

# Fastidious bacteria

## 2



Illustration: Don Smith

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# Topics

## Fastidious gram negative bacili

- *Francisella*
- *Legionella*
- *Brucella*

## Spirochaetes

- *Leptospira*
- *Treponema*
- *Borrelia*

## Common features

- Intracellular parasites
- Culture is not effective for diagnosis
- Systemic infections
- Vector borne (mostly)
- Zoonosis (mostly)

## Rickettsia and rickettsia-like organisms

- *Rickettsia*
- *Anaplasma*
- *Ehrlichia*
- *Bartonella*
- *Coxiella*

# Diagnosis of rare fastidious bacteria is challenging

When infection caused by fastidious pathogen occurs rarely outcome could be worse because:

1. Looking for non fastidious pathogens delays detection of true cause
  - Pathogen will be detected after ruling out more common causes
2. Non fastidious pathogen is taken as true cause
  - Treating wrong pathogen
3. Not using appropriate method
  - Pathogen would not be detected at all

It is important to know fastidious pathogens and their typical symptoms and **risk factors** – it could be life-saving knowledge

# Logistical challenge

Be aware that not all microbiology departments perform detection of fastidious agents

1. It is too rare to be economical to have appropriate method
2. It is too laborious/expensive...
3. It is dangerous (highly infectious agents)

Detection or confirmation of detection of some fastidious agents is performed only by specialised/reference laboratories

# *Francisella tularensis*

## Biology:

Aerobic gram-negative coccobacilli  
facultative intracellular bacterium

Subspecies:

*F. tularensis tularensis*

North America – more virulent

*F. tularensis holarctica*

Europe and Asia – less virulent

Potential bioterrorism agent

## Ecology

Zoonosis

Reservoir: wild animals (rabbits,  
rodents, deer, birds, etc.)

Survive also in water, grass, haystacks,  
animal carcasses

Transmission (entry)

- Respiratory system
- Blood
- Skin contact

Low infection dose = highly infectious

BSL-3

# *Francisella tularensis*

## Clinical significance

Tularemia

Ulceroglandular – through skin contact

Pulmonary

## Dg:

Culture requires cystein for growth

CO<sub>2</sub> and prolonged cultivation  
35–37 °C

Chocolate agar

Symptoms and patient history  
(contact with dead wild animals)

## Rx:

aminoglycosides,  
tetracyclines, or  
fluoroquinolones



*F. tularensis* on chocolate agar

# BIOSAFETY LEVELS

basic classes of laboratory risks from low to high



BSL-1



BSL-2



BSL-3

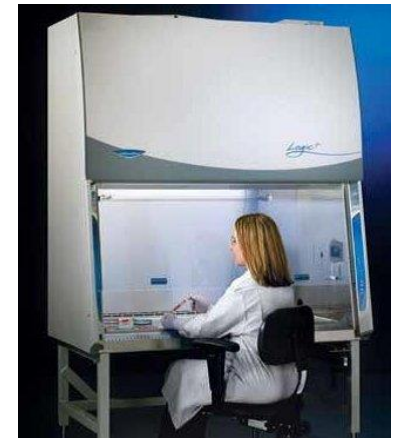


BSL-4

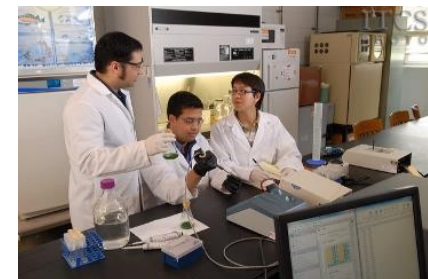


Biosafety Levels			
Biological Safety Levels	Description	Examples	CDC Classification
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	<p>high-risk microbes</p> <p>low-risk microbes</p>
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	

BSL 3 - 4

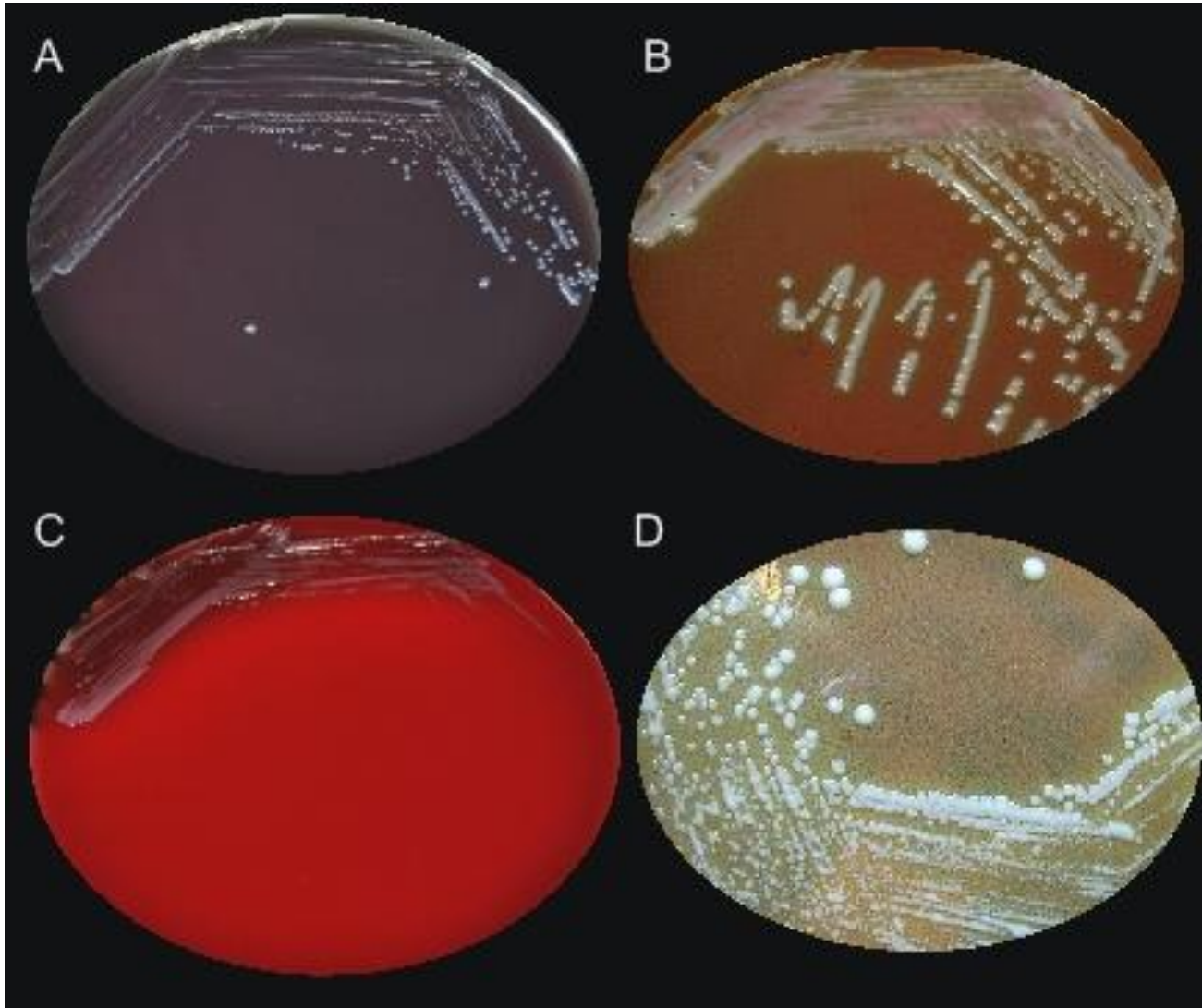


BSL 2



BSL 1

# *Francisella tularensis* - culture



- (A) buffered charcoal yeast extract;
- (B) chocolate agar medium;
- (C) sheep's blood agar;
- (D) cysteine heart agar.

Pathogen profile dictionary  
ppdictionary.com



# Brucella

## Biology:

Strictly aerobic gram-negative cocobacilli  
facultative intracellular bacterium

Species:

*B. abortus* (cattle)

*B. melitensis* (sheep and goat)

*B. suis* (pig)

*B. canis* (dog)

survive in soil, manure and dust  
for weeks or months, and remain viable  
in dead fetal material for even longer

## Ecology

Zoonosis

Reservoir: animals (sheep, cattle, or  
pigs, etc.)

Transmission

- contaminated food (such as unpasteurized milk products, meat products)
- direct contact with an infected animal
- inhalation of aerosols

Low infection dose - level BSL-3

*B. melitensis* is still prevalent in  
Mediterranean countries, the Middle  
East, central and southern Asia, and  
parts of Africa and South America

# Brucella

## Clinical significance

Brucellosis

septicaemic illness, undulant fever. Most human disease is caused by *Brucella melitensis*, *B. abortus* or *B. suis*.

Brucellosis can present as an acute or subacute pyrexial illness that may persist for months or develop into a focal infection that can involve almost any organ system. The characteristic intermittent waves of increased temperature that gave the name *undulant fever* to the human disease are now usually seen only in long-standing untreated cases.

Affects gastrointestinal tract including anorexia, abdominal pain, vomiting, diarrhea, constipation, hepatomegaly, and splenomegaly

Less frequently arthritis (hip, knee, and ankle), spondylitis, osteomyelitis, and sacroiliitis

Rarely endokarditis

Infection in animal has economical impact – in pregnant animals often leads to abortion,

# Brucella

## Dg:

isolation of the organism from blood (blood culture); alternatively serology or PCR

Culture: some species require CO<sub>2</sub>, media with glucose and animal serum

37°C 1 to 6 weeks



***Brucella melitensis***,  
blood agar

Rx: tetracycline, usually in combination with an aminoglycoside or rifampicin

Prevention –  
pasteurization of milk

# Legionella

## Biology:

Gram-negative rods

*L. pneumophila*

Other species less frequent

e.g. *L. longbeachae*

## Epidemiology:

Frequent in water – intracellularly in amoebas (*Acanthamoeba*, *Naegleria*) and other protozoa

Warm water sources

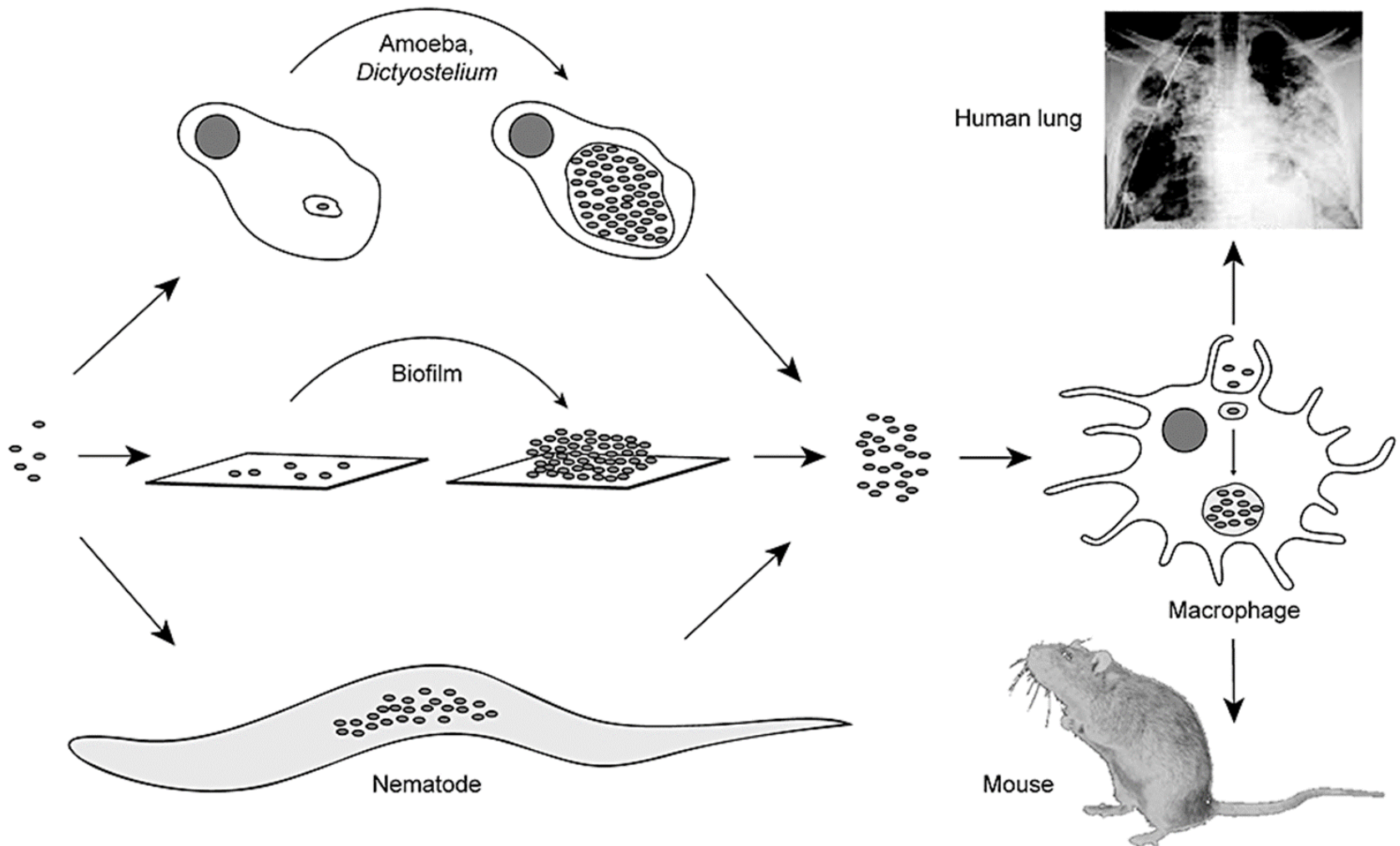
Spread through aircondition and untreated water supply

Inhaling aerosol or droplets containing legionella

Inhaled bacteria are engulfed by monocytes and can survive therein as intracellular parasites



# Legionella



# Legionella

## Clinical significance:

**Legionnaires disease** (mostly *L. pneumophila* serogroup 1)

pneumonia, up to 10% mortality when not treated

high fever, respiratory distress, scanty sputum

confusion, hallucinations

Renal impairment could be present

Potential to cause outbreak (hospitals!!!) –

contaminated water supply

Risk factors: immunosuppression, higher age (over 40y)

Stays in hotels in low income countries

**Pontiac fever** – non-pneumonic, non-fatal, influenza-like symptoms, high attack rate – most of the affected people develop disease

## History

1976 first outbreak of 182 cases of pneumonia, mainly affecting members of the American Legion,

occurred at a convention in Philadelphia = legionnaires disease

Spread through air condition



# Legionella

## Dg:

**Sample:** sputum and other respiratory specimen, lung biopsy – PCR or culture

require cystein and iron grow best on buffered charcoal yeast extract agar (BCYE) with antibiotics

Culture about 1 week, increased CO<sub>2</sub>

Heat stable – sample could be heated to 50°C for 30 min to diminish growth of other bacteria

ATB testing is not performed (too laborious)

**Antigen detection in urine** (ELISA) – commonly used,  
Serology – IG could be detected 8-10 days from start of the symptoms

## Prevention

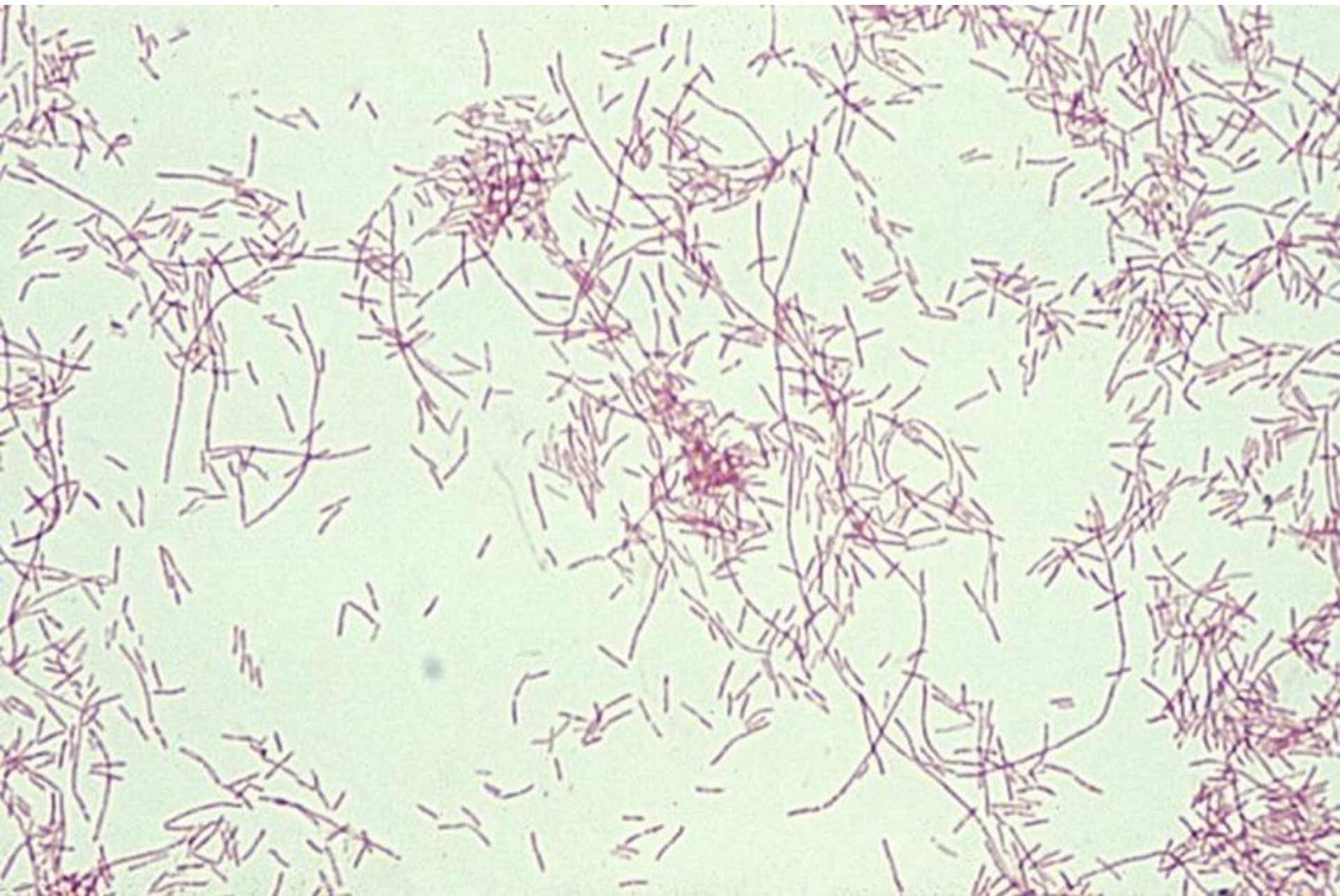
Water treatment:

- heat
- disinfection with chlorine or other biocides, including chlorine dioxide
- copper–silver ionization.

## Rx:

Legionnaires disease – intravenous azitromycin, combined with fluoroquinolones and/or rifampicin in severe cases

***Legionella pneumophila***, Gram stain  
By courtesy of MUDr. Petra Kabelíková





***Legionella  
pneumophila***

BCYE agar

Courtesy of  
MUDr. Petra  
Kabelíková



*Spirochaetes*

# Spirochaetes

Borrelia

Treponema

Leptospira

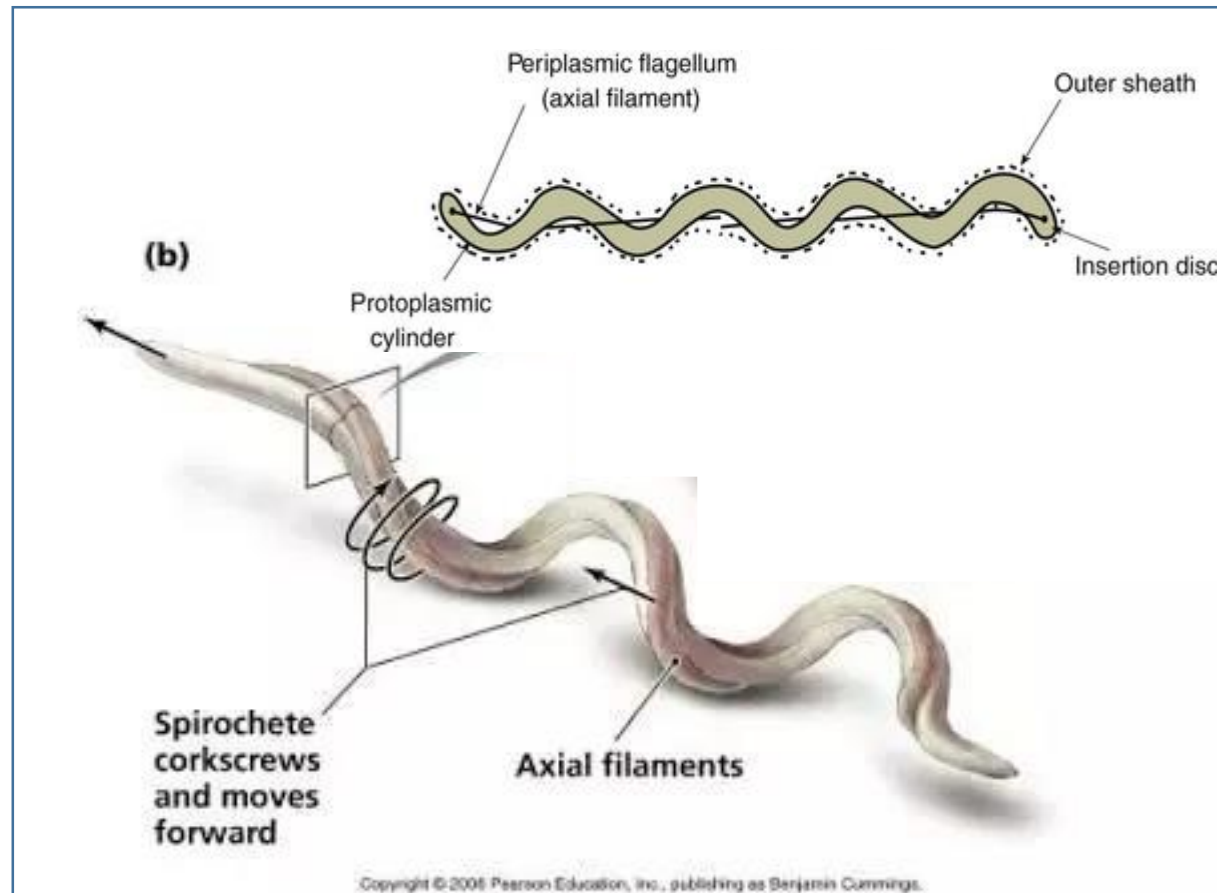
## Biology:

Helical corkscrew –shaped bacteria

Close to gram negatives – have outer and inner membrane but differently organised

flagella in periplasmic space – corkscrew-like movement = tissue penetration

Too thin to be seen by light microscope  
- Dark field microscopy or immunofluorescence



# Different shapes of spirochaetes

*Borrelia*  
and *Brachyspira*



*Leptospira*



*Treponema*



# Borrelia

## Biology:

Microaerophilic spirochaetes

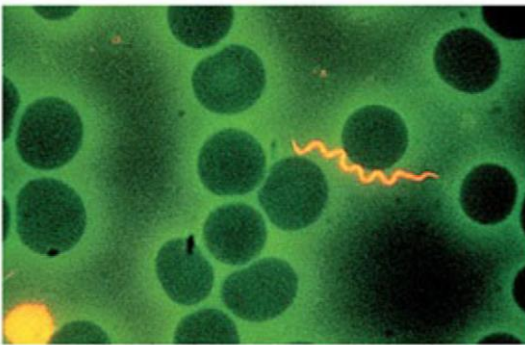
Poorly stained by Gram

*B. burgdorferi sensu lato*

complex of related species:

- *B. burgdorferi sensu stricto*  
– USA and West EU
- *B. garinii*, *B. afzelii* - CZ

*B. recurrentis*



Acridine orange stain of peripheral blood demonstrates *Borrelia* spp.

## Epidemiology:

arthropod vector - Lice or ticks

Relapsing fever (*B. recurrentis*)

– ticks (*Argasidae*) or lice (*Pediculus humanus*)

Lyme disease (*B. burgdorferi*)

- Ixodes ticks - *I. dammini* and *I. pacificus* (USA); *I. ricinus* (EU)



*Ixodes ricinus* developmental stages

# Lyme disease (Lyme borreliosis)

*B. burgdorferi* sensu lato

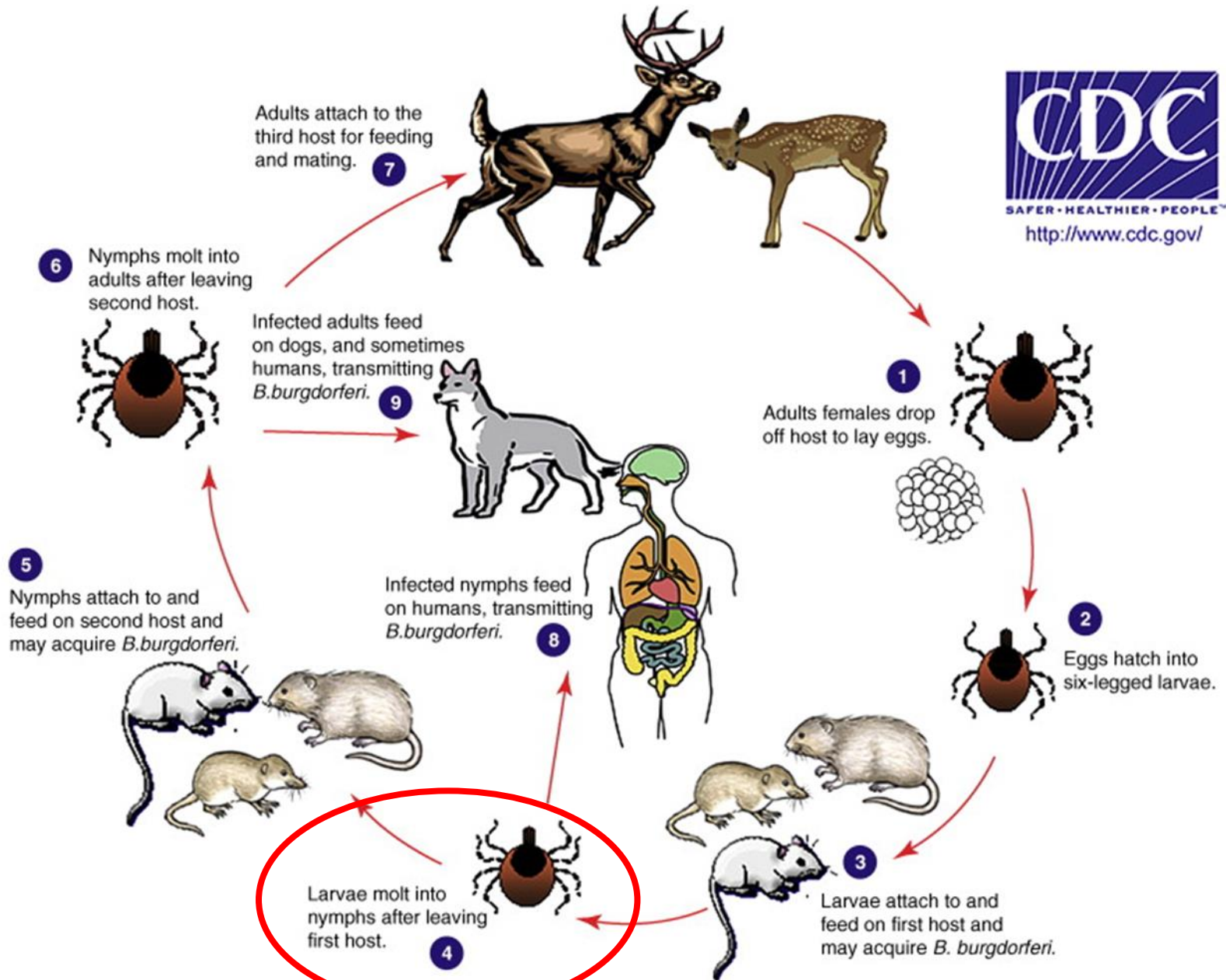
- Reservoir mice and other rodents, deer, sheep, cattle, horses and dogs.
- Transmission through tick bite (*Ixodes* sp.)
- Multisystem infection
  - Non specific symptoms at the beginning
  - Affects skin, joints, heart, and CNS
  - Three stages



Centers for Disease Control and Prevention, <http://phil.cdc.gov/phil/>

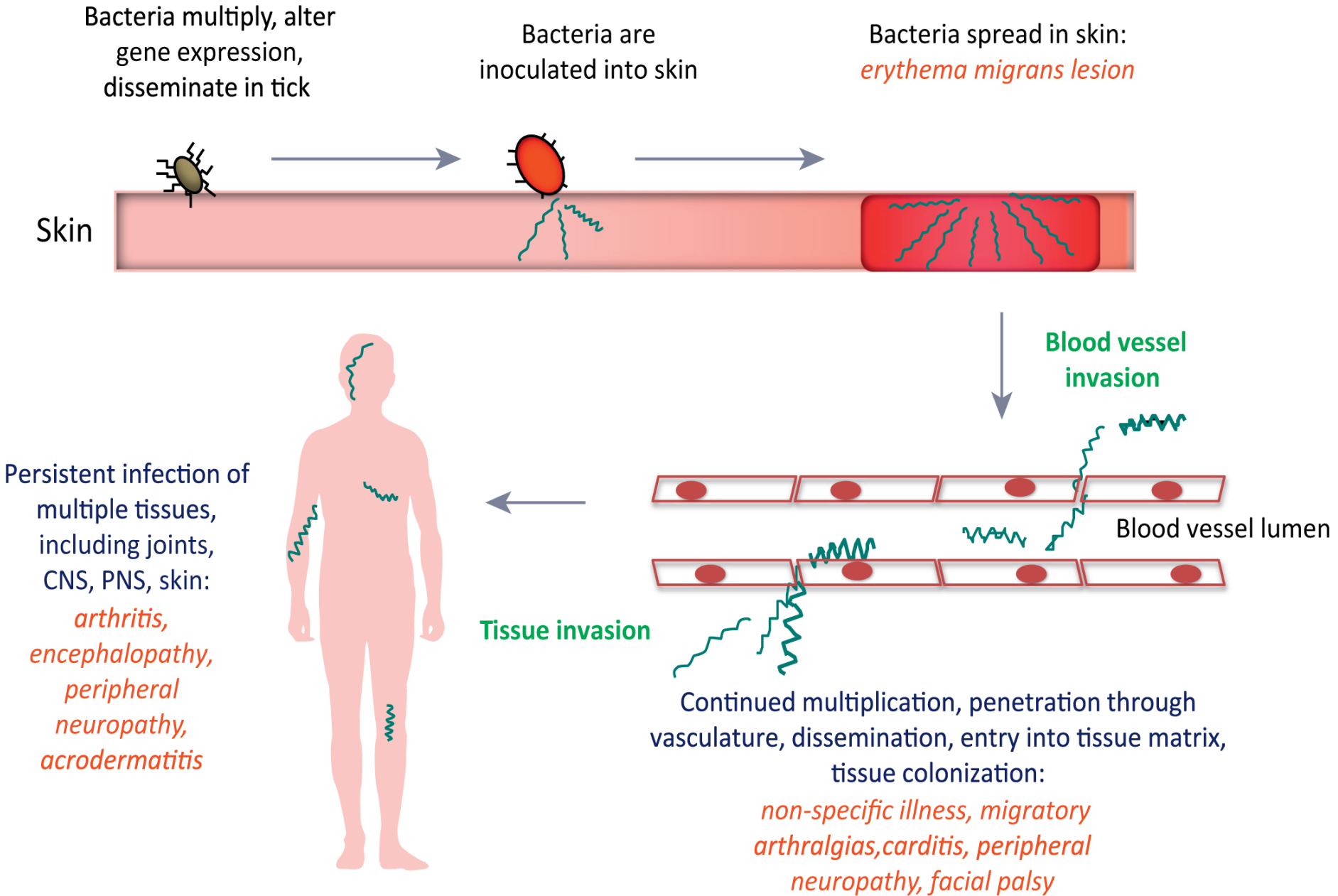
Erythema migrans

Typical symptom but only in about 30% cases



**Nymphs are most common vector in human Lyme disease**

# Lyme disease - pathogenesis





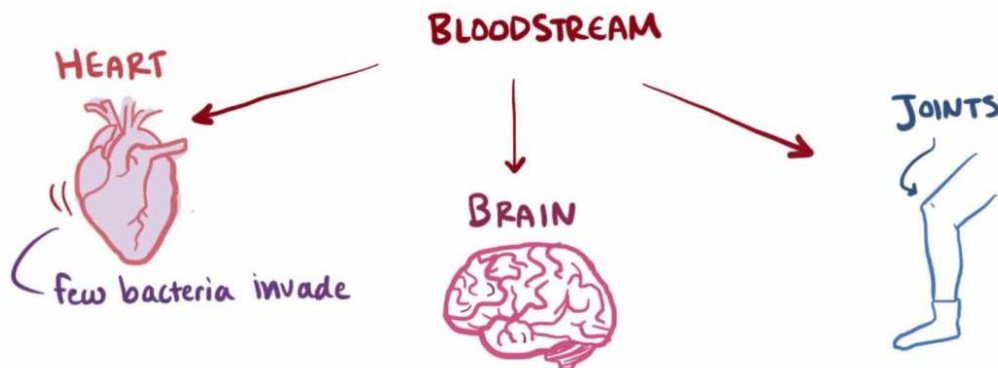
# 3 Stages of Lyme Disease

Early Lyme Disease	Erythema migrans	<ul style="list-style-type: none"> <li>• Most characteristic clinical manifestation</li> <li>• Well demarcated, flat-bordered, blanching erythematous oval patch</li> <li>• Hematogenous spread leads to secondary lesions</li> </ul>
	Constitutional symptoms	<ul style="list-style-type: none"> <li>• Fatigue, myalgia, fever</li> </ul>
	Meningeal irritation	<ul style="list-style-type: none"> <li>• Headache</li> </ul>
	Gastrointestinal symptoms	<ul style="list-style-type: none"> <li>• Hepatitis</li> <li>• Pharyngitis</li> </ul>
Acute Disseminated Infection	Cutaneous	<ul style="list-style-type: none"> <li>• Multiple annular/target-shaped lesions (Early)</li> </ul>
	Neurologic manifestations	<ul style="list-style-type: none"> <li>• Meningoencephalitis</li> <li>• Cranial neuropathy (Bell palsy) (Bilateral in 33%)</li> <li>• Radiculopathy</li> </ul>
	Cardiac manifestations	<ul style="list-style-type: none"> <li>• Occurs 3-5 weeks from erythema migrans</li> <li>• AV block</li> </ul>
Late Lyme Disease	Neurologic manifestations	<ul style="list-style-type: none"> <li>• Fatigue</li> <li>• Chronic encephalopathy</li> <li>• Memory impairment</li> <li>• Hypersomnolence</li> <li>• Psychiatric disturbances</li> </ul>
	Arthritis	<ul style="list-style-type: none"> <li>• Most often affects the knee, can be oligoarticular</li> <li>• Can lead to chronic Lyme arthritis (recurring arthritis)</li> </ul>

Within one month  
Dx: antigen in CSF  
or PCR from CSF or  
skin biopsy

1 to 4 months  
Dx: IgG and IgM in  
serum and CSF

More than 4 months  
Dx: IgG and IgM in  
serum and CSF



Rx: doxycykline,  
amoxicillin, ceftriaxone  
Duration from 2 week  
up to 1 month

# Relapsing fever (typhus recurrentis)

- *Borrelia recurrentis*
- Human is only reservoir
- Transmission: human louse (*Pediculus humanus*)
- Associated with poor hygiene conditions (low income countries, homeless people,...)
  
- Symptoms: Fever returning each 5-10 days, hepatosplenomegaly, ikterus
- Dx: Serology
- Rx: doxycycline

# *Treponema pallidum*

## **Biology:**

Anaerobic to microaerophilic spirochaetes

Intracellular pathogen

Poorly stained by Gram

Sensitive to temperature above 40°C, oxygen etc.

Transmission only through direct contact

Only human to human

Sexually transmitted

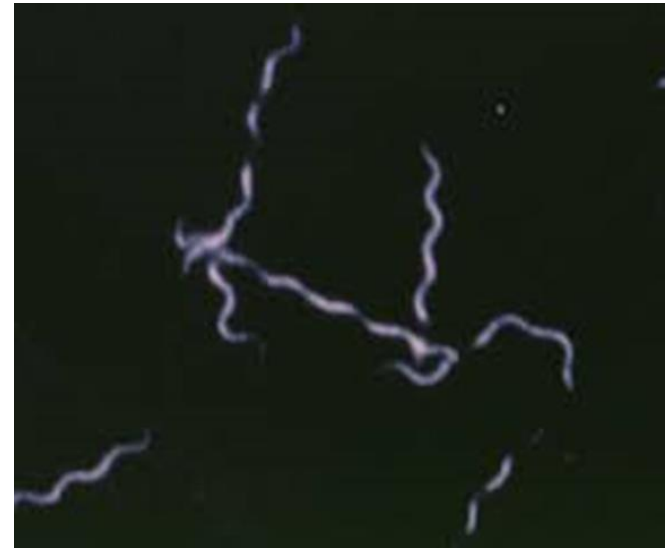
congenital

Subspecies

*T. pallidum pallidum* – cause syphilis

*T. p. endemicum* – bejel or endemic syphilis

*T. p. pertenue* - yaws



*T. pallidum* darkfield microscopy

# *Treponema pallidum*

## Clinical significance:

Syphilis – STD

Penetration through skin lesions or through mucous membranes

Tissue destruction due to immune response

Forms/stages

### 1. Primary

Localised necrosis and ulceration (ulcus durum)  
lymphadenopathy, highly infectious detectable Ig

### 2. Secondary

Skin, mucous epithelia and systemic symptoms,  
CNS (encephalitis), highly infectious, detectable Igs

### 3. Latent – non infectious live long stage

### 4. Tertiary – after 10 to 25 y - cardiovascular and CNS (neurosyphilis) symptoms progressive paralysis

- Congenital

## *Treponema pallidum* subsp. *pallidum*

Stage		Time period	manifestation	diagnostics
early	<b>primary</b>	weeks	ulcum durum and bubo	microscopy, PCR, antibodies
	<b>secondary</b>	weeks - months	Generalisation: skin, condylomata lata	antibodies
	latent		1 year (2 yrs)	none
		many years	none	antibodies
late	<b>tertiary</b>		Organs: neurosyphilis, cardiovascular, gumma	antibodies

# Treponema pallidum

## Dg:

Sample

Urine

Swab (urethra, cervix, vagina)

Swab from skin lesions

Serum

## Methods

Microscopy dark field or fluorescent antibody staining.

PCR – only early stage

## Serology

Nontreponemal (reagin) antibodies

Venereal Disease Research Laboratory (VDRL) test – CSF, flocculation, agglutination

Rapid plasma reagin (RPR) test

Treponemal antibodies

*T. pallidum* indirect hemagglutination (TPHA), agglutination

*T. pallidum* particle agglutination (TP-PA) test, agglutination

ELISA

Treatment - Penicillin

## Prevention

Mandatory screening newborns etc

Monitoring of STDs

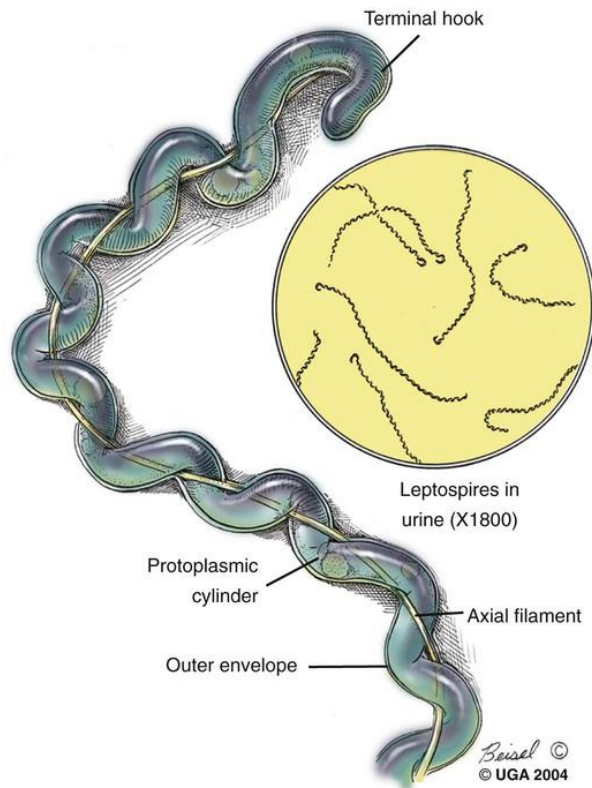
# Leptospira

## Biology:

Obligate aerobic pathogenic or saprophytic spirochaetes

Stable in water environment

*Leptospira interrogans*



## Epidemiology:

Zoonosis

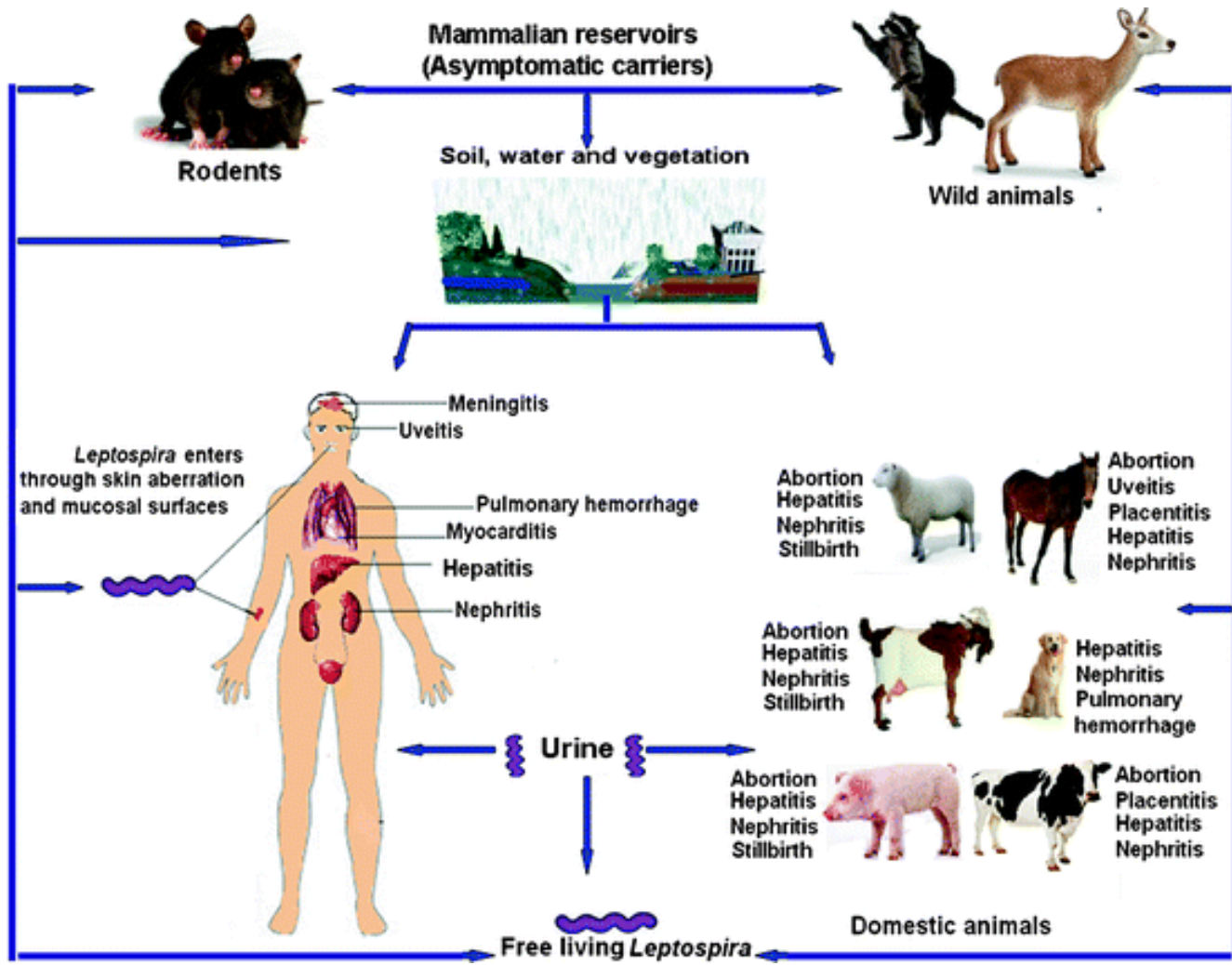
Reservoir wild animals (mammals rodents, etc), lives in kidneys of animals (without causing illness), released with urine

Pathogenic *Leptospira* do not multiply in the environment, but could survive in humid environments like stagnant water or contaminated soil.

Entry through:

Skin abrasion, wound, ingestion, mucous membrane (e.g. Mouth, nose, eye)





Faisal S.M., McDonough S.P., Chang YF. (2012) *Leptospira*: Invasion, Pathogenesis and Persistence. In: Embers M. (eds) *The Pathogenic Spirochetes: strategies for evasion of host immunity and persistence*. Springer, Boston, MA. [https://doi.org/10.1007/978-1-4614-5404-5\\_8](https://doi.org/10.1007/978-1-4614-5404-5_8)



# Leptospira - risk factors

Risk:

- farmers, fishermen, garbage collectors and sewage workers
- Outdoor sport
- floods



# Leptospira

## Clinical significance:

Leptospirosis

Bacteria penetrates bloodstream and spread through body

vary from flu-like to severe illness

=Weil's disease (meningitis, renal failure, icterus, bleeding, could affect liver, shock)

Rx: intravenous penicillin G for severe cases, amoxicillin, doxycycline

## Dg:

Sample: serum, urine, CSF, autopsy

Cultivation is possible pathogenic species grow worse than saprophytic

EMJH medium with rabbit serum, 30°C, 4 - 7 days

## **PCR**

Serology – IgM ELISA

Antigen detection - Latex agglutination etc

**Microscopic agglutination test (MAT)** is the reference test for the diagnosis of leptospirosis patient sera are mixed with different serovars of *Leptospira* – agglutination with IG is observed under microscope

## Symptoms:

### Sources



Rash



Headache



High Fever



Abdominal Pain



Red Eyes



Vomiting



Muscle Pain



Jaundice



Cold



Diarrhea

# *Rickettsia* and related pathogens

Rickettsiales

Rickettsia

Anaplasma

Ehrlichia

Bartonella

Coxiella

# Rickettsia in general

## Biology:

Gram-negative, highly pleomorphic bacteria, obligate intracellular pathogens

Small genome

Primary human pathogen

Sensitive to environmental changes

Spotted fever group

*R. rickettsii*

*R. akari*

Typhus group

*R. prowazekii*

*R. typhi*

## Epidemiology

zoonoses

Animal or human host arthropod vector

Penetrates through skin into blood stream, infects endothelium of bloodstream vessels in multiple organs

## Dg:

Symptom based suspicion

Serology: Weil–Felix test

Mixing patient serum with rickettsial antigen

When antibodies present agglutination appears

PCR

Rx: tetracyclines - doxycycline

# Spotted fever group

## *Rickettsia rickettsii* (Western Hemisphere)

- Rocky Mountain spotted fever
- Tick-borne *Dermacentor* sp = Dog tick
- Reservoir dogs and rodents
- Infection in children during spring (tick activity)
- Sudden fever, headache, myalgia
- Typical rash spreading from limbs to trunk
- Fatal if untreated



Petechial rash on the arm caused by Rocky Mountain spotted fever

## *Rickettsia akari* (USA, former Soviet Union)

- Rickettsialpox
- Mite-borne *Liponyssoides* sp
- Reservoir mice
- Mild disease, nonfatal, self-limiting
- Vesicular rash, fever, lymphadenopathy



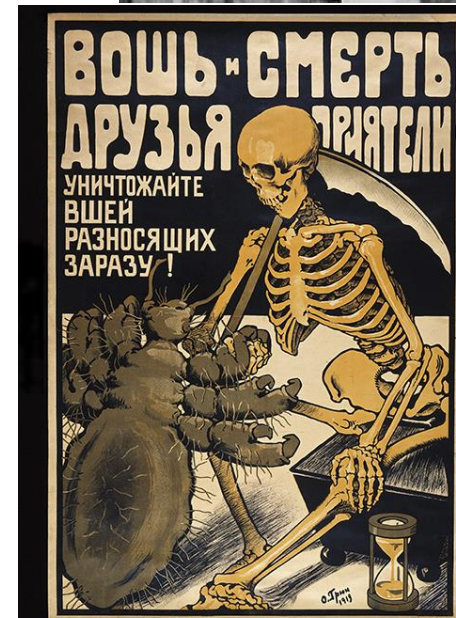
Rickettsialpox rash



# Epidemic/recrudescent typhus



- *Rickettsia prowazekii*
- Vector – human body louse (*Pedicullus humanus*)
- War and poverty
- Africa, South america
- Symptoms: Chills, fever, headache, pain, stupor and delirium
- Signs f severe meningoencephalitis begin with rash
- Rash spreads from trunk to limbs
- Untreated is fatal
- In some people reactivity after many years – recrudescent typhus (Brill-Zinsser disease) – milder symptoms



# Murine/endemic typhus

- *Rickettsia typhi*
- Vector flea (*Xenopsylla* sp)
- Reservoir: Rats
- Symptoms: gradual onset of fever, chills severe headache, generalized pain
- Macular rash spreading from trunk to limbs



# *Ehrlichia* and *Anaplasma*

## Biology:

Obligate intracellular pathogens  
Preference to WBC – mononuclear cells

## Clin. significance

Human monocytic ehrlichiosis (HME)  
*Ehrlichia chaffeensis*  
USA

Human granulocytic ehrlichiosis  
*Anaplasma phagocytophilum*  
USA but also EU and Asia

## Epidemiology

Zoonosis  
Tick borne, reservoir: wild animals and dogs

## Dg:

PCR (blood)  
Serology

Rx: doxycycline



# Bartonella

## Biology:

Gram-negative small rods or cocobacilli

Facultative intracellular parasites

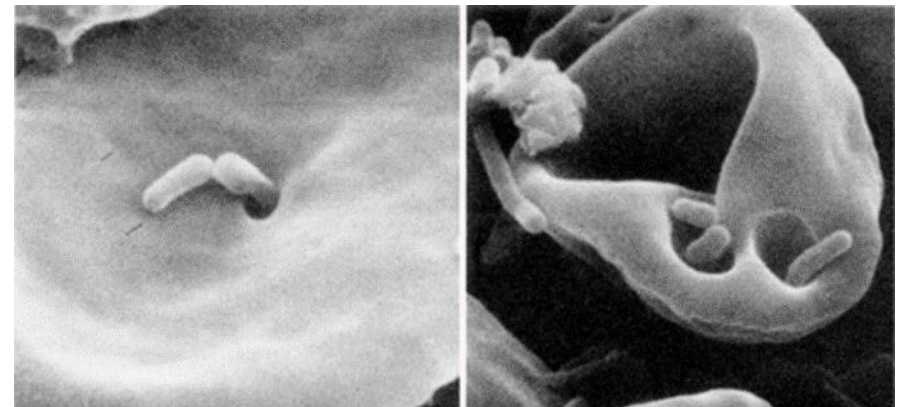
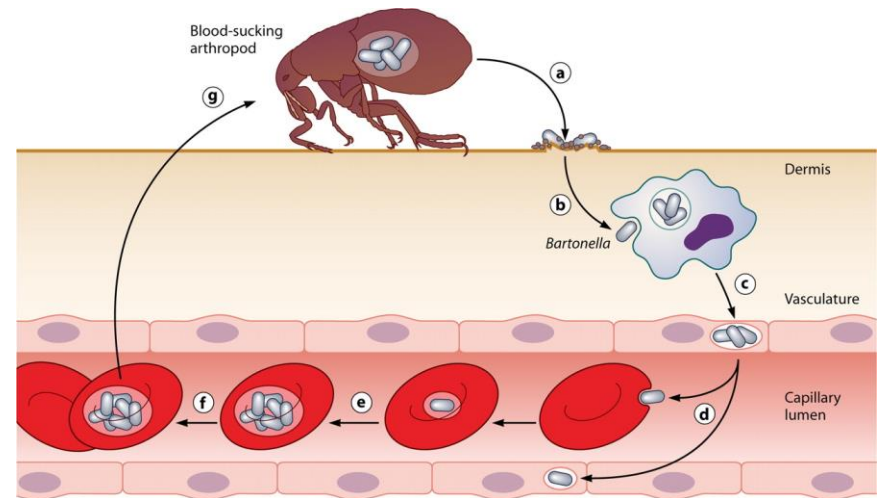
*B. henselae*, *B. quintana*, *B. bacilliformis*

Zoonosis (*B. henselae*)

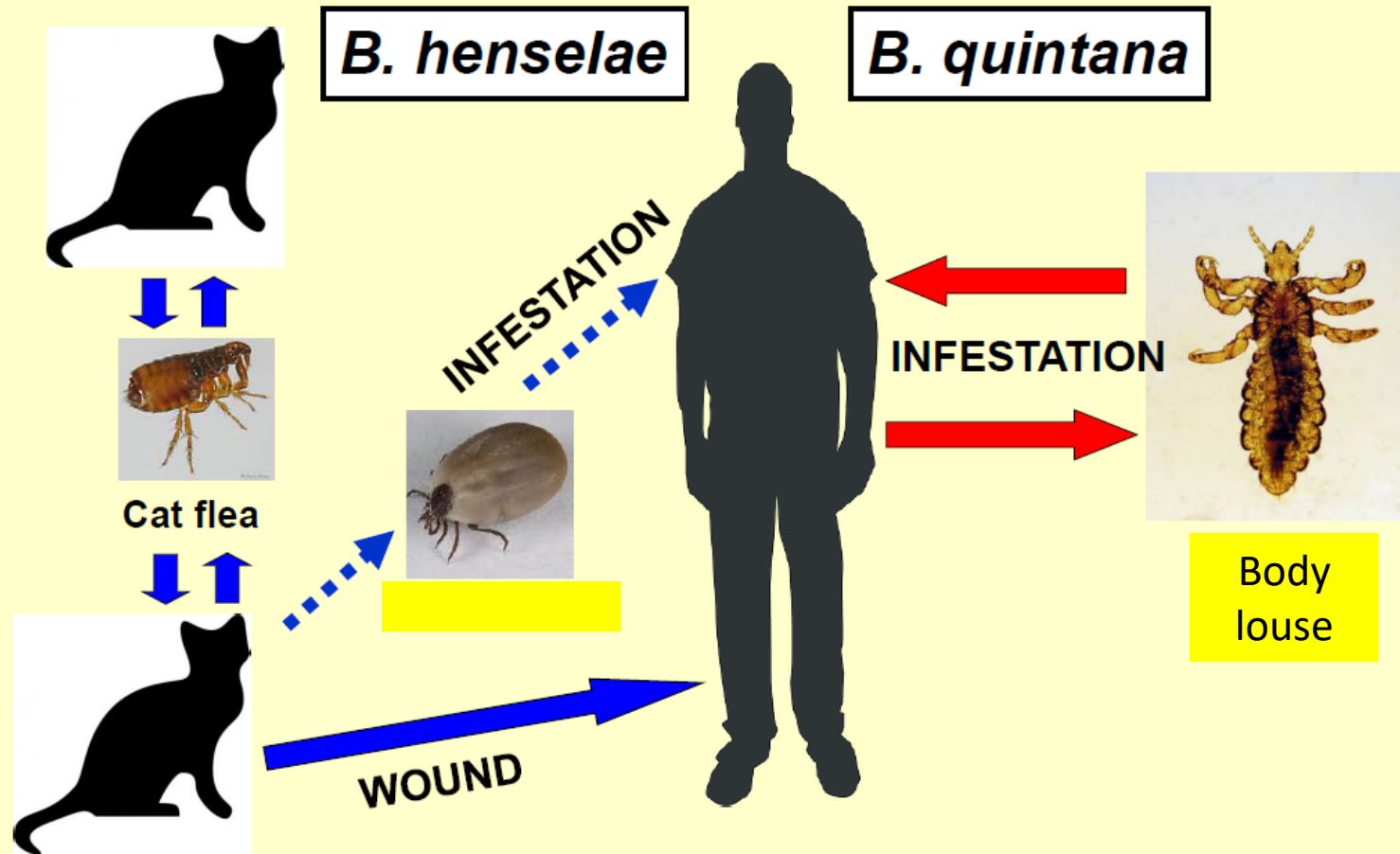
Human as primary host (*B. quintana*, *B. bacilliformis*)

Source: cat, mouse etc.

Transmission: vector borne (ticks, fleas, sand flies, mosquitoes)



# Infectious cycle



# Bartonella

## Clinical significance

- **Cat-scratch disease** – felinosis (*B. henselae*) – local lymphadenopathy, mild disease (fever, headache tiredness), slow recovery – 4 – 12 months, speed up by ATB

**Endocarditis** (*B. henselae*, *B. quintana*)

**Trench fever** (*B. quintana*)

Soldiers (WWI) or today homeless people

**Bacillary angiomatosis** (*B. henselae*, *B. quintana*) – skin lesions (papules or nodules) – vascularised

Present in internal organs – potentially fatal if not treated

## Rx:

Long term therapy (6 weeks and more)  
Tetracyclines, aminoglycosides, macrolides

## Dg.

Culture – CO<sub>2</sub>, at least two weeks, enriched media

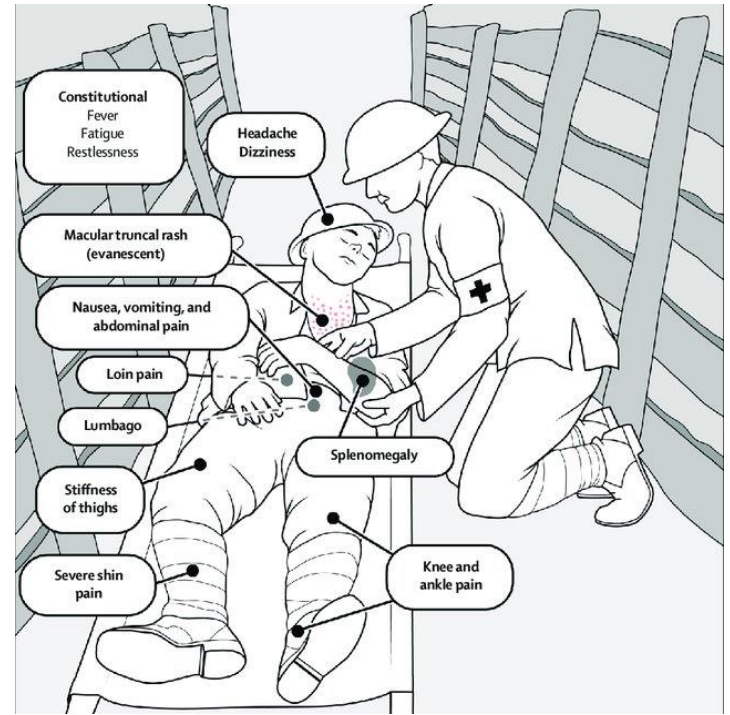
Sample has to be treated with detergent to release intracellular bacteria

Serology – antibody cross reactivity with other bacteria, antibodies are often not produced  
PCR

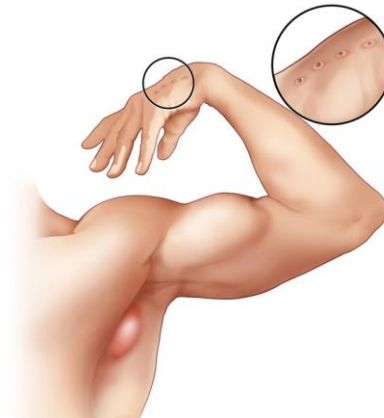




Bacillary angiomatosis



Trench fever symptoms



felinosis

# *Coxiella burnetii*

## **Biology:**

small Gram-negative, coccobacilli  
obligate intracellular bacterial pathogen  
Surviving in phagolysosome of macrophages  
Resistant to environmental conditions  
Highly infectious, primary pathogens for human

## **Clinical significance**

Q- Fever (query – the causing agent was not known for a long time)  
Survive in macrophages, interstitial (atypical) pneumonia, liver and spleen – granuloma, flu-like illness, gastroenteritis  
complications – meningoencephalitis, endocarditis

**Dg: direct** – cultivation using tissue culture, PCR,  
**Undirect** – serology: Weil-Felix reaction

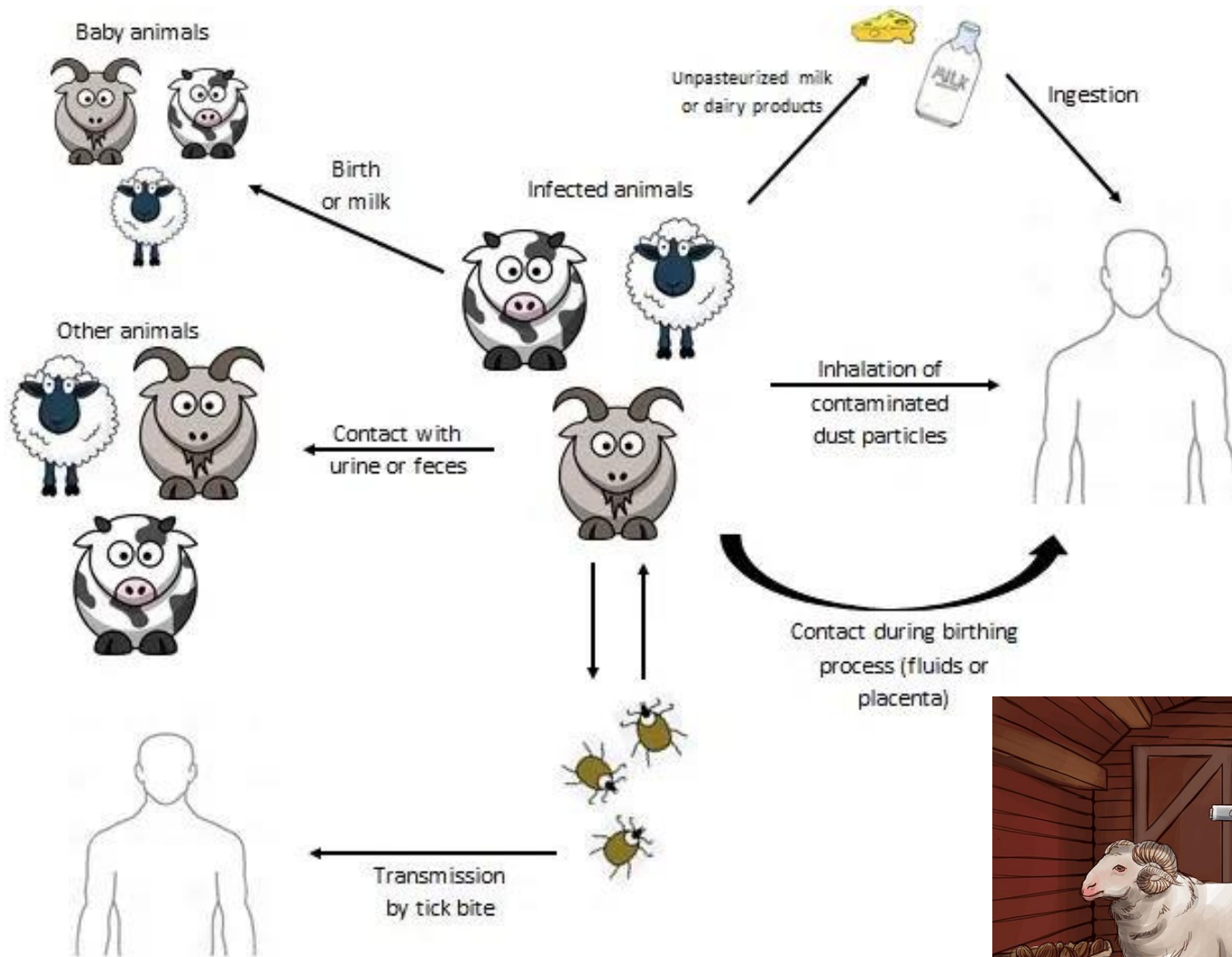
**Rx:** doxycykline+ rifampicin (fluoroquinolones)

## **Epidemiology:**

Zoonosis (cats, dogs, cattle,...)  
source – inhalation of aerosols from infected animals or consumption of milk, or tick-borne transmission  
Risk groups: farmers, slaughter house workers, veterinarians



# Possible transmission paths and potential hosts of *C. burnetii*



# Take home message

Fastidious bacteria (Francisella, Brucella, Legionella, Spirochates, Rickettsia etc.)

- Intracellular
- Culture is not possible or useful – poor sensitivity and long time to result
- Serology! (or PCR, but not always)
- Symptoms (rash, lymphadenopathy, etc) and risk factors!
- Multi-organ infection, long term diseases = long term treatment.
- Zoonosis, vector borne (with exceptions e.g. *T. pallidum*)

Biggest thread in dealing with rare fastidious pathogens is that they could be easily forgotten and not treated in right time.