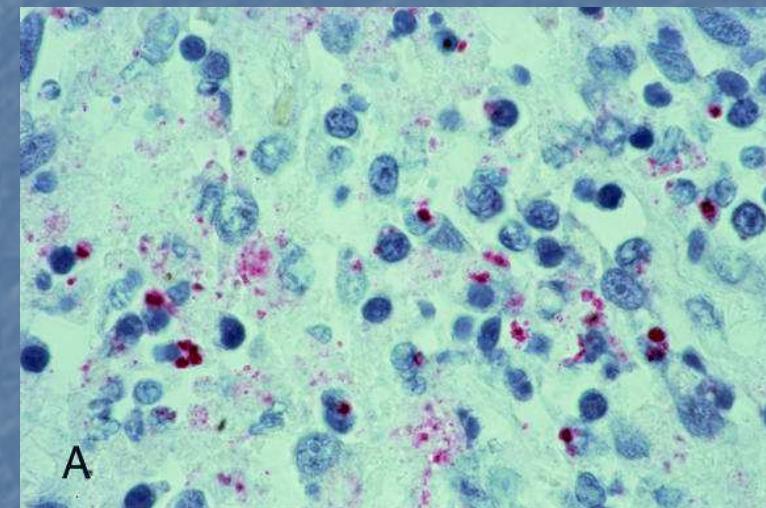
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Ehrlichia chaffeensis (HME),

peripheral blood smears



spleen, lung

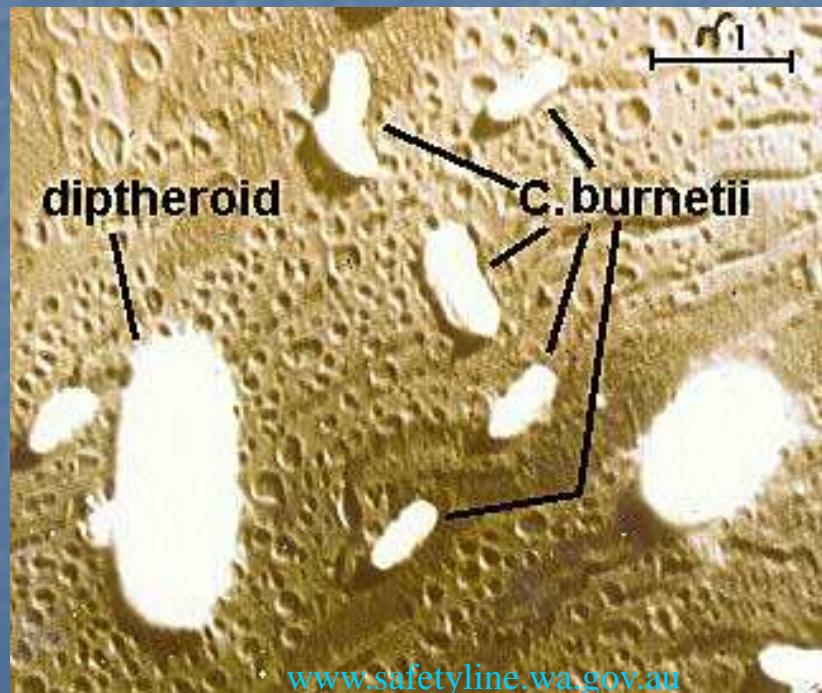


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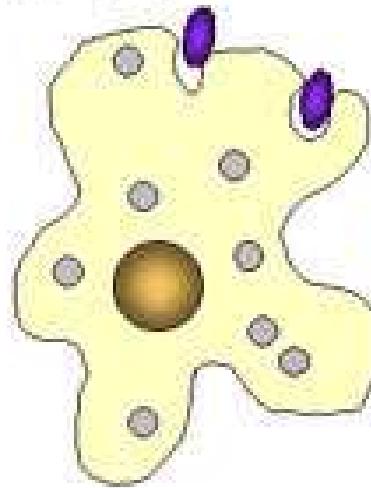
Coxiella burnetii target cells: macrophages, lung parenchyma, liver

- Air-born and food-born spread from infected sheep, goat, cattle, birds (dust)
- very resistant spore-like form in environment

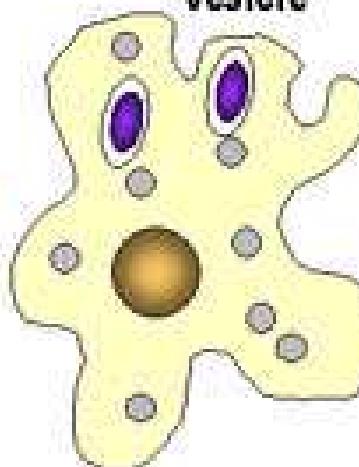


Coxiella burnetii pathogenesis

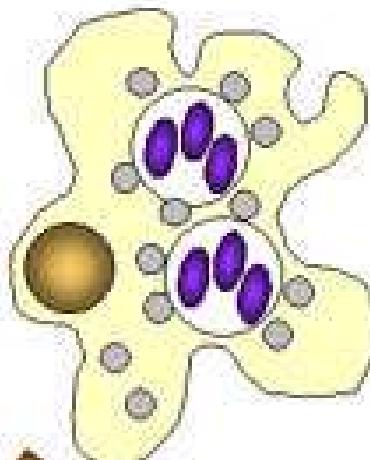
Coxiella infection of a macrophage by phagocytosis



Formation of phagocytic vesicle



Phagosome-lysosome fusion
Bacterium survives and multiplies



Cell and phagolysosome lyses

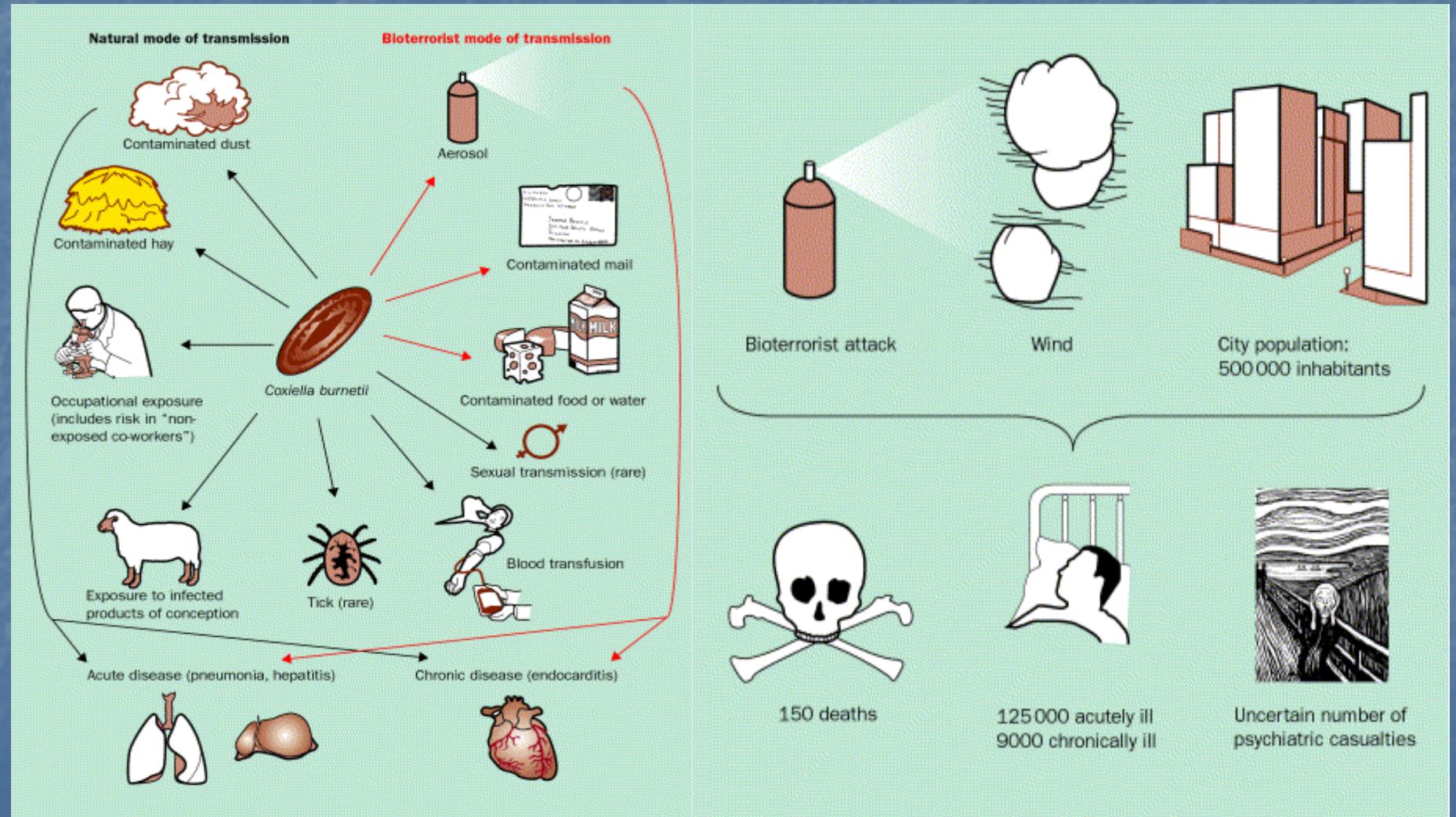


www.med.sc.edu:85/ mayer

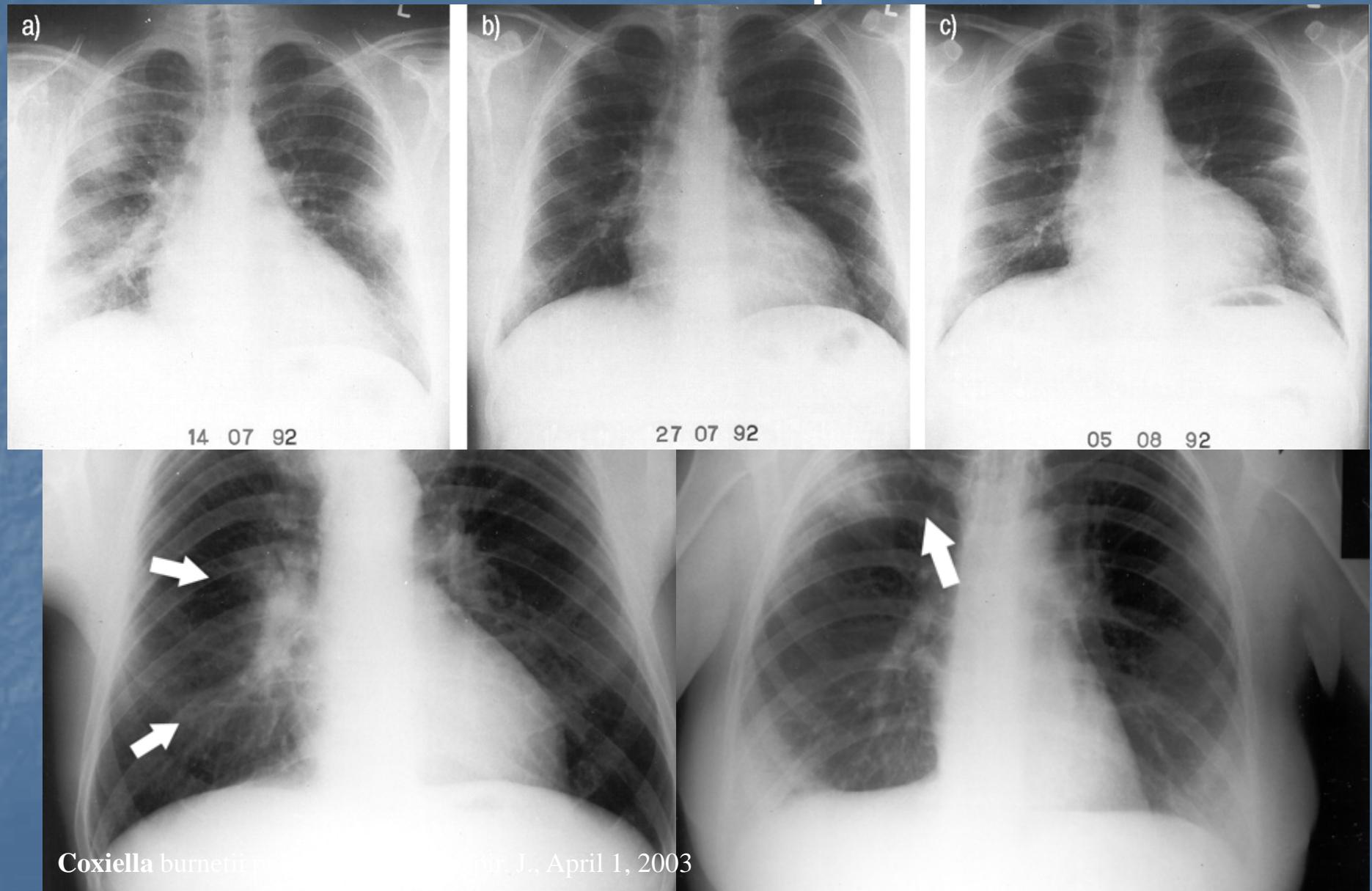
Coxiella burnetii: WHO B cat

Phase I: LPS, complex carbohydrate- week Ag

Phase II: LPS, surface proteins- good Ag



Coxiella burnetii atypical



Q-fever: acute course or chronic granulomatous lesions in lung, liver or endocarditis



Fig 1. — A discrete inflammatory pseudotumor located in the lower lobe of this patient's right lung presents as a cream-colored, rubbery, hemorrhagic mass with irregular borders and measuring approximately 6 cm in diameter.

www.moffitt.usf.edu

**Diagnosis: CF or indirect IF
IgM, IgG of phase I and II Ag-s**

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**Vaccine for non-infected
high risk groups**

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Rickettsial diseases, Ehrlichiosis (HME), Anaplasmosis (HGA), Q-fever therapy and prevention

- Doxycycline, fluoroquinolons, rifampin
10-14 days

