

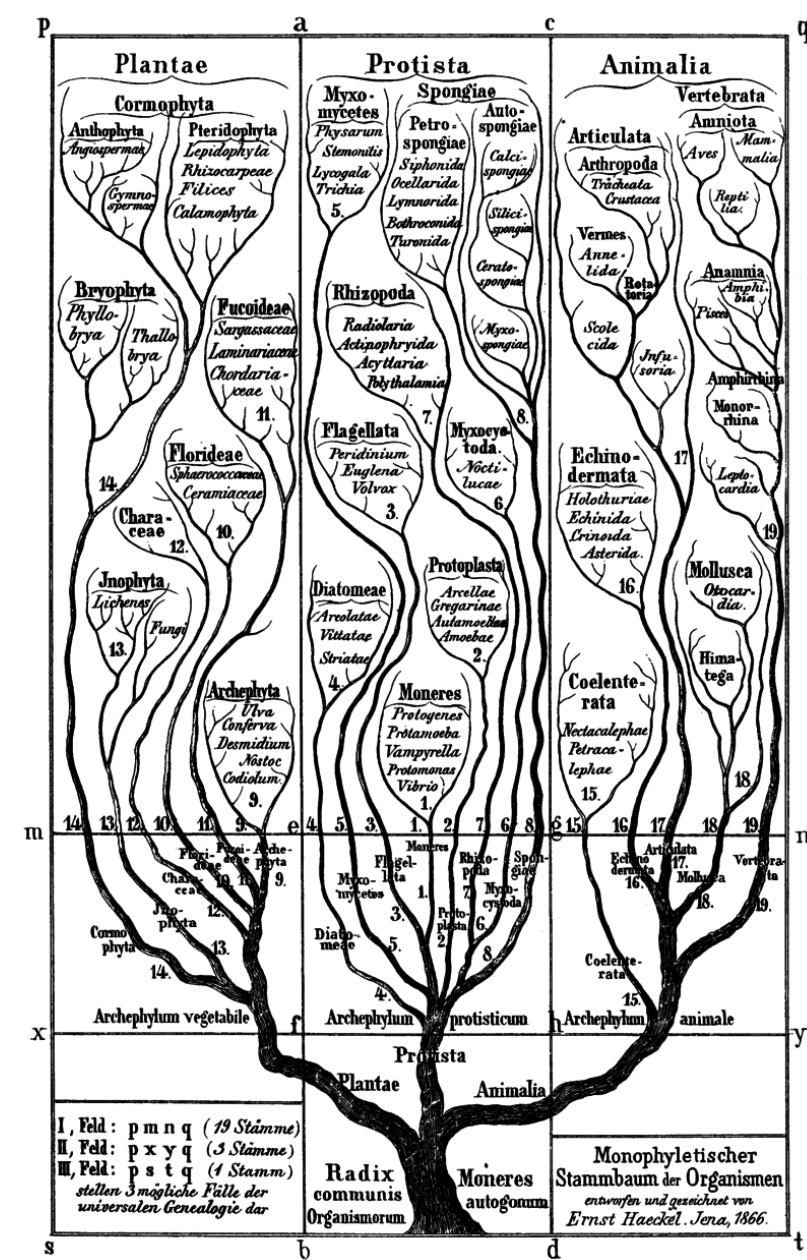
# Bacteriology II

PharmDr. Jakub Treml, Ph.D.



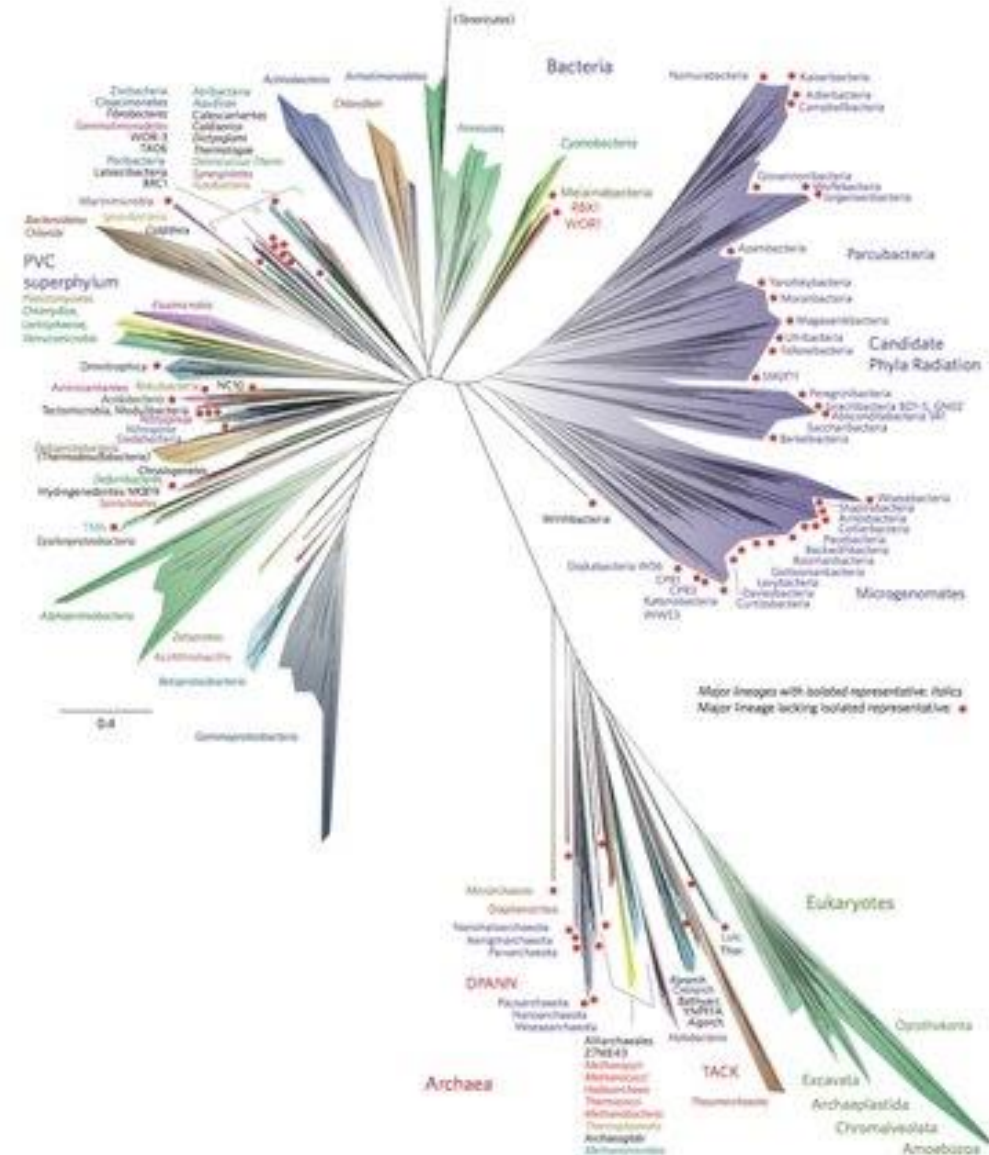
# Bacterial taxonomy

- 1866 – Haeckel: kingdom *Monera*
- 1884 - Gram staining (Danish scientist in Berlin) - *Firmicutes* (G+), *Gratillicutes* (G-) and *Mollicutes* (0)
- according to shape: coccus, bacilus, spirochetes, etc.
- and then came Woese and changed it..
- what is species? (no sex; cca 70% DNA-DNA hybridisation)



# Main bacterial phyla - selection:

- *Proteobacteria*
- *Chlamydiae*
- *Spirochaetes*
- *Bacteroidetes*
- *Cyanobacteria*
- *Deinococcus-Thermus*
- *Actinobacteria*
- *Firmicutes*



# Phylum *Proteobacteria*

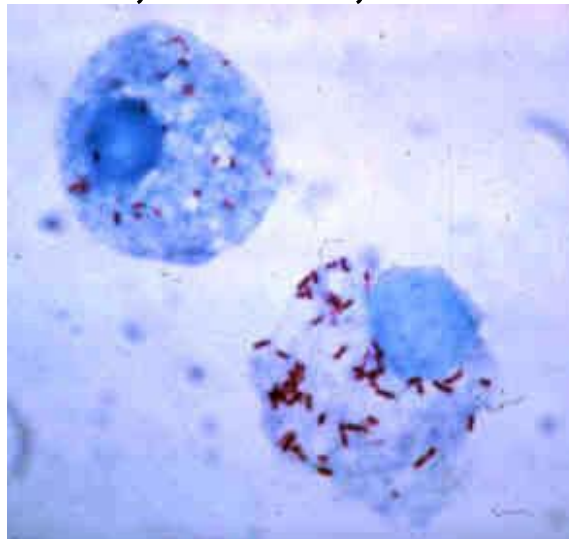
- one of the groups of gramnegative bacteria
- orig. "purple bacteria and relatives" - but very diverse = renamed
- class ***Alphaproteobacteria***: *Rickettsia* (intracel. parasites, typhoid fever); *Rhizobium* (plant symbiosis); ?mitochondria
- class ***Betaproteobacteria***: *Bordetella* (pertusis); *Neisseria* (meningitis, gonorrhoea)

# Phylum *Proteobacteria*

- class ***Gammaproteobacteria***: important families *Enterobacteriaceae*, *Vibrionaceae* and *Pseudomonaceae*
- class ***Deltaproteobacteria***: *Desulfovibrio* (and other sulphate-reducing bacteria)
- class ***Epsilonproteobacteria***: *Campylobacter*, *Helicobacter*

# Class *Alphaproteobacteria* - f. *Rickettsiaceae*

- **obligate intracellular** parasites (monocytes, endothelial cells) – cultivation in cell culture
- **G-** bacteria, nonmotile, **pleomorphic** (round, rod or fibrous)
- transferred by vectors: ticks, louse, mites, fleas

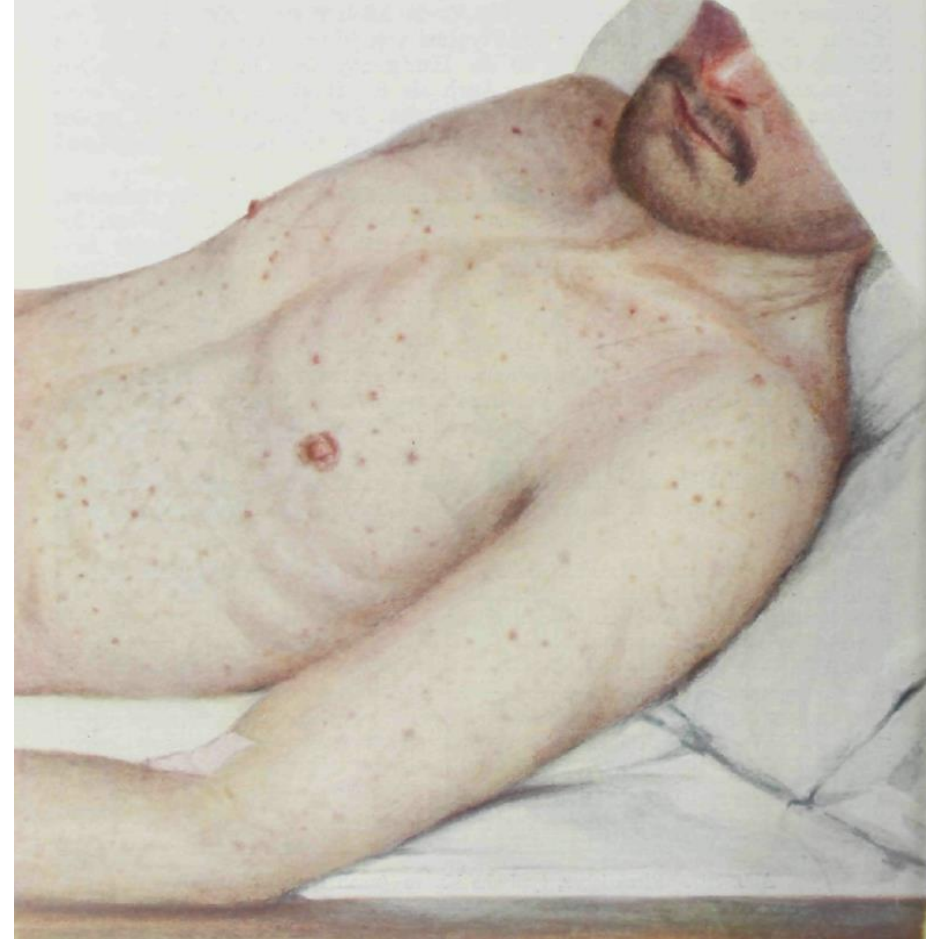


# Endemic typhus

- cause: *Rickettsia prowazekii* – (Stanislaus Prowazek, Czech, died because of this disease)
- high fever, headache, rash
- tachycardia, hypotension, hearing loss, sensitivity to light
- louse (in its feces, into human blood by bites or scratches or by inhalation)
- before ATB mortality 20 – 40%, these days 1%
- th.: chloramphenicol, tetracyclines

# Endemic typhus

- „jail fever“ – e.g. in 1945 – gestapo jail in Terezín
- today mainly in Africa, Andes,...



CC Danvasilis



# Rocky Mountains spotted fever

- cause: *Rickettsia rickettsii* – (Howard Ricketts, Montana, USA)
- vector: tick
- hosts: vertebrates
- symptoms: fever, head and muscle ache, rash
- may be severe, lethality cca 5% even if treated
- Northern and Central America

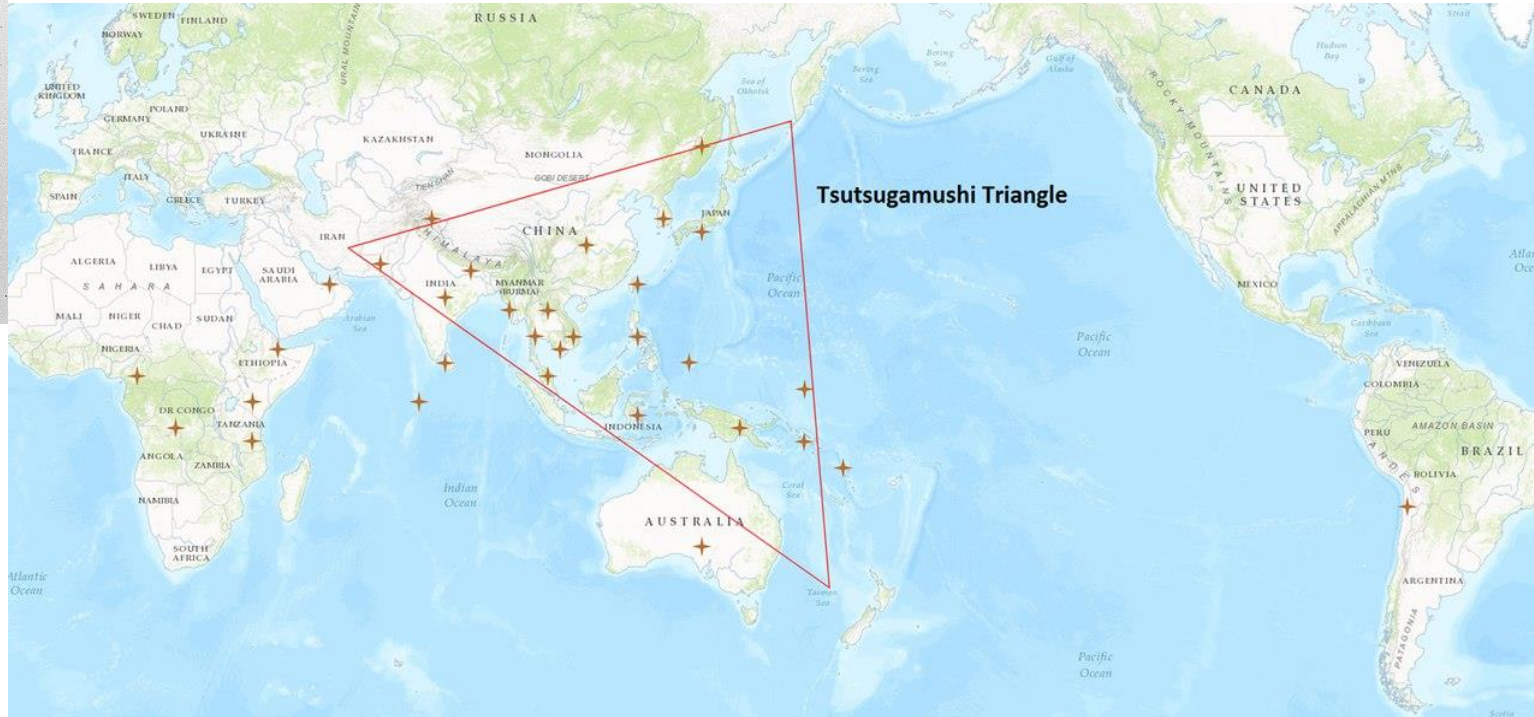
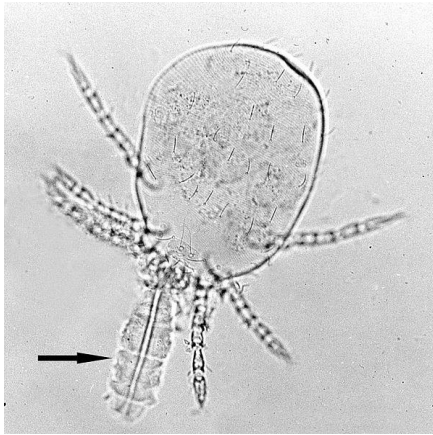
# Rocky Mountains spotted fever



# Scrub typhus

- cause: *Orientia tsutsugamushi* – (jap. *tsutsuga* = „disease“; *mushi* = „insect“)
- vector: mites
- hosts: rodents
- in the place of bite is black eschar (slough), fever, headaches, rash (similar to end. typhus)
- mortality around %, without treatment up to 10% - infection of travellers, rice fields, plantations...
- doxycycline, tetracycline, chloramphenicol

# Scrub typhus



CC Alan R Walker  
CC Chhandama

# Class *Betaproteobacteria*

- order *Burgholderiales*: *Bordetella pertussis*
- order *Neisseriales*: g. *Neisseria*

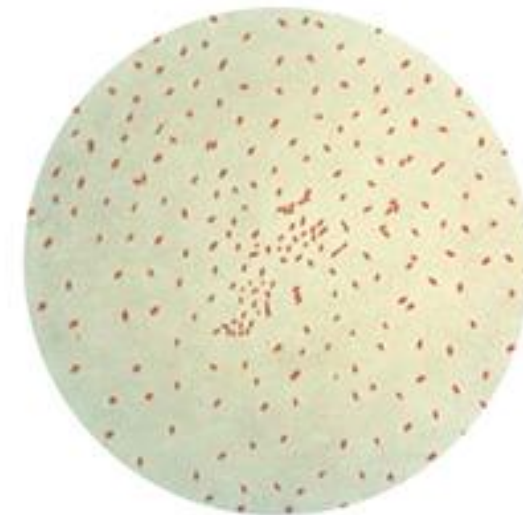
# *Bordetella pertussis*

- G- nonmotile bacillus of ovoid shape (**coccobacillus**)
- strictly aerobic, colonizes RS, **pertussis (whooping cough)**
- swab from nasopharynx, special cultivation media
- airborne droplets - adhesion on mucosa – pertussis toxin (lymphocytosis, inflammation and necrosis); 10-15 days – symptoms of common cold
- blockade of mucociliary trans. => irritant cough, **whooping** sound; apnea, nausea, vomiting, even death (serious for nonvaccinated children!)

# *Bordetella pertussis*

*B. pertussis* – cultivation  
for 7 days – Bordet-  
Gengou agar

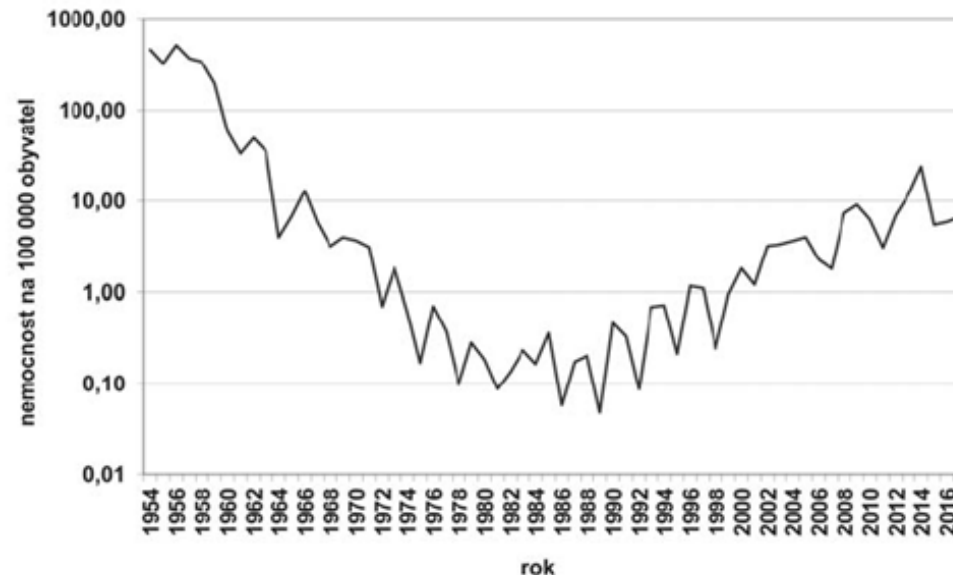
pearlescent shine, narrow  
hemolysis zone



# *Bordetella pertussis*

- th.: 1<sup>st</sup> line – **macrolides; tetracyclines**, cotrimoxazole
- small children and complications = hospital
- since 1958 vacc.; acellular vaccine

Graf 1: PERTUSE, ČR, 1954–2017, HLÁŠENÁ NEMOCNOST (semilogar.)

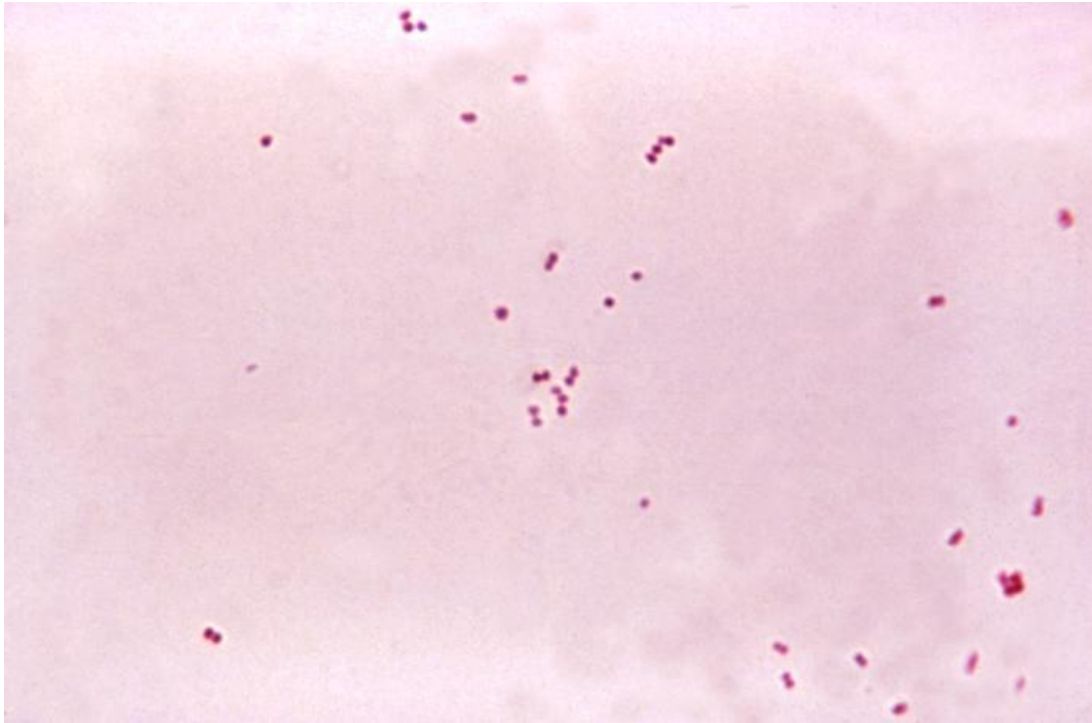




# *Neisseria meningitidis*

- G- coccus, often „**meningococcus**“, strictly aerobic
- nonsporulating, forms diplococci
- airborne droplets
- main ag. is **capsule polysaccharide**; different serogroups (in Europe mainly B and C) – modifications
- causes **invasive meningococcal disease** (IMD)

# *Neisseria meningitidis*



cerebrospinal fluid

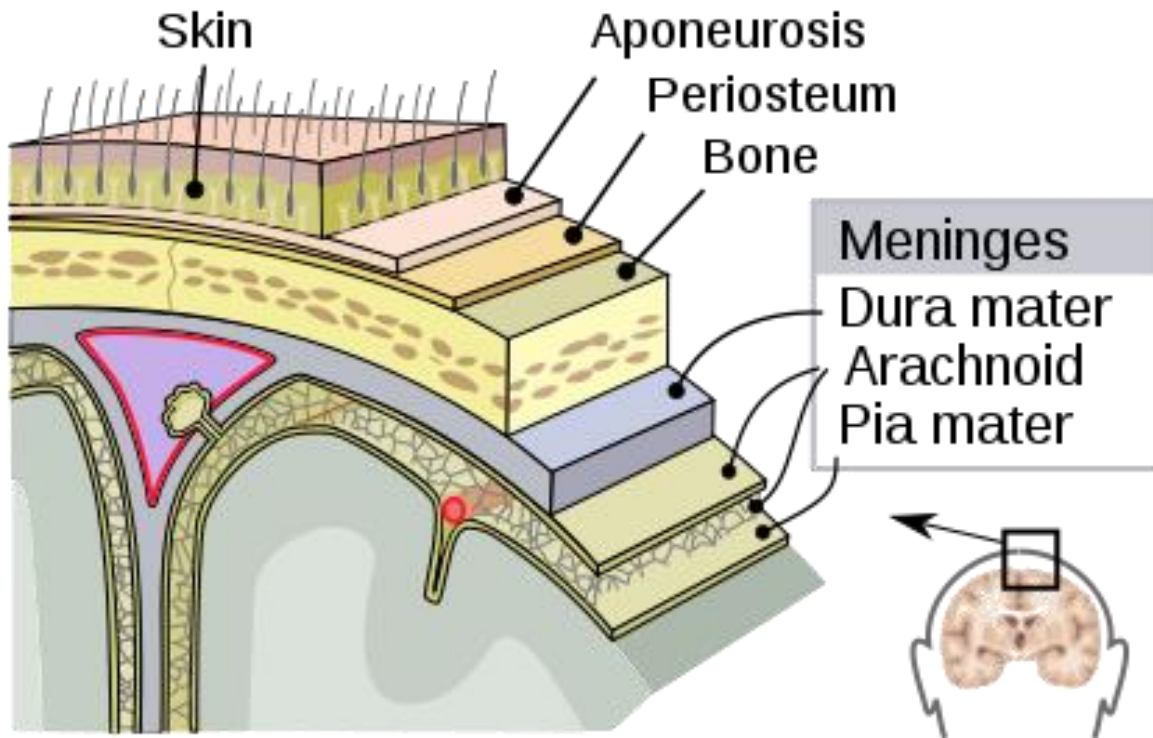


N.Y.C. agar (peptone,  
starch, horse blood)

# *Neisseria meningitidis* - IMD

- incubation time 1 – 8 days; **very sudden** (hrs.)
- most often in **youngsters**
- *N. meningitidis* (in CZ cca 10% asympt. carriers) nasopharynx - blood circulation (fever; endotoxins – headache, joint ache, rash – **septicaemia** and shock, multiorgan failure; letality 25%)
- most severe form: Waterhouse-Friedrichsen sy (DIS)
- +/- **meningitis** – disturbances of consciousness, meningeal symptoms (neck stiffness, vomiting)

# *Neisseria meningitidis* - IMD



1. **dura mater** (thick, connective tissues, veins)
2. **arachnoid** (CSF is beneath)
3. **pia mater** (attached to brain)

# *Neisseria meningitidis* - IMD

- blood, CSF - lumbal puncture, PCR
- th.: *N. m.* v CZ sensitive to **PEN** i.v.
- in case of suspicion of IMD **cephalosporines III.** generation (the cause can be also another bacteria, resistant to PEN)
- **vaccination:** not in compulsory list, but recommended (children 2 months – 2 years; 13 – 15 years)
- tetravaccine (serogroups A, C, W-135 and Y)
- vaccine against serogroup B (variable, covering cca 74%)

# *Neisseria gonorrhoeae* - gonococcus

- yellow-whiteish discharge from penis without erection (gr. gonos = semen)
- G- diplococcus attached on mucosa of urogenital tract
- STD; children (from mother) or because of bad hygiene
- ♂: more often sympt. - urethritis, prostatitis (discharge, burning)
- ♀: often asympt. - urethritis up to cervicitis, ~1 % blood dissemination
- extragenital: conjunctivitis, rectum, nasopharynx (similar to angina)
- th.: ceftriaxone, ciprofloxacin
- incidence, most cases in Prague 23/100 000 (age 15 - 34)

# Class *Gammaproteobacteria*

- family *Enterobacteriaceae*: *Salmonella*, *Escherichia coli*, *Shigella*
- family *Yersiniaceae*: *Yersinia pestis*
- family *Vibrionaceae*: *Vibrio cholerae*
- family *Pseudomonaceae*: *Pseudomonas aeruginosa*



MacConkey agar: bile acids, cryst. viol., pH, lactose

Lac<sup>+</sup>: *E. coli*, *Klebsiella* (pinkish)

Lac<sup>-</sup>: *Salmonella*, *Shigella*, *Pseudomonas*

# Salmonellosis (enteritis)

- diarrheal disease – caused by *S. enteritidis* a *typhimurium*
- anthropozoonosis - contaminated food (insufficiently heat treated eggs, mayonnaise, meat, etc.) - inf. dose  $10^6 - 10^9$
- incub. time 10 - 12 hod. → watery stool, fever, complications with bacteremia and endocarditis
- th.: rehydration, minerals; intestinal disinf., adsorbents
- NO ATB – prolongate excretion of bac. (weeks up to months...)
- incidence CZ: 100/100 000 per year



# *Escherichia coli*

- Theodor Escherich – 1886 – *Bacterium coli* – normal microbiota, but can be pathogenic (G-, motile)
- **ETEC (enterotoxigenic)**: non-invasive, traveller's diarrhea, enterotoxin
- **EIEC (enteroinvasive)**: bloody diarrhea, high fever
- **EHEC (enterohemorrhagic)**: bloody diarrhea, hemolytic-uremic sy.; serotype O157:H7 (antigenes LPS and flagelin)
- **UPEC (uropathogenic)**: 90% IMC – attachment via fimbriae

# Shigellosis (bacterial dysentery)

- acute diarrhea – blood in stool, fever, cramps - very infective
- mostly *Shigella dysenteriae* – G- non-motile bacillus (i.d.:  $10^2$ )
- shiga toxin: necrosis of colon epithelium
- disease of dirty hands: from a patient, contaminated food
- in CZ 400 cases per year - children, summer
- th.: rehydration, ATB (co-trimoxazole, fluoroquinolones)

# *Yersinia pestis*

- G- bacillus, nonmotile, facultative anaerobic
- Virulent proteins encoded in **plasmids**
- facultative intracellular parasite
- cause of **plague** – without ATB therapy high mortality (with therapy cca 10%)
- virulence factors:
  1. **V and W proteins:** encoded in plasmids, septicemia
  2. **Yops:** Yersinia outer proteins – inh. of phagocyte migration, cytotoxicity
  3. **F-1 antigen:** antiphagocytosis
  4. **coagulase and activator of plasminogen:** first forms microthrombus, second enhances hematogenic spread of infection



# Plague

- there are three forms: bubonic, septicemic and pneumonic
- **bubonic**: spread by fleas (*Pulex irritans*, *Xenopsylla cheopis*) from infected rodents (rats) – lumps (buboes) swollen lymph nodes – inflammation, necrosis, gangrene
- to other organs - lungs, blood circulation (disseminated coagulopathy, bacteremia – **septicemic**)
- **pneumonic**: airborne droplets, severe pneumonia



# Plague

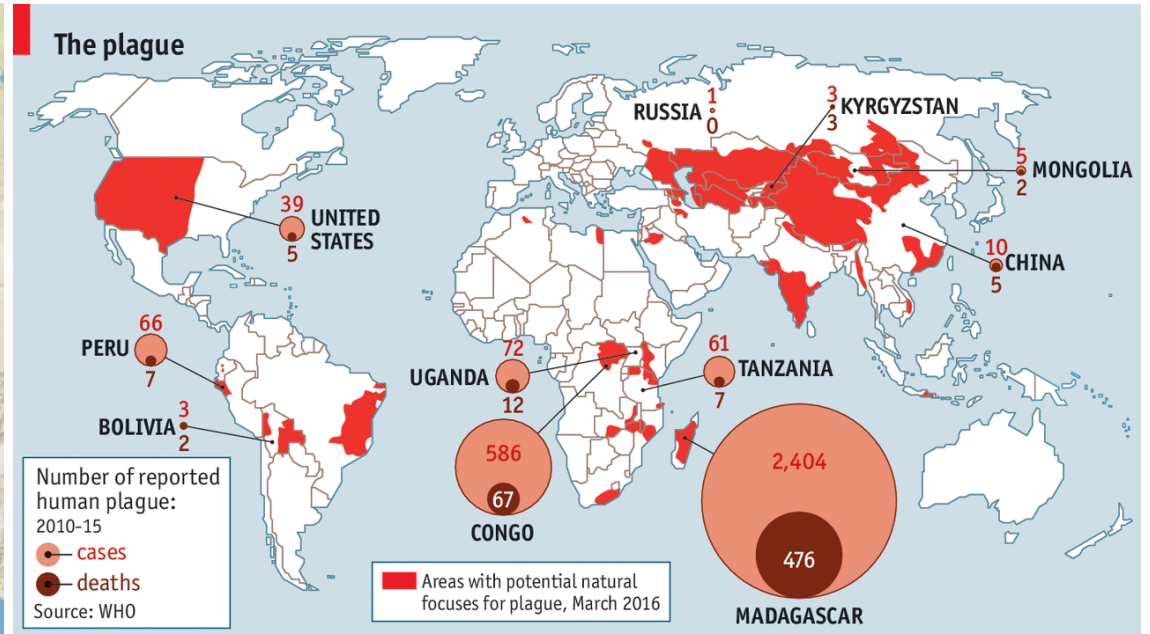


1346 1347 1348 1349 1350 1351 1352 1353

--- Approximate border between the Principality of Kiev and the Golden Horde - passage prohibited for Christians.

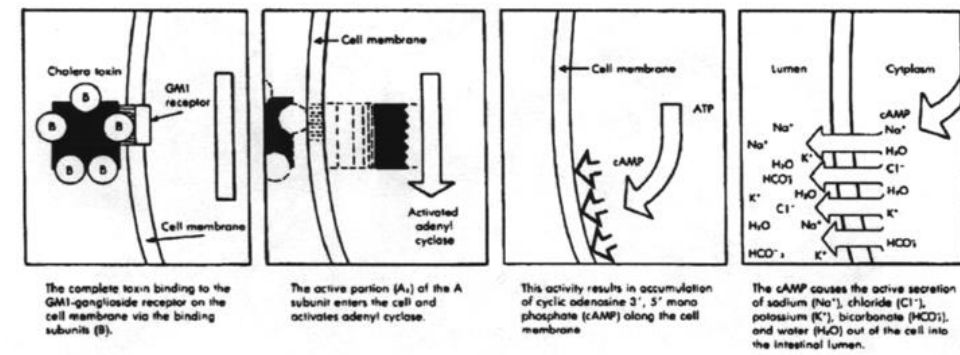
↔ Land trade routes

↔ Maritime trade routes



Economist.com

# *Vibrio cholerae*



- G- bacteria (comma shape) - motile, halophilic, f. anaerobic
- cause of **cholera** (non-invasive; cholera toxin - watery diarrhea "rice water with flakes" - dehydration, ion. disbalance)
- fecal-oral; bad hygiene, contam. water → rehydration, ATB
- prevention: hygiene and sanitation, heat over 60°C
- SE Asia (+Yemen – WHO: very sever, due to war) incidence worldwide 5 milions and 150 000 deaths



*Allivibrio fisheri* – marine bacteria - symbiosis with sepia, bioluminescence, quorum sensing

[https://www.researchgate.net/publication/237485394\\_Milky\\_Seas\\_A\\_New\\_Science\\_Frontier\\_for\\_Nighttime\\_Visible-Band\\_Satellite\\_Remote\\_Sensing](https://www.researchgate.net/publication/237485394_Milky_Seas_A_New_Science_Frontier_for_Nighttime_Visible-Band_Satellite_Remote_Sensing)

# Class *Epsilonproteobacteria*

## *Campylobacter jejuni*:

- G- curved bacillus, microaerophilic, thermophilic (42°C)
- campylobacterial enteritis – incub. time 1 – 7 days
- contaminated food, contact with infected animal
- bloody diarrhea with slime, pain in right lower quadrant
- th.: rehydration, minerals; in severe cases ATB

# Class *Epsilonproteobacteria*

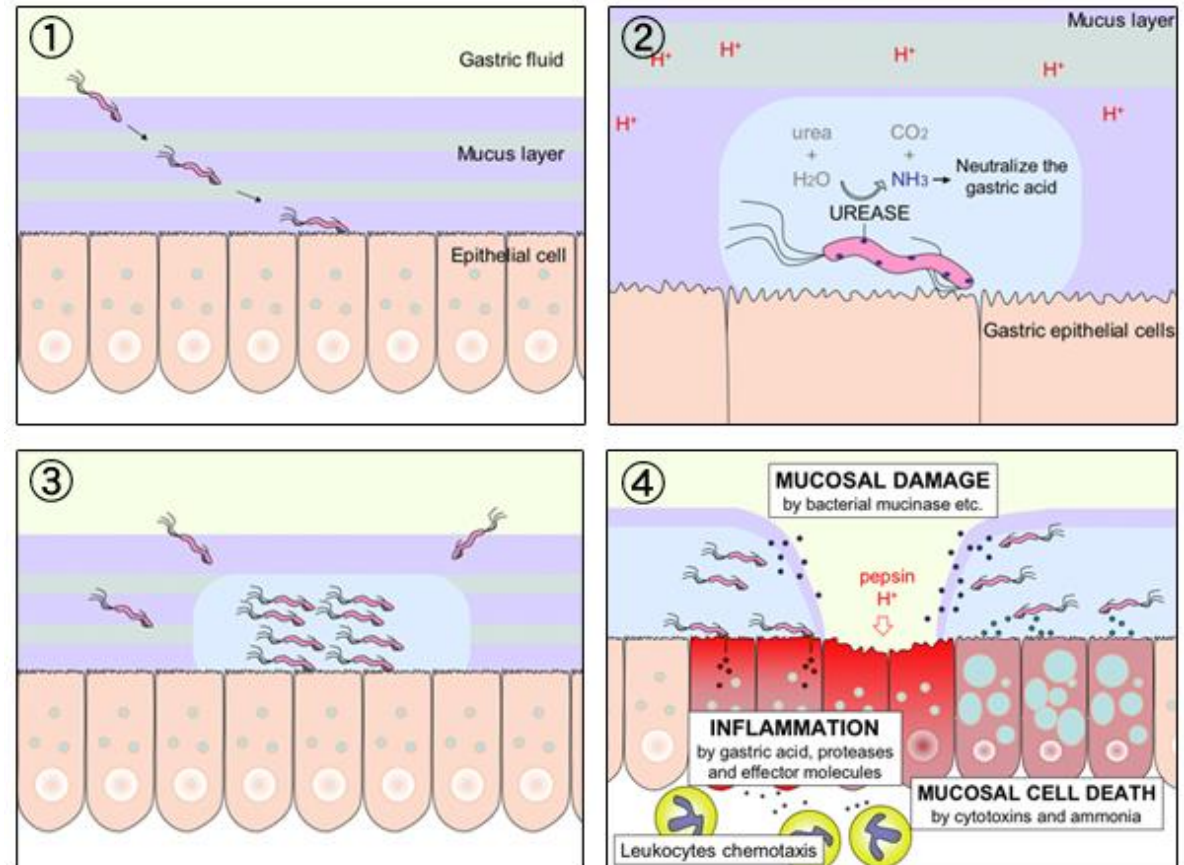
## *Helicobacter pylori*:

- G- **spiral** microaerophilic bacillus; stomach mucosa of humans
- motile; prevalence in population 30 – 55%
- infection in 90% pac. with duodenal ulcer, in 60 – 80% with stomach ulcer
- oro-oral or oro-fecal (mostly infection from mother to child..)
- th.: golden std. triple combination **omeprazol** (PPI) + **ampicilin** + **claritromycine** (or another macrolide)
- if allergy to PEN – metronidazole
- + bismuth nitrate, citrate – quadrutherapy in case of resistance



# Class *Epsilonproteobacteria*

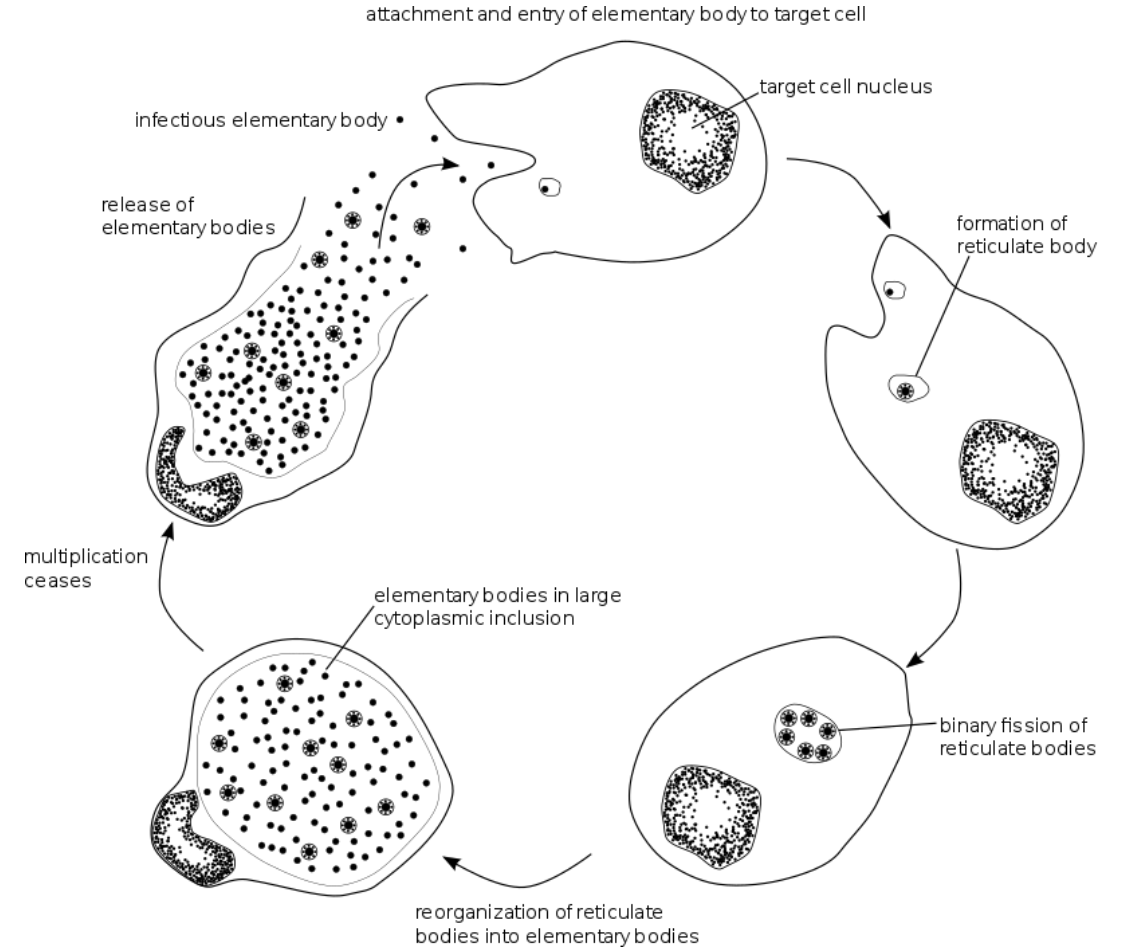
- chron. gastritis
- ulcers (abusus of NSAID, protective/aggressive factors) even cancer
- dg.: antigen *H.p.* in stool; detection of  $^{13}\text{C}$  v breath (labelled urea)



CC Y tambe

# Phylum *Chlamydiae*

- obligate intracellular parasites
- stained as G-; contain peptidoglycan (in past it was denied)
- cause *chlamydiosis*
- life cycles abnormal for bacteria (like viruses) - **reticular bodies** (metab. active, bin. fission), **elementary** (no; but infectious)



# Phylum *Chlamydiae*

*Ch. trachomatis*: STD – urogenital infection, lymphogranuloma venereum (pustule) - proof of inf. after 7 days (ELISA, PCR) - treatment for BOTH partners

*Ch. pneumoniae*: inf. of respiratory tract - atypical pneumonia – spread by contact - ATB (fluoroquinolones)

# Phylum *Spirochaetes*

- stain weakly as G-; cell wall composition similar to diderms (peptidoglycan + outer membrane and LPS-like)
- spiral or helical shape (corkscrew)
- motile: endoflagella attached in periplasmatic space – movement by rotation along the axis or by shrinkage
- clin. important: *Borrelia*; *Treponema*

# *Borrelia burgdorferi sensu lato*

- microaerophilic (cca 2 - 10% O<sub>2</sub>); special media (AAs, nucleotides,..); hosts are arthropods
- 4 – 30 μm long
- cause of **Lyme disease (borreliosis)** spread by ticks *Ixodes ricinus*
- species **complex** encompassing *B. burgdorferi* s. s.(N. Am.), *B. garinii* a *afazellii* (Europe and Asia)
- incomplete metabolic equipment = depend on host
- proteins on surface – **Osp** – variability, escape from IS
- change of morphology – spheroplasts (different proteins)

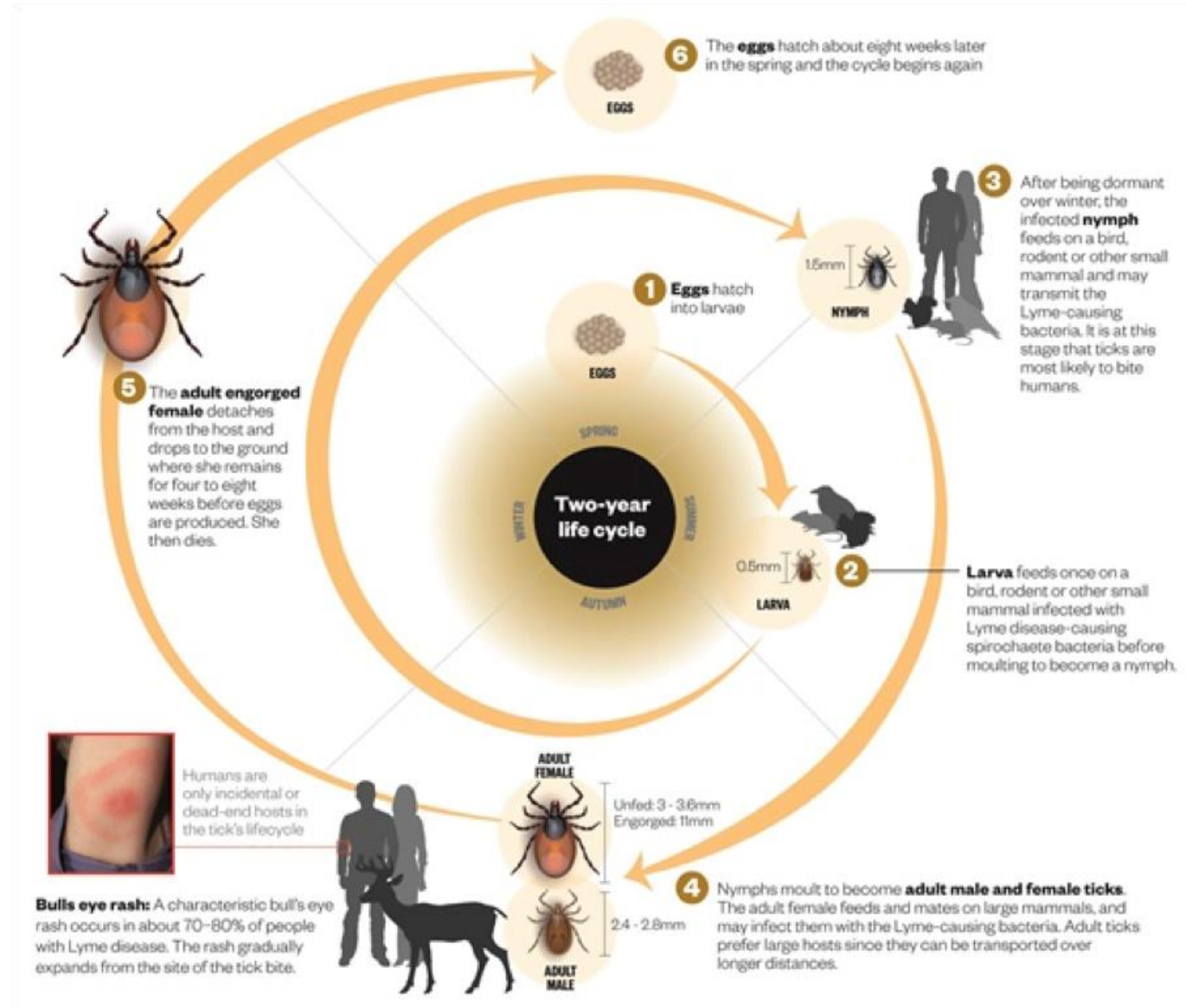
# *Borrelia burgdorferi sensu lato*

- „escape“ from IS into CNS and intracellular persistence
- production of immunosuppressant cytokines



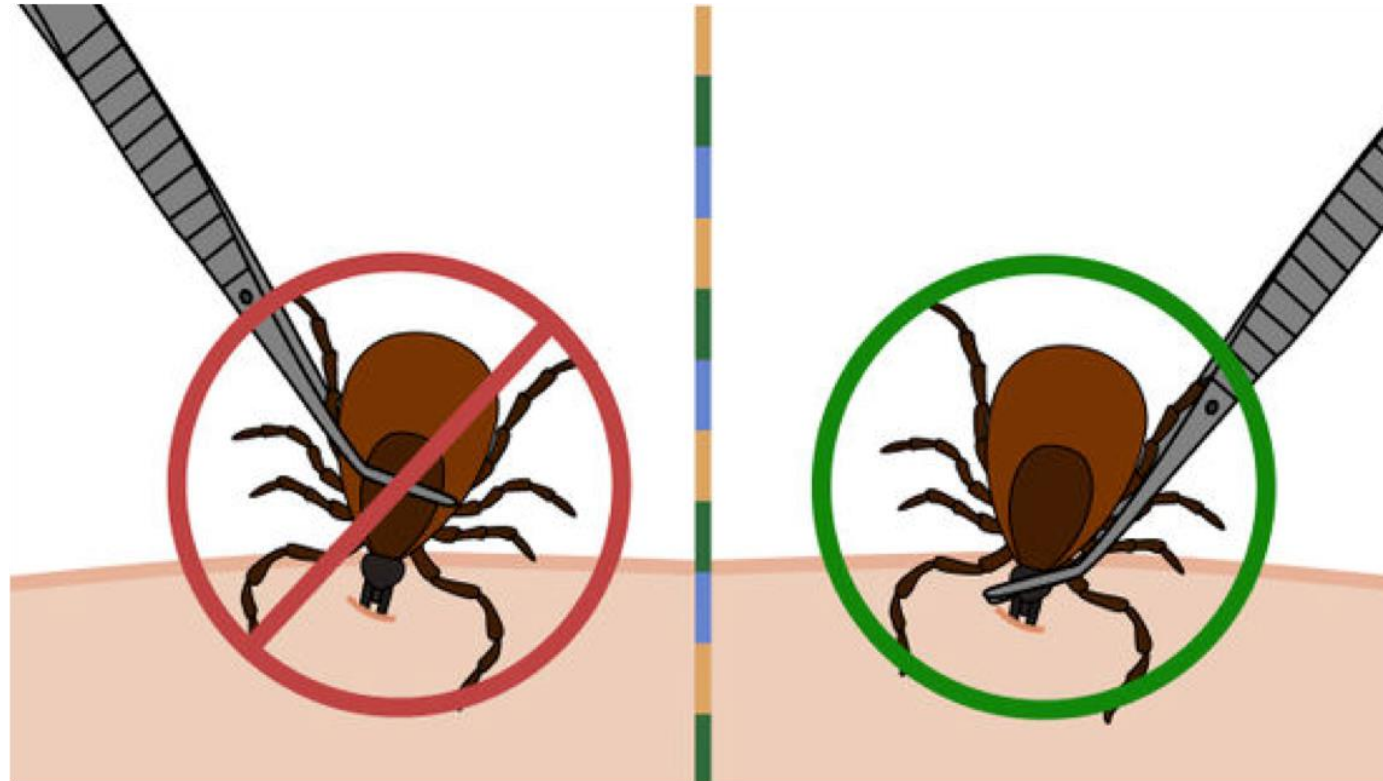
CC James Lindsey

# Life cycle of a tick



<https://www.pharmaceutical-journal.com/news-and-analysis/features/treating-lyme-disease-when-will-science-catch-up/20200978.article?firstPass=false>

# How to remove a tick?

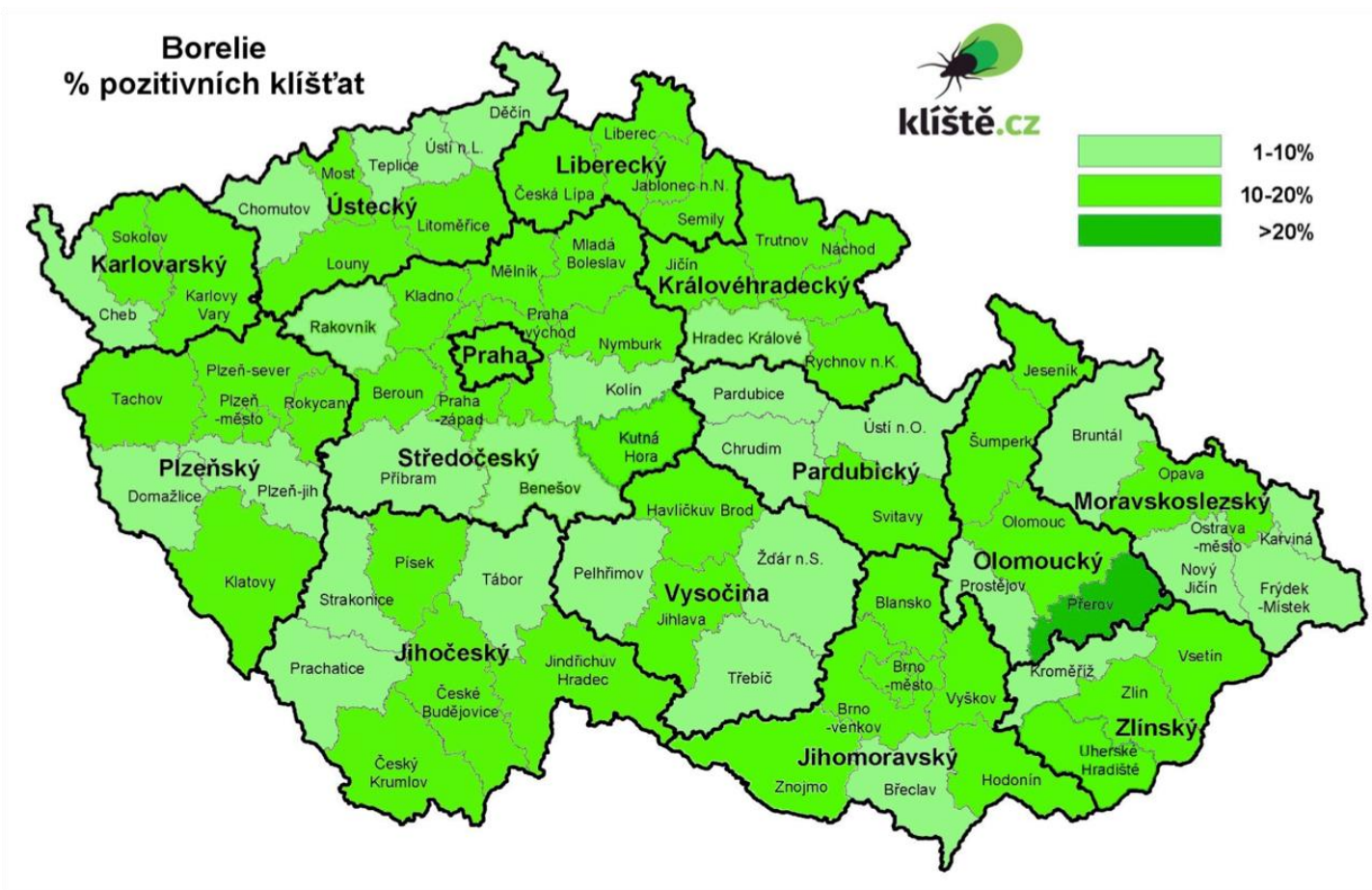


<http://doggonewalking.ca/5-steps-removing-tick/>

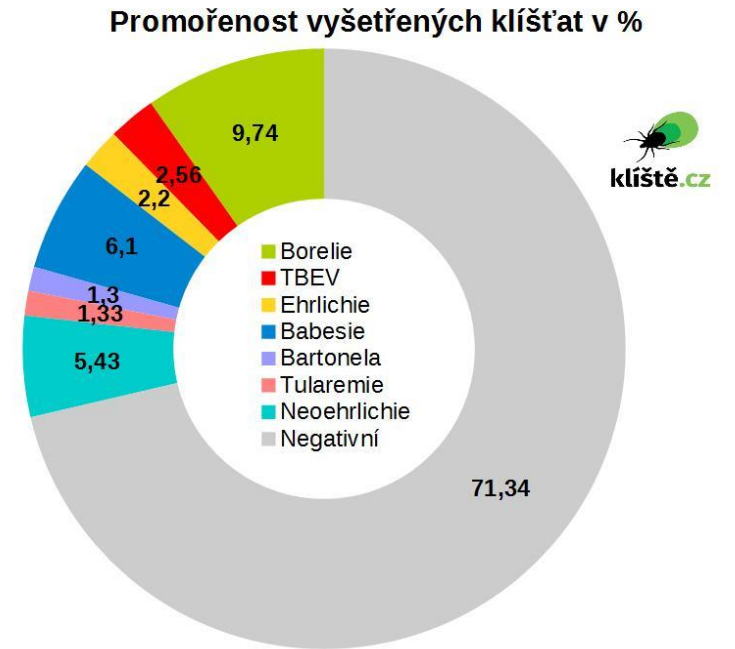


# Ticks

Percentage of positive ticks



Immunity rate of ticks



# Lyme disease

- borrelia transfer to body after 36 – 48 h since bite
- spread to other organs, including CNS

## 1<sup>st</sup> stage (days and weeks after infection):

- erythema migrans („bull's eye“, painless, sometimes absent, disappear and show in different place..)
- "flu-like" symptoms: fatigue, mildness, head-, joint-, backache

## 2<sup>nd</sup> stage (weeks and months):

- borrelial lymphocytoma: purplish lump
- facial nerve palsy; borrelial meningitis



# Lyme disease

## 3<sup>rd</sup> stage (years):

- chronic arthritis, encephalomyelitis
- acrodermatitis chronica atrophicans (degeneration of subcutis)
  
- dg.: **IgM** (3<sup>rd</sup> to 6<sup>th</sup> weak), later **IgG**
- mere antibody positivity is not an indication for ATBs
- cultivation is difficult; PCR of syn. fluid, CSF or skin biopsy
- th.: **penicilines** (amoxiciline+clavulanate), **doxycycline**
- Incidence in CZ: 4000 cases/year
- tick positivity 5 – 10 %