2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA

Univerzita Karlova

FN M+H Fakultní nemocnice Motol a Homolka

Národní institut virologie a bakteriologie

Seminar: *Brucella* and *Francisella*. Diagnostics of G-rods III

Jakub Hurych

Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University
& Motol and Homolka University Hospital

Microbiology I.
Practical sessions

Brucella and Francisella

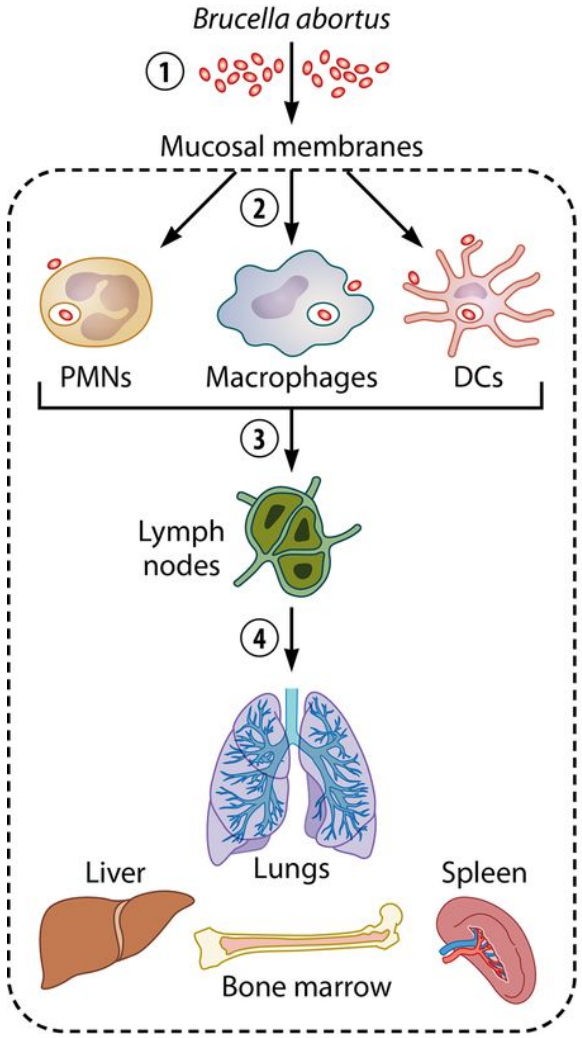
Created by Daniela Lžičařová

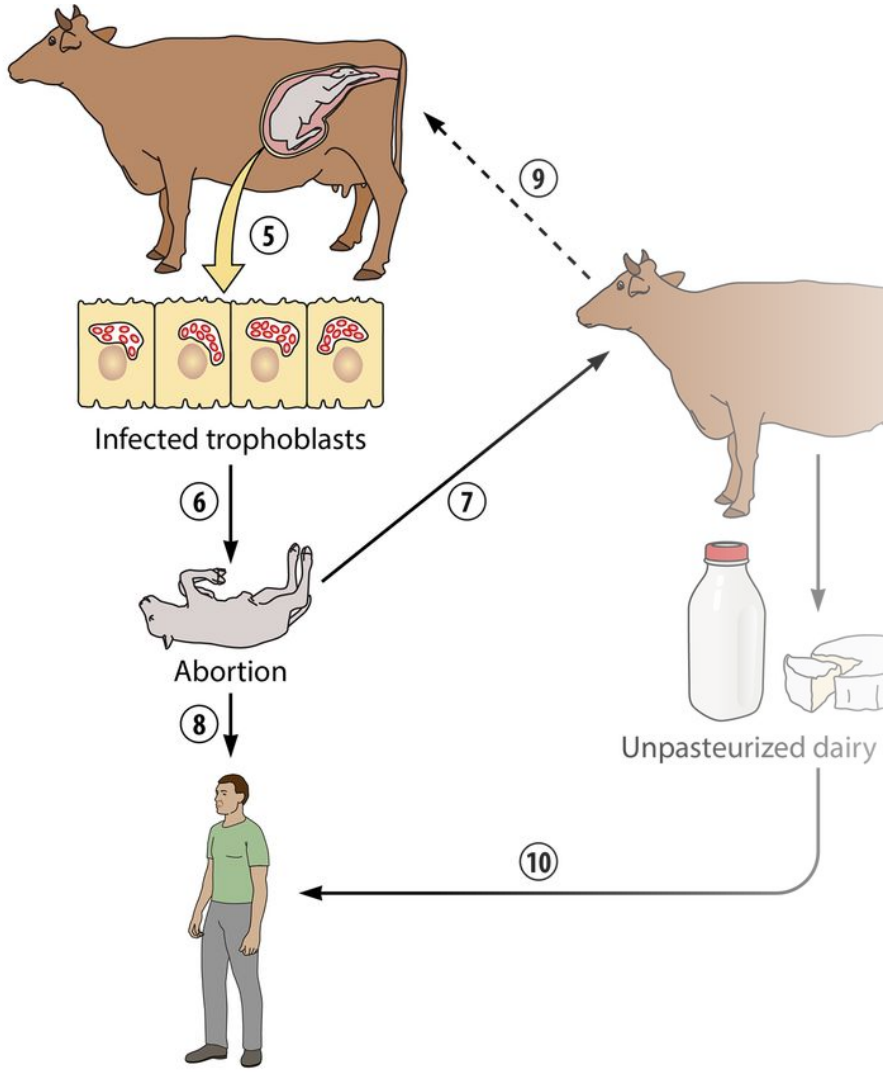
Brucella

- The causative agent of brucellosis – a zoonosis; isolated imported cases in the Czech Republic; 500,000 cases worldwide per year

- Infection in cattle in the Czech Republic has been eradicated through rigorous veterinary control

- **Source of infection**
  - *Brucella abortus* – cattle, camels, yaks, bison
  - *Brucella melitensis* – goats, sheep, camels
  - *Brucella suis* – domestic pigs, wild boar, reindeer
  - *Brucella canis* – dogs





Route of transmission

- Contact of broken skin or the conjunctiva with tissues and body fluids of infected animals – **occupational infection**
- Consumption of raw, unpasteurised milk and dairy products
- Inhalation of infected aerosols
- Laboratory transmission

Human-to-human transmission is extremely rare, but has been reported (mother-to-child transmission, sexual transmission)

Incubation period usually **2–4 weeks**

Brucellosis – pathogenesis

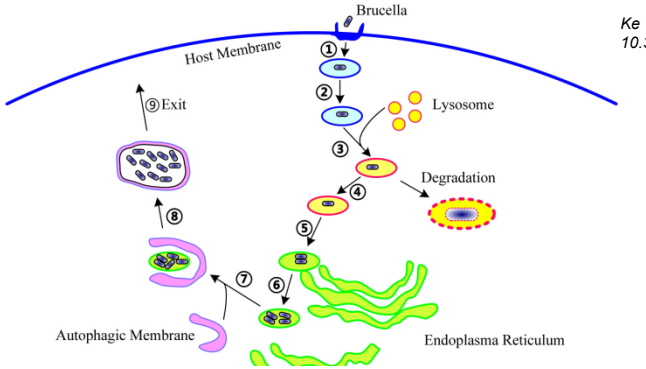
Intracellular pathogen, dissemination within the body inside cells of the reticuloendothelial system

Ability to survive and replicate in **macrophages and monocytes**

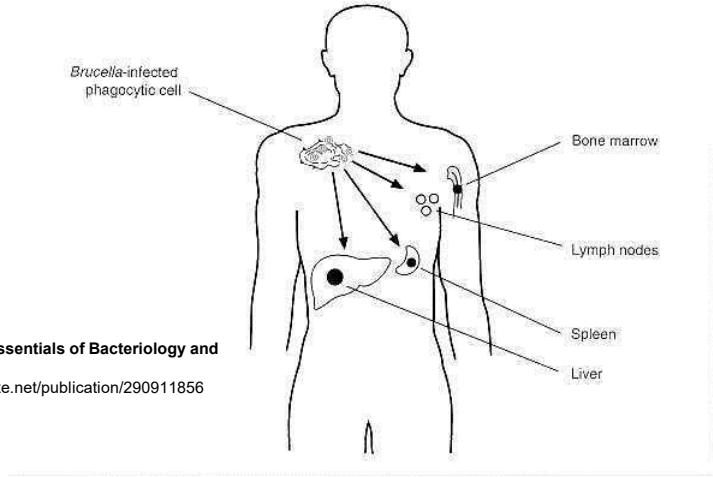
Spread to the spleen, liver, bone marrow, lymph nodes and kidneys

Granulomatous inflammation, tissue destruction.

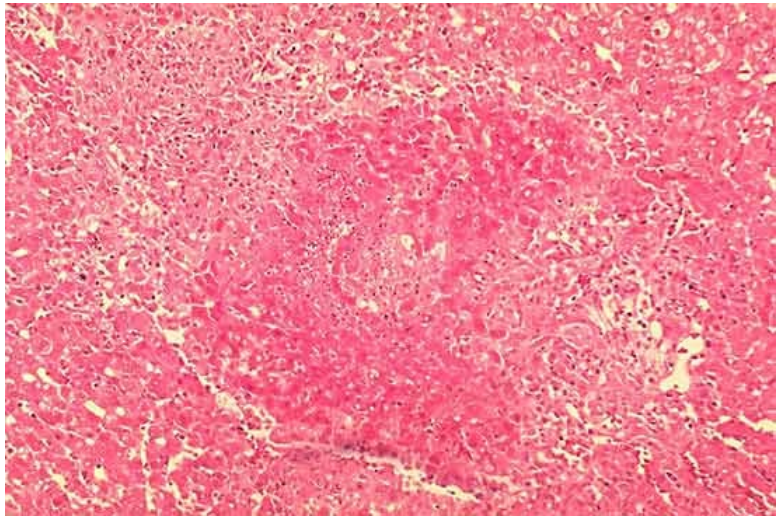
Course ranging from hyperacute to chronic depending on the species



Ke Y. Front. Cell. Infect. Microbiol. 5:72. doi: 10.3389/fcimb.2015.00072



Kamel FH, Jarjez SF. **Essentials of Bacteriology and Immunology,** https://www.researchgate.net/publication/290911856



britannica.com

Principles of diagnosis

- **1. Clinical presentation and medical history –** epidemiological (contact with animals)
- **2. Serology** (first-line laboratory method)
- **3. Confirmation** (culture/PCR)

Brucellosis – clinical presentation

A systemic disease that can affect any organ

Initially, non-specific symptoms typical of a systemic infection: **fever of unknown origin, joint/muscle pain, sweating**

Bang's disease, caused ***by B. abortus*** – chronic course, musculoskeletal system, typically sacroiliac joints, mortality 1–2%

Maltese fever, caused ***by B. melitensis*** – acute at onset, progresses to a chronic phase, frequent organ complications, mortality 5–10%

Brucellosis caused ***by B. suis*** – severe septic course, formation of abscesses in the liver, spleen and other organs, mortality 30%

Endocarditis

Risk **of relapse** following inadequate treatment, within 3–6 months

Treatment

Doxycycline + rifampicin, doxycycline + gentamicin, children: co-trimoxazole + rifampicin

Min. 6 weeks

Brucella melitensis, culture on blood agar

Sukumaran ST, Thayyil SS, Ambramoli SK. Acute brucellosis caused by *Brucella melitensis*: A case report. J Acad Clin Microbiol 2013;15:66-8

Brucellosis – laboratory diagnosis

Serology

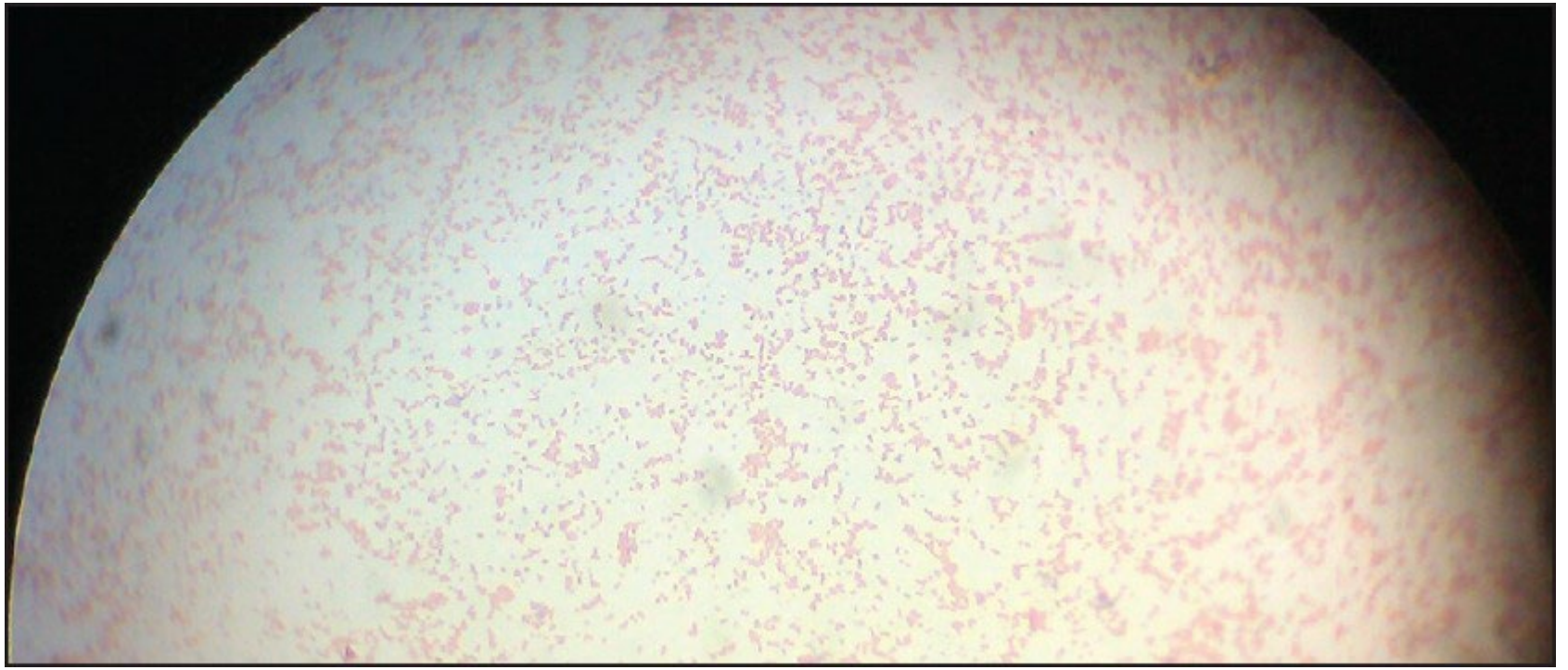
- **Agglutination, microagglutination** in acute cases; diagnosis is confirmed by a fourfold increase in titre when testing paired sera; a high titre in a single sample (dilution 1:160 or higher) is highly suspicious
- **ELISA**, indirect Coombs test, indirect immunofluorescence (IFA) – detection of non-agglutinating/incomplete antibodies in chronic and complicated cases

Direct detection

- **PCR** – tissue, serum, cerebrospinal fluid

- **Culture** – the gold standard, requires BSL-3 biosafety level
- Biological material – tissue, blood, cerebrospinal fluid, bone marrow, exudates (joint, pleural), abscess contents
- Culture media – blood culture bottle, solid enriched media (chocolate agar, media for detecting gonococci, brucella agar with horse blood), culture for 6 weeks, 37 °C, elevated CO_2 tension$_2$

Brucella melitensis, Gram stain



Sukumaran ST, Thayyil SS, Ambramoli SK. Acute brucellosis caused by *Brucella melitensis*: A case report. J Acad Clin Microbiol 2013;15:66-8

It’s not always Brucella

- Haematological oncology patient
- Egypt: camel milk
- Blood culture: *Ochrobactrum* from MALDI

A bone marrow examination revealed no haematooncological disease. Extensive microbiological examinations did not confirm any of the infectious diseases considered, the result of brucellosis serology was evaluated as negative according to the manufacturer’s instructions. A polymorphic gram-negative bacillus was detected in blood culture taken on 14 August after two and a half days of incubation and identified as *Ochrobactrum* spp. by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using Microflex LT/SH Smart in positive mode with MBT Compass 4.1.100 software including MALDI Biotyper library v.13–12,438 MSPs (Bruker Daltonics GmbH, Bremen, Germany). The result was evaluated at a score of 1.38–1.41. This very slowly growing bacteria in aerobic and anaerobic conditions was sensitive to cotrimoxazole, rifampicin, aminoglycosides, ciprofloxacin and tigecycline (based on the disc diffusion testing). The pathogen with the same characteristics was found in other cultures taken on 21 and 29 August.

PET-MRI, indicated to localise the focus of infection, showed spondylitis of the Th12 and L1 vertebral bodies. As spondylitis is a common complication of brucellosis, whereas *Ochrobactrum* spp causes it rarely, a re-evaluation of the agent was performed. Repeated serological testing (Latex agglutination in microtiter plate and Rose Bengal test) have already conclusively confirmed positive antibody titres. Additional use of archived spectrum—via MBT Compass Explorer v. 4.1.100 software with Database-CD BTyp2.0-Sec-Library 1.0 (referred to as “MBT SR – security relevant library”)—consists of 104 MSPs, clearly identified the pathogen as *B. melitensis* (score value 2.28).

Adequate antibiotic therapy (cotrimoxazole and gentamicin) was started after almost 4 months of clinical symptoms. Due to the patient’s intolerance to i.v. therapy,

Folia Microbiologica (2025) 70:235–239
https://doi.org/10.1007/s12223-024-01231-7

ORIGINAL ARTICLE

Prolonged fever in a 13-year-old girl misdiagnosed as *Ochrobactrum* spp. bacteremia—the pitfalls of diagnostic *Brucella* spondylitis in a non-endemic country

Dita Smíšková[1] · Petr Smíšek[2] · Otakar Nyč[3]

Received: 5 August 2024 / Accepted: 28 November 2024 / Published online: 17 December 2024

Table 1 Time line—key clinical and laboratory data

Date of medical examination	Main symptoms and results of laboratory and imaging tests
2023–05–18 GP	Fever up to 39 °C, sore throat. Treated with macrolide antibiotic for positive rapid strep test. Fever lasted 2 weeks, fatigue persisted
2023–06-01 GP	Fatigue, no fever – *leukopenia (WBC 4.0× 10^9/L), anaemia (HGB 103 g/L), CRP 12.9 mg/L (normal to 5.0 mg/L). EBV and CMV serology negative*
2023–06–12 Emergency dpt	Recurrence of fever, new back pain in lumbar region. Back pain resolved after 5 days of analgesic therapy, fever lasted 10 days – *CRP 26.0 mg/L. Splenomegaly of 158mm on abdominal and pelvic ultrasound. Urinary tract infection excluded*
2023–06–27 GP	Recurrence of fever, lasted 10 days, fatigue – *leukopenia (WBC 3.7× 10^9/L), CRP 6.3 mg/L*
2023–08-01 GP	Fever up to 40 °C for 5 days, fatigue, headache and sore throat. Body mass loss of 5 kg over 10 weeks, night sweats and transient swelling of right knee after increased activity. Referred to tertiary care centre – *Splenomegaly, WBC 4.0×10^9/L, HGB 101 g/L, PLT 135×10^9/L, CRP 54.1 mg/L. EBV and CMV serology negative again. Paranasal sinuses and chest X-rays with normal results*
2023–08–11 HOPD	Persistent fever above 38 °C, fatigue. Autoimmune diseases unlikely according to the specialist, use of non-steroidal anti-inflammatory drugs recommended. Travel history was completed – *CRP (43 mg/L), ESR 35 mm/h, anaemia (HGB 98g/L). Bone marrow aspiration with signs of non-specific activation, no evidence of lymphoproliferative disease. Leishmania, TBEV, Borrelia, Y. enterocolitica, F. tularensis, T. gondii serology negative.* ***Brucella antibodies titer 1:80****, evaluated as negative. QuantiFERON TB Gold negative*
2023 –08–21 HOPD	*No fever for 7 days (on medication Ibuprofen 500 mg 3 t.d.)* – ***Positive blood culture—Ochrobactrum* spp.** *Bacteria confirmed in three blood cultures*
2023–09-01	– *PET MRI—spondylitis of Th12 and L1 vertebrae, no signs of spondylodiscitis and paravertebral abscesses*
2023–09-04 IDD	Spondylitis therapy started, *Ochrobactrum* spp considered etiological agent. Due to the close relationship between *Brucella* spp. and *Ochrobactrum* spp., reevaluation of the agent was performed
2023–09-06 IDD	Antibiotic therapy for *Brucella* spondylitis initiated – ***B. melitensis antibodies titer 1:1280, B. abortus 1:640; B. suis 1:640, Rose Bengal Test (RBT) positive***
2023–11-06	– ***B. melitensis antibodies titer 1:640***
2023–11–28	ATB therapy finished after 12 weeks
2024–05-02	Patient has no clinical or laboratory evidence of disease relapse – ***B. melitensis antibodies titer 1:320. RBT positive***

Brucellosis – sources

Jorgensen JF, Pfaller MA: Manual of Clinical Microbiology, 11th Edition, ASM Press 2015

Manďáková Z. Brucellosis – basic information about the disease, http://www.szu.cz/tema/prevence/bruceloza-1

Murray, PR. Medical microbiology, eighth edition, Elsevier 2016

Beneš J. Infectious Diseases, Galén Prague 2009

Brucellosis Reference Guide: Exposures, Testing, and Prevention. CDC 2017, www.cdc.gov

Francisella

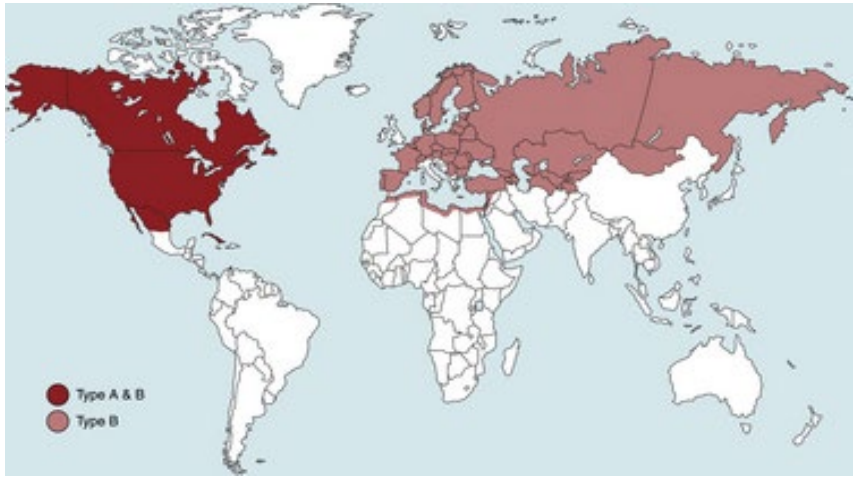
Francisella tularensis
- in Europe, the *holarctica* subspecies (type B),
- in North America, the subspecies *tularensis* (A) – higher virulence than *holarctica*

Causative agent of tularemia, a zoonosis, a disease with natural foci

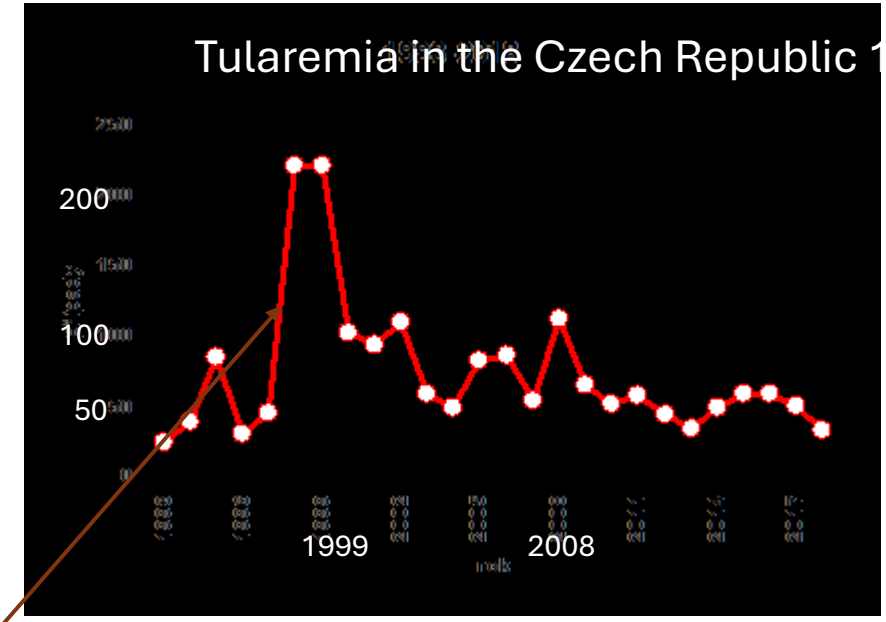
Highly dangerous agent (bioterrorism)

In the Czech Republic, there are approximately 50 cases per year

The main cause was widespread transmission among wild animals, particularly in brown hares and small rodents (field voles)



Veteriankey.com



Dr Kateřina Fabiánová, PhD, Department of Infectious Disease Epidemiology, CEM, SZÚ, Prague; July 2019, revised

Francisella tularensis – Central Europe

Source of infection – wild mammals (rodents, lagomorphs, carnivores)

Transmission
- Contact with an infected animal, live or dead – broken skin, conjunctiva
- Vector – blood-sucking arthropods (*Ixodes, Dermacentor*)
- Inhalation of infectious aerosol
- Ingestion of infected water or food

- **High risk of laboratory infection**

- No human-to-human transmission has been reported

Very low infectious dose (10 organisms)
Incubation period 1–21 days

www.cdc.gov

absa.org

ecdc.europa.eu

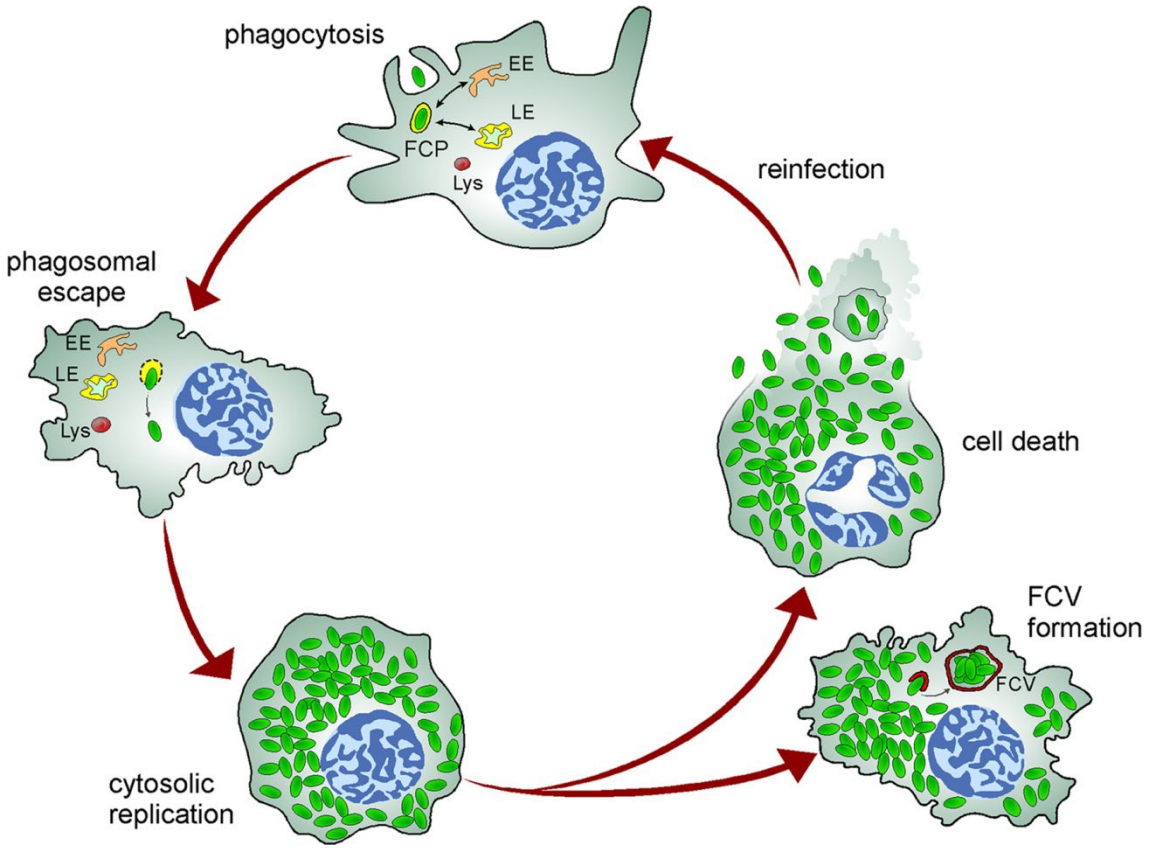
Still from the Czech film Vaterland, 2004

Tularemia – pathogenesis

Intracellular survival and replication: macrophages, neutrophils, epithelial cells, endothelial cells

Inhibition of phagosome-lysosome fusion

Polysaccharide capsule preventing complement activation and phagocytosis



Chong A, Celli J. Front. Microbiol., 28 December 2010
https://doi.org/10.3389/fmicb.2010.00138

Principles of diagnosis

- **1. Clinical presentation and medical history –** epidemiological (contact with animals)
- **2. Serology** (the laboratory method of first choice)
- **3. Confirmation** (culture/PCR)

Tularemia – clinical presentation

Always systemic symptoms (fever, fatigue, headaches, muscle and joint pain)

Clinical forms:

- **Ulceroglandular** – the most common
- Oculoglandular (infection enters via the conjunctiva)
- Glandular (lymphadenitis)
- Oropharyngeal (transmission via food)
- Pulmonary (aerosol transmission)
- Typhoid (systemic infection, unclear primary focus)

Type B – *holarctica* – minimal mortality, lower virulence; type A (*tularensis* – mortality 2% even with targeted treatment available

Treatment: **gentamicin, doxycycline**, (ciprofloxacin, chloramphenicol)

Tularemia – laboratory diagnosis

A minimum of BSL-2/3 biosafety level is required for direct detection – only selected laboratories

absa.org

Collection of biological material – direct detection

From the site of infection depending on the clinical form, after surface decontamination; liquid material, tissues (biopsy) and blood for haemoculture are most suitable (all clinical forms; risk of false negatives at the onset of infection)

llnl.gov

If a swab is taken, then in transport medium

Rapid transport at room temperature; for longer transport, at 2–8 °C

Notify the laboratory in advance

For indirect detection (antibodies), collect 2 samples of clotted blood – the first as soon as possible after the onset of symptoms, the second 3–4 weeks later

Tularemia – laboratory diagnosis

Indirect detection – detection of antibodies from paired serum samples

- Agglutination (tube or micro-agglutination in a plate)
- ELISA + Western blot

Direct detection

- **PCR** – targeting specific *F. tularensis* genes, 16S (genus-specific)
- **Culture – only in** a **BSL-3** laboratory, **cysteine-enriched** media (from more common media, e.g. buffered charcoal yeast extract (BCYE) and GC Agar II with 1% haemoglobin and 1% IsoVitaleX) – aerobically for at least 48 hours at 37 °C.

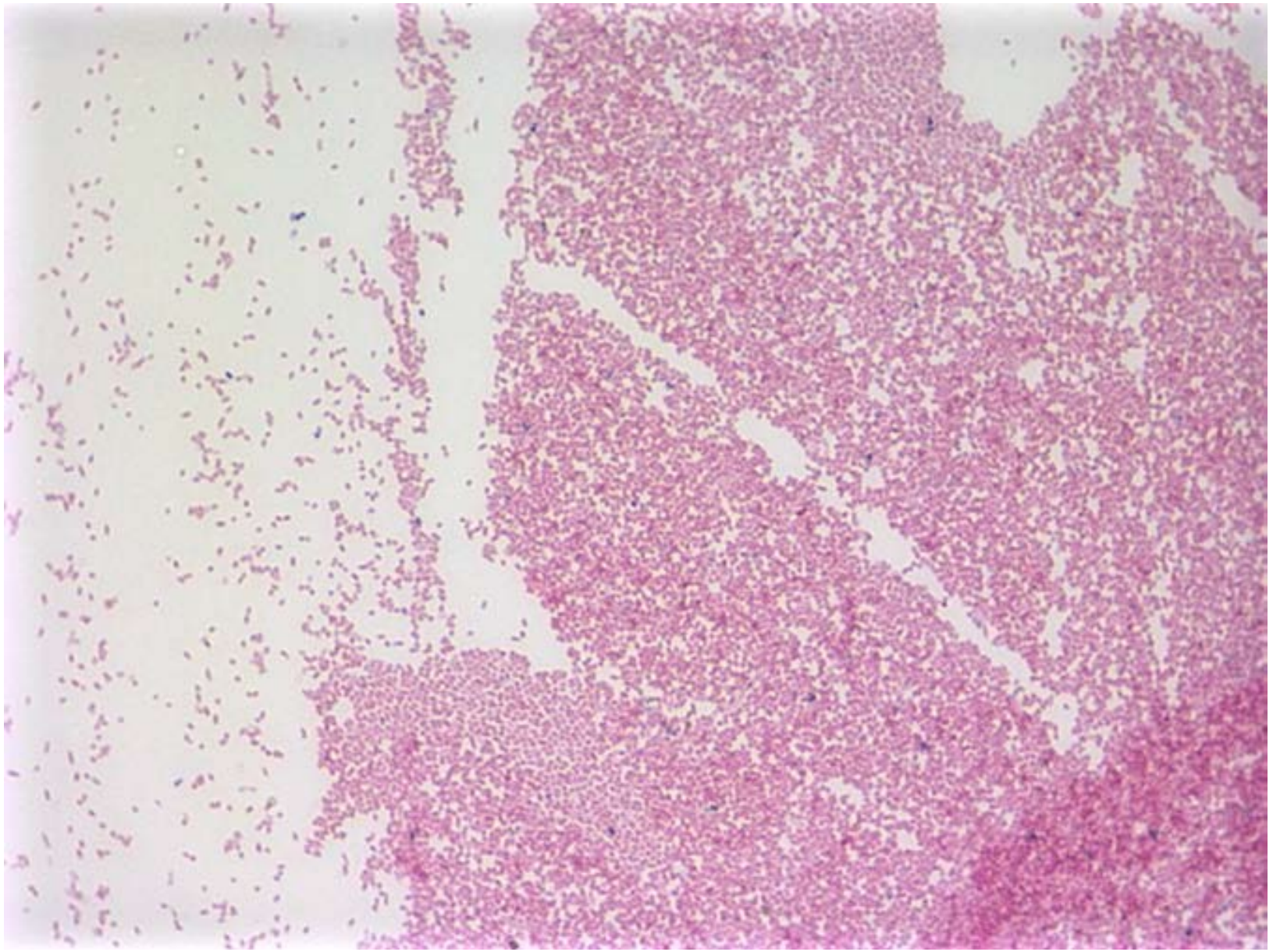
F. tularensis can also grow from tissue or fluid samples on standard media (blood or chocolate agar…) and in haemoculture flasks – caution is required!

- **Antigen detection** (using a fluorescently labelled antibody directly in the material)

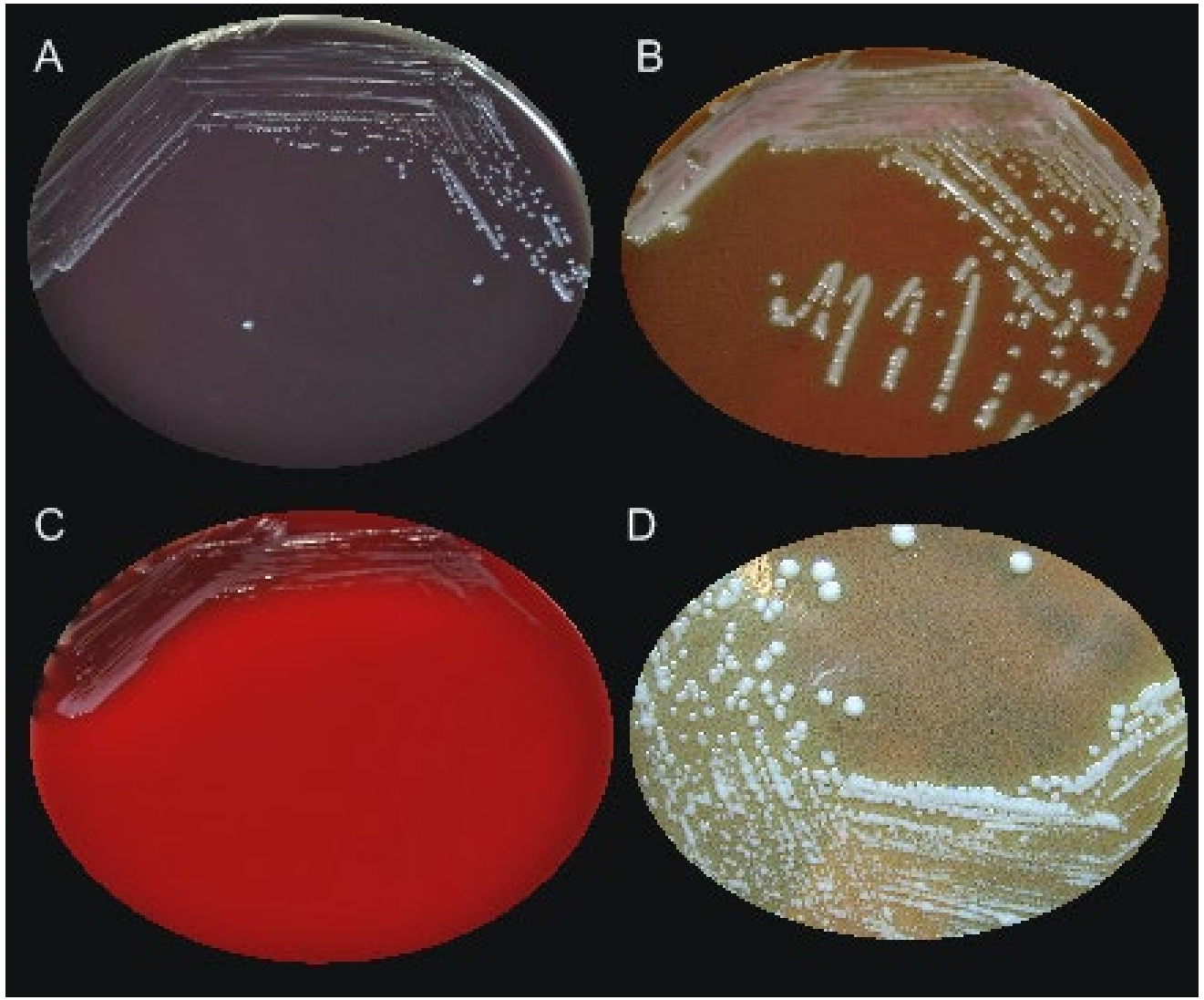
Identification: MALDI-TOF

Microscopy of minimal significance; easily overlooked in clinical material, but weakly G-stains; morphologically very small G-cocobacilli – smaller than Haemophilus

Francisella tularensis – Gram stain



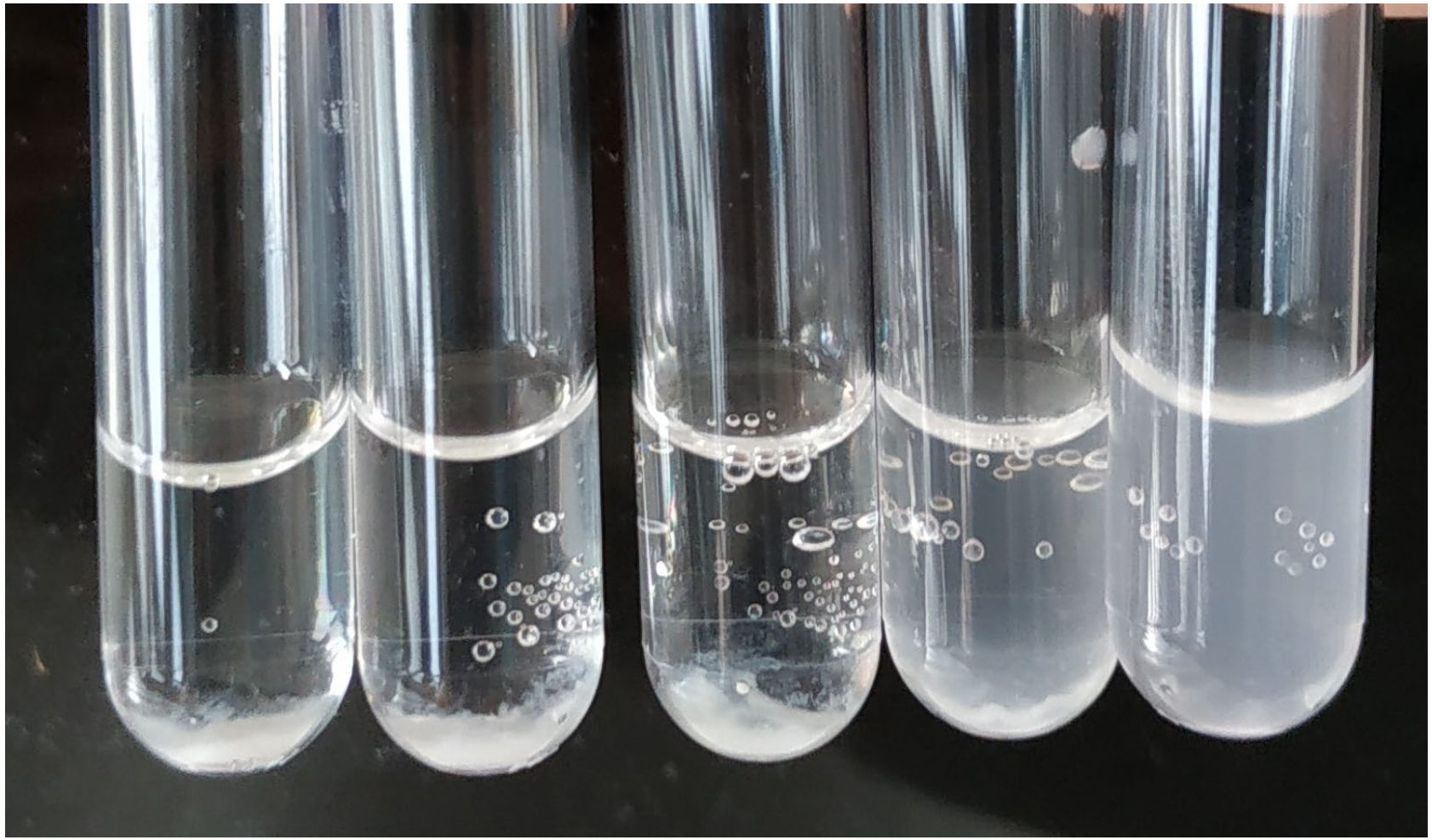
Francisella tularensis - cultivation



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

Pathogen profile dictionary
ppdictionary.com

Tularemia – detection of antibodies by agglutination reaction



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Tärnvik A et al. WHO Guidelines on Tularaemia, WHO 2007

Carvalho CL et al. Tularaemia: A challenging zoonosis. **Comparative Immunology, Microbiology and Infectious Diseases**, Volume 37, Issue 2, March 2014, Pages 85–96 https://doi.org/10.1016/j.cimid.2014.01.002

Diagnostics of G-bacteria III

Fastidious bacteria

Created by Anežka Gryndlerová

Contents

- *Neisseria sp.*
- *Haemophilus sp.*
- *Bordetella sp.*
- *Legionella pneumophila*

- *Moraxella catarrhalis*

Neisserie

Neisseria spp.

Protokol: X-23-2423 --- Konečný výsledek ---
Vzorek: **Aspirát DCD** endotracheální (ET)
Vyšetření: DCD - kultivace

MIKROSKOPICKY

Preparát z klinického materiálu: buněčná drť, řasinkové epitelie, leukocyty 10-25, g+koky , g-tyčinky zcela ojediněle

PRIMOKULTIVACE

Nález 1: **Klebsiella pneumoniae**

Nález 2: **Neisseria subflava**

- G-aerobic diplococci, coffee bean-shaped
- Representatives?
  - *Neisseria gonorrhoeae* (gonococcus)
  - *Neisseria meningitidis* (meningococcus)
  - *Neisseria subflava, flavescens, perflava*, …
    - Common microbiota of the oral cavity and HCD
- Sensitive to environmental influences, difficult to culture - ?
  - *N. gonorrhoeae, meningitidis*

Neisseria gonorrhoeae – the disease

- Gonorrhoea
  - Urethritis, cervicitis – whitish discharge
  - Epididymitis, prostatitis, salpingitis
  - Anorectal gonorrhoea, tonsillopharyngitis
  - Pelvic inflammatory disease (PID)
    - Involvement of the pelvic organs (uterus, adnexa)

Gonorrhoea – diagnosis

- Sample collection?
  - Urine (first sample), cervical swab, urethral swab
  - Throat swab, rectal swab
- Transport of samples
- Culture (CA, selective enriched agar)
  - MALDI-TOF (*Neisseria sp.*) → biochemical analysis (species identification)
- PCR

NEISSERIAtest									Dodatkové testy		
Jamka:	H	G	F	E	D	C	B	A			Thayer--Martinův agar
Test:	NEC	GLU	MLT	FRU	SUC	GGT	TRB	SPS	ONP	DNA	
N. gonorrhoeae	–	+	–	–	–	–	–	–	–	–	+
N. meningitidis	–	+	+	–	–	+	–	–	–	–	+
N. lactamica	–	+	+	–	–	–	–	–	+	–	+
N. polysaccharea	–	+	+	–	d	–	–	+	–	–	+
N. sicca+ N. mucosa+	–	+	+	+	+	d	–	+	–	–	–
N. subflava	–	+	+	d	d	d	–	d	–	–	–
N. flavescens	–	–	–	–	–	–	–	+	–	–	–
N. cinerea++ N. elongata++	–	–	–	–	–	–	–	–	–	–	–
M. (B.) catarrhalis	–	–	–	–	–	–	+	–	–	+	–

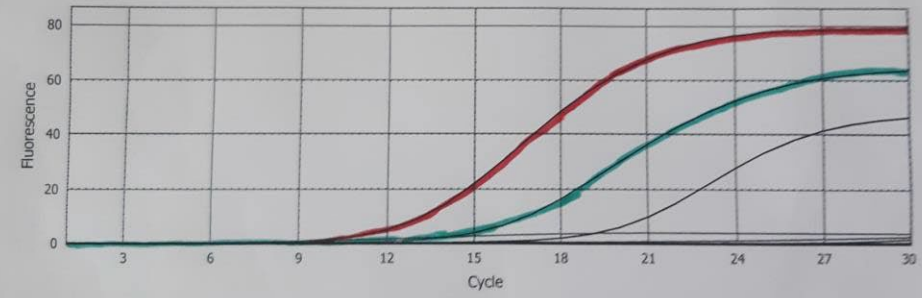
AusDiagnostics

MT-PCR Sample Report - Sample 25225775

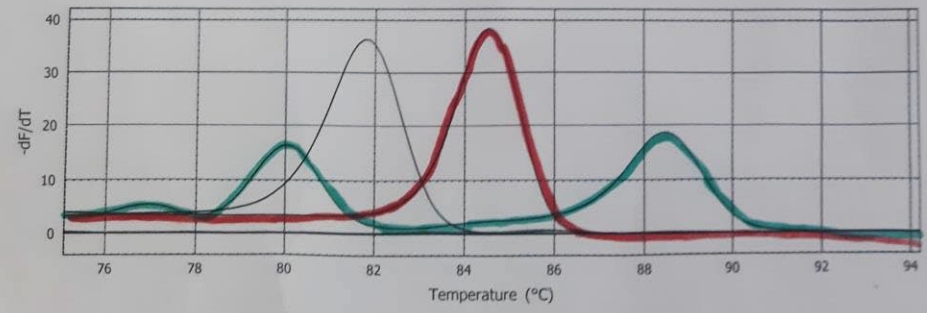
No.	Colour	Gene	Call	Concentration	Corrected Melt	Take-off
I1		Chlamydia				
I2		LGV				
I3		Gonorrhoeae	Present (opaJ,opaH)	759 749	80,03 88,49	12,17
I4		RUO: Ceftriaxone R				
I5		T.vaginalis				
I6		U.urealyticum				
I7		M.hom, U.parvum				
I8		M.genitalium				
I9		Fluoroquinolone R				
I10		Mgen 23S				
I11		Human DNA	Present	5 178 649	84,53	9,18
I12		SPIKE	Present	50 000	81,8	16,41

Operator: MA, Sample Volume: 10µl, Step 1 Sensitivity: NA extract or swab (18 cycles), Analysis Sensitivity: Normal
MasterMix cartridge batch: C264550DL, Step 1 reaction tube batch: S2654311
File: I:\1567 - Urinogenital and Resistance 12 well 2022-12-15 1429.epx

Cycling Curves



Melt Curves



Protokol: LMM-22-3972 --- Konečný výsledek ---
Vzorek: **Výtěr z pochvy**
Vyšetření: PCR STD

Sexuálně přenosné choroby - vyšetření metodou PCR
*(V závorce uvedena relativní kvantita nabývající hodnot od * [nejmenší] do ***** [největší])*

Chlamydia trachomatis: negativní
Neisseria gonorrhoeae: POZITIVNÍ (*****)
Mycoplasma genitalium: negativní
Mycoplasma hominis: POZITIVNÍ
Ureaplasma parvum: POZITIVNÍ

Pozn. Při současné pozitivitě *M. hominis* a *U. parvum* systém neumožňuje každý z nálezů odděleně kvantifikovat. Pozitivita agens s vyšší kvantitou (nelze říci, o které z těchto dvou se jedná) je: *****

Ureaplasma urealyticum: negativní
Trichomonas vaginalis: negativní

Telefonické hlášení:
12.09.2022-14:14 MUDr. Kelčík Roland Mgr. Dratvová Barbora
PCR pozitivita N.gonorrhoeae, M. hominis a U. parvum.

Protokol: K-23-4015 --- Konečný výsledek ---
Vzorek: **Výtěr z krku**
Vyšetření: HCD - kultivace

PRIMOKULTIVACE
Nález 1: viridující streptokoky, neisserie

Protokol: K-23-1088 --- Konečný výsledek ---
Vzorek: **Výtěr z krku**
Vyšetření: HCD - kultivace, Neisseria gonorrhoeae - kultivace

PRIMOKULTIVACE
Nález 1: viridující streptokoky, neisserie

Kultivace cílená na Neisseria gonorrhoeae
Nález: negativní

Kódy pro pojišťovnu: 82017(1), 82025(1), 82031(1)

- Testing for gonorrhoea from the throat or rectum – special request

Neisseria gonorrhoeae – the disease

- Disseminated gonococcal infection
  - Purulent arthritis
  - Maculopapular rash
  - Rarely, sepsis, meningitis
  - (Specimen collection: blood culture, joint aspirate)

- Neonatal conjunctivitis
  - Conjunctival swab
  - Prevention – disinfectant solution

https://www.msdmanuals.com/en-jp/home/infections/sexually-transmitted-diseases-stds/gonorrhea
https://www.researchgate.net/publication/225977492_The_Impact_of_Infection_During_Pregnancy_on_the_Mother_and_Baby/figures?lo=1
https://www.drmax.cz/ophthalmo-septonex-gtt-opht-1x10ml?gad_source=1&gclid=CjwKCAjw3NyxBhBmEiwAyofDYX-8LAgju5GTl2oW5lWaeWepwJ3R7vSiruWJqPxNCu_lZ1qenzC7XRoC0m4QAvD_BwE

Therapy

- Beta-lactams
  - Basic penicillins – frequently resistant (beta-lactamase)
  - Third-generation cephalosporins

- Alternatives (but more frequent resistance)
  - Tetracyclines
  - Macrolides
  - Fluoroquinolones

Neisseria meningitidis

- Colonisation of the pharyngeal mucosa
- Polysaccharide capsule
  - Serotypes - ?
    - A
    - B
    - C
    - W135
    - Y

Neisseria meningitidis – disease

- Invasive meningococcal disease (IMD)
  - Preceded by flu-like symptoms (fever, headache, muscle pain,
    nausea, vomiting)
  - Sepsis
  - Purulent meningitis
  - Mixed form

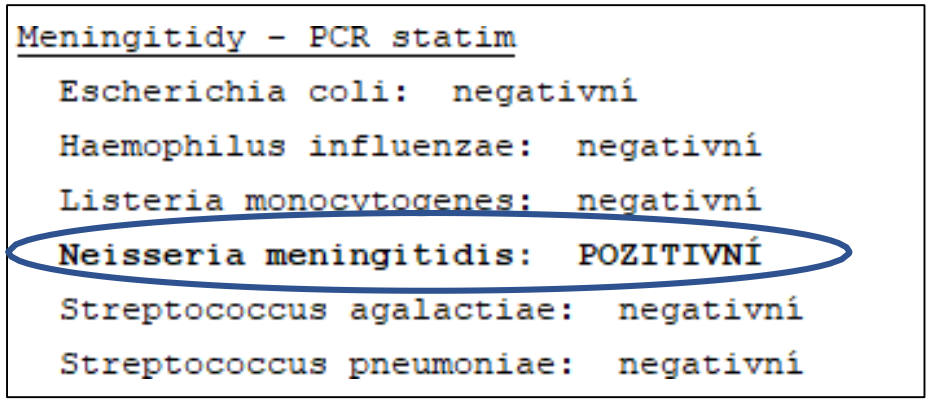
- Lipooligosaccharide – endotoxin
  - Sepsis
  - DIC (disseminated intravascular coagulation)

- Incidence: mainly children under 2 years of age, adolescents

https://www.researchgate.net/publication/258080215_Short_Report_Severe_Myalgia_of_the_Lower_Extremities_as_the_First_Clinical_Feature_of_Meningococcal_Purpura_Fulminans/figures
https://www.canada.ca/en/public-health/services/immunization/vaccine-preventable-diseases/invasive-meningococcal-disease/health-professionals.html

Neisseria meningitidis – diagnosis

- Sample collection?
  - cerebrospinal fluid, blood culture, necrotic tissue
- Microscopy
- Culture (CA, KA)
  - MALDI-TOF (*Neisseria sp.*) → biochemical analysis (species identification)
- PCR (cerebrospinal fluid)



Neisseria meningitidis – treatment

- Third-generation cephalosporins (immediately), penicillin G

- Prevention – vaccination (optional)
  - A, C, W135, Y
  - B

Haemophiliacs

Haemophilus spp.

- Commensals of the oral cavity and
  pharynx
- Difficult to culture
  - Sensitivity to external factors
  - Nutritional requirements
    - factor V (NAD), factor X (hemin)
- Representatives - ?
  - *Haemophilus influenzae*
  - *Haemophilus parainfluenzae*
  - *Haemophilus haemolyticus*
  - *Haemophilus ducreyi (*chancroid)

Protokol: HEM-23-3089 --- Konečný výsledek ---
Vzorek: **Aspirát DCD fibroaspirát**
Vyšetření: DCD - kultivace

MIKROSKOPICKY
Preparát z klinického materiálu: buněčná drť, erytrocyty, dlaždicové epitelie, g+koky

PRIMOKULTIVACE
Nález 1: **viridující streptokoky, neisserie**
Nález 2: **Rothia mucilaginosa**
(g+ koky ve dvojicích a tetrádách, fyziologická součást mikroflóry orofaryngu)

POMNOŽENÍ
Nález: **dtto**

Mikroaerofilní kultivace
Nález 1: **Haemophilus parahaemolyticus**

Screening na kvasinky a plísně
Nález: **negativní**

Výsledek prodloužené mykologické kultivace sdělíme v případě pozitivity dodatečně - celková délka kultivace je 5 dní.

https://www.altmeyers.org/en/dermatology/molle-ulcer-121527

Haemophilus influenzae

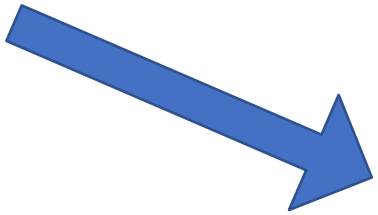
- G- (very) short rods, facultative anaerobes
- Polysaccharide capsule (some strains)
  - a-f
  - Subtype b
- Origin of the species name?

Haemophilus influenzae – a disease?

- HCD infection
  - Otitis media, sinusitis
  - Epiglottitis
- Pneumonia
- Conjunctivitis
- Meningitis

Haemophilus influenzae – diagnosis?

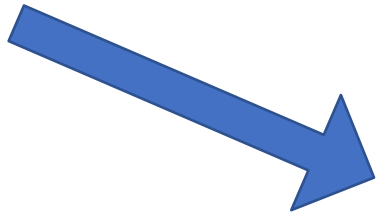
- HDC infection
  - Otitis media, sinusitis
  - Epiglottitis
- Pneumonia
- Conjunctivitis
- Meningitis



- puncture fluid, pus, (swab)

Haemophilus influenzae – diagnosis?

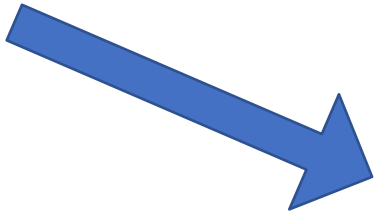
- HDC infection
  - Otitis media, sinusitis
  - Epiglottitis
- Pneumonia
- Conjunctivitis
- Meningitis



- blood culture

Haemophilus influenzae – diagnosis?

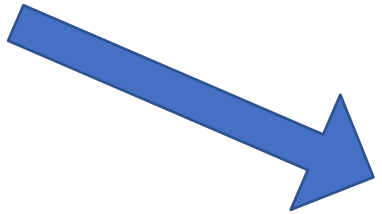
- HDC infection
  - Otitis media, sinusitis
  - Epiglottitis
- **Pneumonia**
- Conjunctivitis
- Meningitis



- sputum, blood culture, ~~throat swab~~

Haemophilus influenzae – diagnosis?

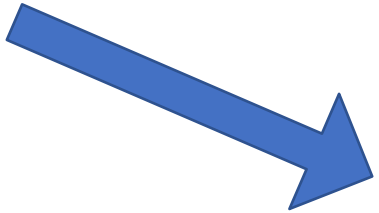
- HDC infection
  - Otitis media, sinusitis
  - Epiglottitis
- Pneumonia
- **Conjunctivitis**
- Meningitis



- Conjunctival swab

Haemophilus influenzae – diagnosis?

- HDC infection
  - Otitis media, sinusitis
  - Epiglottitis
- Pneumonia
- Conjunctivitis
- Meningitis



- Cerebrospinal fluid, blood culture

Haemophilus influenzae – diagnosis

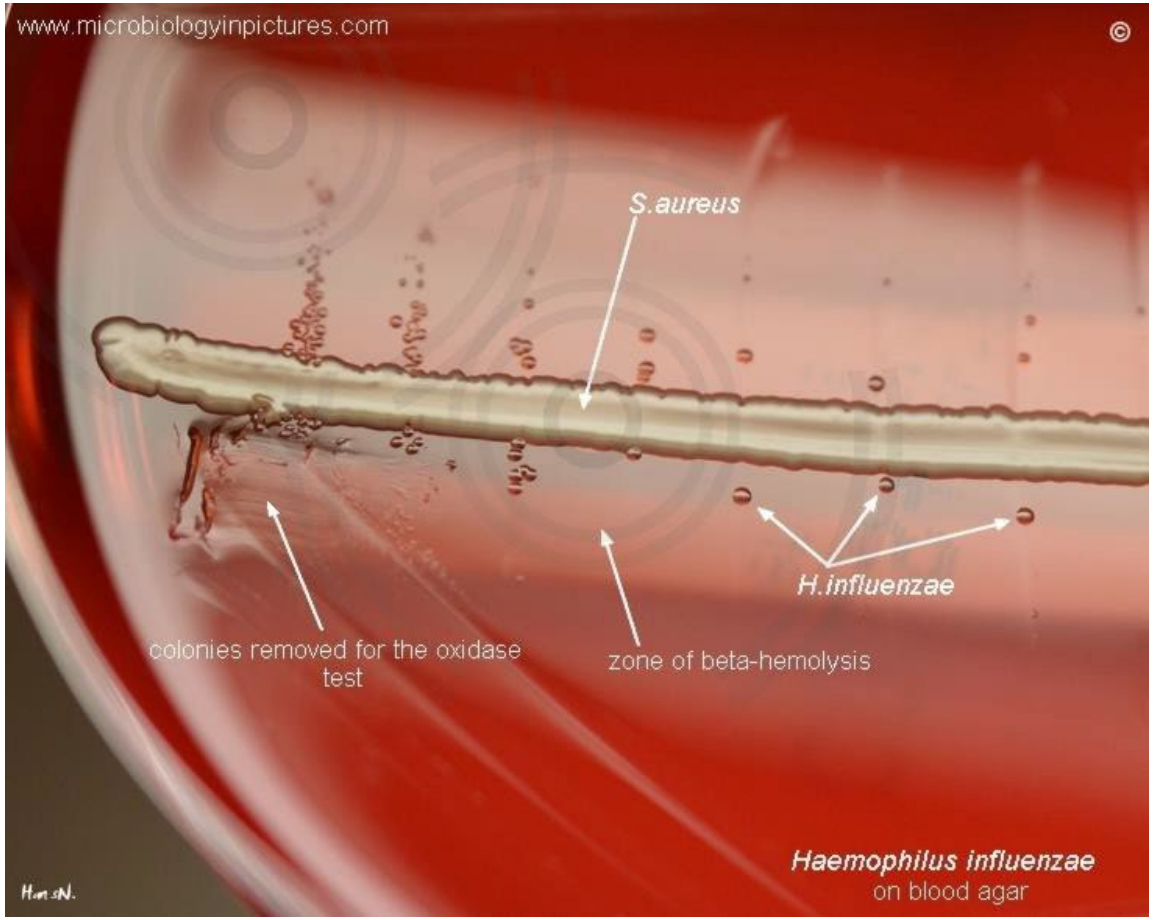
- Culture
  - CA, KA with staphylococcal streak – satellite phenomenon
- CSF
  - Culture + microscopy, PCR

- The next 5 most common causes of bacterial meningitis?

Protokol: LMM-23-2027 --- Konečný výsledek ---
Vzorek: **Likvor** z lumbální punkce
Vyšetření: Bakteriální meningitidy - PCR

Neuroinfekce - vyšetření metodou PCR

Streptococcus pneumoniae:	negativní
Haemophilus influenzae:	negativní
Neisseria meningitidis:	negativní
Listeria monocytogenes:	negativní
Escherichia coli:	negativní
Streptococcus agalactiae:	negativní


www.microbiologyinpictures.com
©
S.aureus
H.influenzae
colonies removed for the oxidase test
zone of beta-hemolysis
Haemophilus influenzae
on blood agar

H. influenzae – resistance to beta-lactams

- Beta-lactamase (primary penicillins, aminopenicillins)
- Other resistance mechanisms (mutations in the PBP gene)

Nález 1: **Haemophilus influenzae**

ANTIBIOGRAM (disková difuzní metoda)

ampicilin.....	C	tetracyklin.....	C
amoxicilin /klavulanát...	C	nalidixová kyselina.....	C
cefuroxim.....	C	chloramfenikol.....	C
cefotaxim.....	C	kotrimoxazol.....	C

Nález 1: **Haemophilus influenzae**

ANTIBIOGRAM (disková difuzní metoda)

ampicilin.....	R	nalidixová kyselina.....	C
cefuroxim.....	C	chloramfenikol.....	C
cefotaxim.....	C	kotrimoxazol.....	C
tetracyklin.....	C		

ANTIBIOGRAM – MIC v mg/l (E-test)

ampicilin /sulbactam.....	1	C	cefotaxim.....	0,023	C

Nález 2: **Haemophilus influenzae**

ANTIBIOGRAM (disková difuzní metoda)

ampicilin.....	R	tetracyklin.....	C
amoxicilin /klavulanát...	R	nalidixová kyselina.....	C
cefuroxim.....	R	chloramfenikol.....	C
cefotaxim.....	C	kotrimoxazol.....	R

H. influenzae – treatment

- Meningitis, epiglottitis
  - Third-generation cephalosporins
- Aminopenicillins, extended-spectrum aminopenicillins

- Prevention – vaccination (Hib – hexavalent vaccine)

od započatého 9. týdne (2 měsíce)	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
2.–3. měsíc	
4 měsíce	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
4.–6. měsíc	
11.–13. měsíc	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
12.–15. měsíc	
13.–18. měsíc	Spalničky, zarděnky, příušnice
od dovršení 5. do dovršení 6. roku věku dítěte	Spalničky, zarděnky, příušnice
	Záškrt, tetanus, černý kašel
od dovršení 10. do dovršení 11. roku	Záškrt, tetanus, černý kašel, dětská obrna

The HACEK Group

- G-negative rods, microbiota of the lower respiratory tract and pharynx
- Less common causes of infective endocarditis
- *Haemophilus spp.*
- *Aggregatibacter spp.*
- *Cardiobacterium cpp.*
- *Eikenella corrodens*
- *Kingella kingae*
  - + osteomyelitis - infants, toddlers

https://microbe-canvas.com/Bacteria/gram-negative-rods/facultative-anaerobic-3/no-growth-on-mcconkey-agar-without-salt-1/catalase-negative-3/oxidase-positive-5/eikenella-corrodens.html

Bordetella

Bordetella

- G-aerobic rods
- Extremely sensitive to drying, toxins and toxic substances

- Representatives?
  - *Bordetella pertussis*
  - *Bordetella parapertussis*

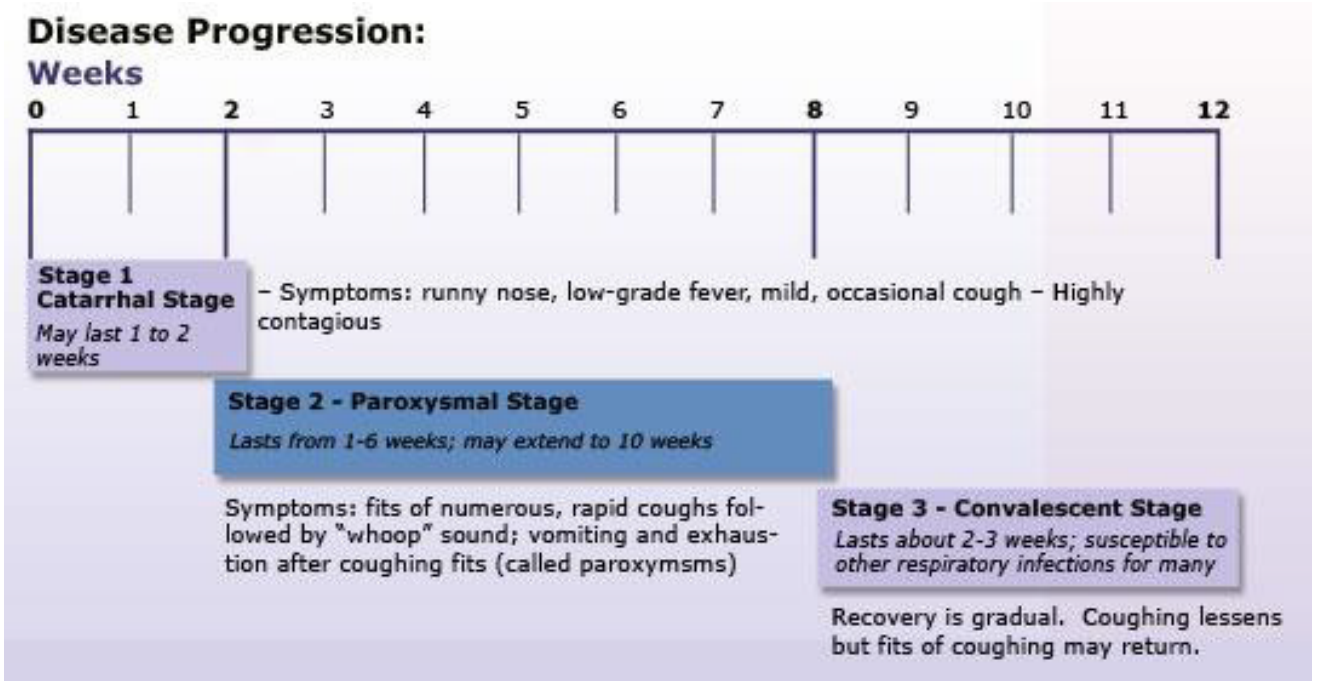
B. pertussis – virulence factors (examples)

- Filamentous haemagglutinin, pertactin, fimbriae
  - Adhesion factors
- Pertussis toxin
  - Influence on the immune response, leukocytosis with lymphocytosis, …

- → Contained in vaccines

Bordetella pertussis

- Pertussis – whooping cough
  - At-risk population – infants
  - Catarrhal stage
  - Paroxysmal stage
    - https://www.youtube.com/watch?v=S3oZrMGDMMw
  - Convalescent stage
- Atypical course
  - Other age groups



https://www.cdc.gov/pertussis/about/signs-symptoms.html

Bordetella pertussis – diagnosis

- Sample collection
  - material from the lower respiratory tract (sputum), nasopharyngeal swab
  - ~~Blood culture~~ (non-invasive)
- Cultivation
  - First stage of the disease
  - Extended culture (5 days), Bordet-Gengou agar and others
- PCR (higher sensitivity)
- Serology
  - Antibodies against pertussis toxin

Protokol: SER-23-1143 --- Konečný výsledek ---
Vzorek: **Sérum**
Vyšetření: Bordetella pertussis - protilátky , Bordetella parapertussis - protilátky, Mycoplasma pneumoniae - protilátky, Chlamydophila pneumoniae - protilátky, Chlamydophila pneumoniae - konfirmace, Chlamydia trachomatis - protilátky, Chlamydia trachomatis - konfirmace, Stáčení séra

---

Chlamydia trachomatis screening

Chlamydia trachomatis IgA: negativní
Chlamydia trachomatis IgG: negativní
Chlamydia trachomatis West.Blot IgA: negativní
Chlamydia trachomatis West.Blot IgG: negativní

Chlamydophila pneumoniae (ELISA)

Chlamydophila pneumoniae IgA: 73,26 U/ml - POZITIVNÍ
Chlamydophila pneumoniae IgG: >320 U/ml - POZITIVNÍ
Chlam. pneum. West.Blot IgA: hraniční
Chlam. pneum. West.Blot IgG: POZITIVNÍ

Mycoplasma pneumoniae (ELISA)

Mycoplasma pneumoniae IgM: negativní
Mycoplasma pneumoniae IgG: negativní

Pertusse toxin

Pertusse toxin IgA: 19,5 IU/ml - POZITIVNÍ
Pertusse toxin IgG: negativní

Parapertusse

Bordetella parapertussis IgA: negativní
Bordetella parapertussis IgG: 3,05 index - POZITIVNÍ

Závěrečné hodnocení:
Chlamydophila pneumoniae - nejspíše se jedná o postakutní fázi infekce nebo anamnestické protilátky, k potvrzení výsledku doporučuji kontrolní vyšetření za 2-3 týdny.
Pertuse toxin - k rozlišení počáteční fáze infekce a nespecifické aktivace IgA protilátek je nutné vyšetření párového séra v odstupu cca 10 - 14 dní.
Parapertuse - nejspíše se jedná o anamnestické protilátky, k potvrzení doporučuji kontrolní vyšetření párového séra v odstupu cca 10 - 14 dní.

Bordetella pertussis – treatment

- Macrolides – good penetration into the mucous membranes
- Catarrhal phase – greatest effect

- Prevention – vaccination
  - Hexavalent vaccine
  - Pertussis toxoid, filamentous haemagglutinin, pertactin
  - (+ fimbriae antigens)
  - 3rd trimester of pregnancy

od započatého 9. týdne (2 měsíce)	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
2.–3. měsíc	
4 měsíce	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
4.–6. měsíc	
11.–13. měsíc	Záškrt, tetanus, černý kašel, dětská obrna, virová hepatitida B, onemocnění vyvolaná Haemophilus influenzae b
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13.–18. měsíc	Spalničky, zarděnky, příušnice
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	Záškrt, tetanus, černý kašel
od dovršení 10. do dovršení 11. roku	Záškrt, tetanus, černý kašel, dětská obrna

Bordetella pertussis

- https://www.cdc.gov/vaccines/pregnancy/hcp-toolkit/tdap-vaccine-pregnancy.html

These first few months of life are when infants are at the greatest risk of contracting pertussis and having severe, potentially life-threatening complications from the infection. To help protect babies during this time when they are most vulnerable, women should get the tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) during **each** pregnancy. A strong recommendation from you may be what most influences whether or not your patient's newborn is protected against pertussis.

5 Facts about Tdap and Pregnancy

1. **Tdap during pregnancy provides the best protection for mother and infant.**
    - Recommend and administer or refer your patients to receive Tdap during every pregnancy.
    - Optimal timing is between 27 and 36 weeks gestation (preferably during the earlier part of this period) to maximize the maternal antibody response and passive antibody transfer to the infant.
    - Fewer babies will be hospitalized for and die from pertussis when Tdap is given during pregnancy rather than during the postpartum period.

B. parapertussis

- Disease
  - parapertussis, pertussis-like syndrome
- Does not produce pertussis toxin
- Cannot be prevented by vaccination

Legionella pneumophila

Legionella pneumophila

- Origin of the name?
- G-aerobic rods
- Pontiac fever
  - Flu-like symptoms
- Legionnaires' disease
  - Severe ‘atypical’ pneumonia
- At-risk groups
  - Elderly people, immunocompromised individuals

https://depts.washington.edu/molmicdx/mdx/tests/ambpcr.shtml

Legionella pneumophila – diagnosis

- Sample collection
  - Sample from the DDC
  - Nasopharyngeal swab (PCR)
  - Urine
- Culture
  - 5 days, enriched media
- PCR
- Ag testing of urine (type 1)

- → macrolides, fluoroquinolones

http://www.biomerieux-culturemedia.com/product/95-legionella-agar-with-l-cysteine--bcye--and-without-l-cysteine--bcy-

Protokol: LMM-23-2099 --- Konečný výsledek ---
Vzorek: **Výtěr z nazofaryngu**
Vyšetření: atypické pneumonie - PCR

Atypické pneumonie - vyšetření metodou PCR
*(V závorce uvedena relativní kvantita nabývající hodnot od * [nejmenší] do ***** [největší])*

Mycoplasma pneumoniae: negativní
Chlamydophilla pneumoniae: negativní
Chlamydophilla psittaci: negativní
Legionella pneumophila: negativní
Legionella longbeachae: negativní
Pneumocystis jiroveci: negativní
Cryptococcus neoformans: negativní

Závěrečné hodnocení:

Upozornění: Pro správnou interpretaci nálezu je vhodné pamatovat na to, že byl v tomto případě **vyšetřen materiál z horních cest dýchacích** (HCD). **Pozitivní výsledek** může často znamenat přítomnost daného agens i v dolních cestách dýchacích (DCD), ale někdy jen pouhou kolonizaci HCD (př. *Pneumocystis*). **Negativní výsledek** zcela nevylučuje možnou přítomnost daného agens v DCD (př. *Legionella*). V takovém případě (při klinickém obrazu svědčícím pro atypickou pneumonii) doporučujeme výsledek verifikovat vyšetřením vzorku odebraného z DCD.

Moraxella catarrhalis
Formerly Branhamella catarrhalis

Moraxella catarrhalis

Formerly *Branhamella catarrhalis*

Moraxella catarrhalis

- G-aerobic diplococcus
- Commensal of the mucous membranes of the upper respiratory tract
- Sinusitis, otitis
- Potentiated aminopenicillins
  - Beta-lactamase

- Colonies can be ‘moved’ on agar

Summary

- *Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae, Bordetella pertussis, Legionella pneumophila*
- The above-mentioned pathogens can be cultured in routine clinical practice. However, this often requires special enriched media and the culture takes longer (compared to, for example, staphylococci, enterobacteria, etc.).
- PCR can be used to diagnose the above-mentioned pathogens (depending on the sample taken and the microorganism detected)
- In some cases, serological testing for antibodies (pertussis) can be performed.