MICROBIOLOGY

INTRODUCTIOM TO MEDICAL MIKROBIOGY BASIC CHARACTERITICS OF BACTERIA, VIRUSES, FUNGI AND PARAZITES

MEDICAL PARASITOLOGY

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PRACTICAL BLOCKS 1 & 2, summer semester 2020 GROUPS TIME SCHEDULE

Dentistry - 2020			
Wee k	Date	Work group	Teacher
2	02.03.2020	2151	Bobek
9	20.04.2020	2151	Pavlík

Start: 8 a.m.



PRACTICAL BLOCK Rescheduling due to illness is possible only as agreed with dr. Jan Bobek (jan.bobek@lf1.cuni.cz)

EXAMS, PREREQUISITES, ETC. Mgr. Kateřina Petříčková, Ph.D. (katerina.petrickova@lf1.cuni.cz) MUDr. Emil Pavlík, CSc. (pavlik.emil@seznam.cz)



HISTORY

c. 4 BC Fundamental medical procedures

till 15th century lack of information on causes of infectious diseases (e.g., in cholera, black death it was assumed to be caused by miasma = a noxious form of "bad air")



Hippocrates (born 460 BC, Kos, Greece) Father of Medicine - founder of the Hippocratic School of Medicine



Source: https://en.wikipedia.org/wiki/Miasma_theory

A representation by Robert Seymour of the cholera epidemic of the 19th century depicts the spread of the disease in the form of poisonous air.

HISTORY

4. st. BC Fundamental medical procedures

- till 15th century lack of information on causes of infectious diseases (e.g., in cholera, black death it was assumed to be caused by miasma = a noxious form of "bad air")
- 1553 G. Fracastoro: a hypothesis on epidemic diseases are caused by small particles or "spores" which spread by
 - direct contact
 - indirect contact
 - without direct contact for long distances



Girolamo Fracastoro 1478-1553

Robert HOOKE (1632-1723)

Probably, the first observer of microorganisms.



Hooke's microscope, from an engraving in *Micrographia*. Source: https://en.wikipedia.org/wiki/Robert_Hooke



Memorial portrait of Robert Hooke at Alum Bay, Isle of Wight, his birthplace, by Rita Greer (2012).

Source:https://en.wikipedia.org/wiki/Robert_Hooke#/media/File :Memorial_portrait_of_Robert_Hooke_at_Alum_Bay,_IoW_for_ Carisbrooke_Museum.JPG

Antonie van LEEUWENHOEK

(1635 - 1703)

Often considered to be the first acknowledged microscopist and microbiologists. He is best known for his pioneering work in the field of microscopy.





A replica of a microscope by van Leeuwenhoek.

Sources:

https://en.wikipedia.org/wiki/Antonie_van_Leeuwenhoek#/media/Fil e:Leeuwenhoek_Microscope.png

BACTERIOLOGY

Ferdinand Julius COHN

(1828 - 1898)

• the first who classify algae as plants, and defined what distinguishes them from green plants

 established classification of bacteria into four groups based on shape (sphericals, short rods, threads, and spirals) – still in use today

• described the life-cycle of *Bacillus* spp.



Source: https://en.wikipedia.org/wiki/Ferdinand_Cohn#

Edward JENNER

(1749 - 1823)





Source:http://wellcomeimages.org/indexplus/obf_i mages/63/06/f922c4b86c952df78a555cd9eba8.jpg

The steps taken by Edward Jenner to create vaccination, the first vaccine for smallpox. Jenner did this by inoculating James Phipps with cowpox, a virus similar to smallpox, to create immunity, unlike variolation, which used smallpox to create an immunity to itself.

The terms "vaccine" and "vaccination" are derived from Variolae vaccinae (small-pox of the cow), the term devised by Jenner to denote cowpox. He used it in 1796 in the long title of his *Inquiry into the Variolae vaccinae known as the Cow Pox*, in which he described the protective effect of cowpox against smallpox.

Ignác Fülöp SEMMELWEIS

Ignaz Philipp Semmelweis (1818-1865)



Source: https://en.wikipedia.org/wiki/

An early pioneer of antiseptic procedures.

- discovered that the incidence of puerperal fever (also known as "childbed fever") could be drastically cut by the use of hand disinfection in obstetrical clinics.



<u>Puerperal fever</u> yearly mortality rates for the First and Second Clinic at the Vienna General Hospital 1841-1846. The First Clinic evidently has the larger mortality rate.



Puerperal fever monthly mortality rates for the First Clinic at Vienna Maternity Institution 1841–1849. Rates drop markedly when Semmelweis implemented chlorine hand washing mid-May 1847.

Louis PASTEUR

(1822 - 1895)

He is regarded as one of the three main founders of bacteriology together ith F. Cohn and R. Koch.

Remembered for his remarkable discoveries of the principles of vaccination, microbial fermentation and pasteurization, i.e. for breakthroughs in the causes and prevention of diseases.



• Provided direct support for the germ theory of diseases and its application in clinical medicine.

principles of vaccination remarkable breakthroughs in the causes and prevention of diseases reduced mortality from puerperal fever created of the first vaccines for rabies and anthrax

- discovered pasteurization (warming of milk to stop the bacterial contamination) prevention for TBC, samonellosis, brucellosis, diphteria, etc.
- discovered microbial fermentation



Although pathogens are the principal cause of diseases, environmental and hereditary factors often influence the severity of the disease, and whether a potential host individual becomes infected when exposed to the pathogen.

Joseph LISTER (1827-1912)

Pioneer of antiseptic surgery.

Championed the use of carbolic acid as an antiseptic, so that it became the first widely used antiseptic in surgery, i.e. he successfully introduced carbolic acid (now known as phenol) to sterilize surgical instruments and to clean wounds.









Lister spraying phenol over patient.

Heinrich Hermann Robert KOCH (1843-1910)

1892: Identification and cultivation of the causative agents tuberculosis (TB) *Mycobacterium tuberculosis*



Mycobacterium tuberculosis

Source of pictures: Wikipedia.org







M. tuberculosis in the tissue

Heinrich Hermann Robert KOCH (1843-1910)

1876: Identification of *Bacillus anthraxis* causative agent of anthrax



Source: Wikipedia







So

http://textbookofbacteriology.net/Anthrax.html

Heinrich Hermann Robert KOCH

(1843 - 1910)

- began to utilize agar to grow and isolate pure bacterial cultures
- implemented the staining of bacteria by aniline
- performed the first photos of bacteria
- his research led to the creation of Koch's postulates (series of four generalized principles linking specific microorganisms to specific diseases)





Sourcej: www.herbarium.usu.edu

Alexander FLEMING

(1881 - 1955)

Scottish physician, microbiologists and pharmcologists Saint Marys, London;coworker: A.Wright

1922: baktericide enzyme - lysozyme

1928 Discovery of benzyl peniciline (Peniciline G) – from the mould of *Penicillium chrysogenum* (syn. *P. notatum*)

Howard Walter FLOREY (1989-1968) Ernst Boris CHAIN (1906-1979)

1940: isolation and concentration of **the germ-killing agent in penicillin.**

Since 1941: PNC aplication in treatment

In the CR: after 2nd World War







Sources: https://upload.wikimedia.org/wikipedia/commons/, www.heritage.anu.edu.au

ERNEST DUCHESNE (30.5.1874 – 12.4.1912)





In 1987, 32 years before A. Fleming, discovered the antibiotic properties of penicilin, but his research went unnoticed.



Dmitri losifovich IVANOVSKY

(1864-1920)

1892: investigation on tobacco disease causing great damage to plantations – discovery of viruses

Martinus BEIJERINCK

(1851-1931)

1898: filtration experiments demonstrating that tobacco mosaic disease is caused by an infectious agent smaller than a bacteria

Stanislaus Josef Mathias von PROWAZEK

(1875-1915)

- 1913: with L. Halberstaedter described inclusion bodies (Halberstaedter-Prowazek bodies) of *Chlamydia trachomatis*, the causative agent of trachoma
- 1913: with H. da Rocha Lima discovered *Rickettsia prowazeki,* the agent of epidemic typhus









1915, Frederick W.TWORT 1917, Félix d,HÉRELLE

Discovery of viruses that infect of bacteria = bacteriophages



 \rightarrow **1930:** cultivation of viruses – experimental animals

1931: cultivation on chicken embryos



1949: J.F.ENDERS, T.H.WELLER, F.Ch.ROBBINS: discovery that poliomyelitis virus is capable of growing in **cultures of different tissues**



CZECH REPUBLIC

August Josef CORDA

(1809-1849)

• under the microscope, observed algae, ciliates under and fungi

Vilém Dušan LAMBL

(1824-1895)

• identified Giardia lamblia



Jaroslav HLAVA

(1855-1924)



Giardia lamblia

Founder of Czech bacteriology

Ivan HONL (1866 – 1936)

Besides other things, one of the early researchers on antibiotics and serological examinations.



INFECTIOUS DISEASES AND PATHOGENS



Germ that that can produce a **DISEASE**

PATHOGENICITY the ability of an organism to cause disease and harm the host

- ability represents a genetic component of the pathogen
 - pathogenic species
 - non-pathogenic species
- the overt damage done to the host is a property of the host-pathogen interactions
- lack of ability to cause disease in primarily healthy individuum is typical for
 - commensals
 - opportunistic pathogens

PATHOGENICITY AND VIRULENCE OF MICROORGANISMS



Germ that that can produce a **DISEASE**

PATHOGENICITY the ability of an organism to cause disease and harm the host

determined by

VIRULENCE FACTORS

i.e., pathogen abilities to infect or damage a host

Molecules produced by particular agent that add to their effectiveness and enable them to achieve the following:

- to settle ("colonization") at the host niche (this includes attachment to cells)
- immunoevasion, evasion of the host's immune response
- immunosuppresion, inhibition of the host's immune response
- entry into and exit out of cells (if the pathogen is an intracellular one)
- obtain nutrition from the host

INNATE PATHOGENICITY

Includes mode of transmission which is necessary for natural spreading of a disease in a population

- obligate (primary) pathogen: induce a disease in a healthy individual
- facultative (opportunistic) pathogen: induce a disease under particular (i.e., defined) conditions only

OBLIGATE PATHOGENS

- Causal agent of "classical" infections: Corynebacterium diphtheriae (diphteria); Salmonella Typhi (typhoid fever); Neissseria gonorrhoeae (gonorrhoea)...
- Differences between various species/strains (Yersinia pestis is more pathogenic than Streptococcus pyogenes)
- Host specificity specilization on
 - •• **one** host species only (e.g., *Shigella* spp., gonococci humans only)
 - •• various host species (stafylococci, Lyssavirus virus humans as well as other vertebrates)

FACULTATIVE PATHOGENS

INDUCTION OF INFECTION IS BASED ON PARTICULAR CONDITIONS

• change of natural occurrence (Escherichia coli:

intestine x urogenital tract, blood system...)

immunosuppresion

(diabetes, corticosteroids, antibiotics, tumours, presence of strange units)

The presence of pathogenic bacteria on a body surface (like on the skin, mouth, intestines or airway) **without causing clinical evidence** of infection in the person.

Example: S. aureus on the skin

PATHOGENS INFECTIOUS AGENTS THAT CAUSE DISEASE



helmints

arthropods

MULTICELLULAR ORGANISMS

THE RANGE OF SIZES SHOWN BY PROKARYOTES, RELATI-VE TO THOSE OF OTHER ORGANISMS AND BIOMOLECULES


DIFFERENCES BETWEEN MICROOGRANISMS

I. NUTRITION

- autotroph
- heterotroph

II. OXYGEN REQUIREMENTS

- aerobes
- facultative aerobes
- microaerophiles
- aerotolerant
- obligate anerobes

III. METABOLISM

- fermentation
- aerobic respiration
- anaerobic respiration

IV. TEMPERATURE REQUIREMNTS

- psychrophiles
- mesophiles
- thermophiles

= factors influencing the settlement in a particular host organism

NUTRITION

autotrophs ("self-feeding" organisms) produce organic compounds from simple anorganic substances present in its surrounding; they do not need a living source of energy or organic carbon (e.g., algae, come bacteria

 heterotrophs – inable to produce organic compounds from anorganic; their nutrition depends on the existence of autotroph organisms



Source: https://en.wikipedia.org/wiki/;Matthewiparker NASA Earth

en.wikipedia.org/wiki/; Norman Kuring



GROUPS OF ORGANISMS AND THEIR METABOLISMS

GROUP OF ORGANISMS	OXYGEN REQUIREMENTS	METABOLISM
obligate aerobes	survive and grow only in an oxygenated environment	aerobic respiration
facultative aerobes	can tolerate different concentration of oxygen, but in the presence of oxygen grow better	aerobic and anaerobic respiration, fermentation
microaerophiles	require oxygen for energy production, but in lower than atmospheric concentration	aerobic respiration
facultative anaerobes	do not use oxygen, but are not harmed by it	fermentation
obligate anaerobes	do not use oxygen which can kill them	fermentation or anaerobic respiration

TEMPERATURE

Psychrophiles

- survive under temperatures of below 20 °C

Mesophiles

- survive under temperatures of 20°- 45° C

Thermophiles

- survive under temperatures of 45° - 60 °C

PSYCHROPHILES

- survive under temperatures of below 20 °C

Toleration to extreme cold: *Athrobacter* spp., *Pseudomonas* spp. *Hypho-monas* spp., etc.

Pseudomonas spp. - known for its ubiquity, can cause disease in plants and animals, including humans



Pseudomonas aeruginosa: Gramnegative, rod shaped bacterium of medical importance – multidrug resistant pathogen associated with serious illnesses – hospital acquired infections (ventilator-associated pneumonia, various sepsis syndromes)



Sources of pictures: wikipedia.org

MESOPHILES

- survive under temperatures of 20°- 45° C;

grow optimal at 37 °C

Acc. V. Spot Magn_Det_WD 1 µm

Under a very high magnification of 20,000x, this scanning electron micrograph (SEM) shows a strain of *Staphylococcus aureus* bacteria taken from a vancomycin intermediate resistant culture (VISA).

Escherichia coli



Sources of pictures: https://en.wikipedia.org/wiki

Staphylococcus aureus

THERMOPHILES

- survive under temperatures of 45° - 60°C

Occurrence: geothermally heated regions and deep see hydrothermal vents, as well as decaying plat matter (peat bogs, compost)



Grand Prismatic Spring and Midway Geyser Basin, Yellowstone National Park: brigth color produced by thermophils

Sources : https://en.wikipedia.org/wiki

Why to know these properties?

REQUIREMENTS OF MICROORGANISMS

NUTRITION, ENERGY, TEMPERATURE, OXYGEN

OTHER FACTORS INFLUENCING GROWTH AND REPRODUCTION (.g., hydrostatic pressure; concentration of hydrogen ions; etc.)

CULTIVATION OF MICROORGANISMS IN VITRO → IDENTIFICATION OF INFECTIOUS AGENT Wikinedia STUDY \rightarrow **ADEQUATE THERAPY** PREVENTION OF INFECTION

BASIC GROUPS OF MICROBIAL PATHOGENS



PRIONS

Abnormal transmissible pathogenic agents (PrPSc)

- ability to induce abnormal folding of specific normal cellular proteins called prion proteins (PrPc)
 - - abnormal folding leads to tissue-brain damage

Prion diseases (transmissible spongiform encephalopathies, **TSEs**)

- rare progressive neurodegenerative disorders in humans, animals
- long incubation periods
- characteristic spongiform changes associated with neuronal loss
- failure to induce inflammatory response.



Sponge-like lesions in the brain tissue of a classic CJD patient. Source: CDC

Creutzfeldt-Jakob Disease (CJD) Variant Creutzfeldt-Jakob disease (vCJD) Bovine spongiform encephalopathy (BSE) Chronic wasting disease (CWD)

VIRUSES

- Small infectious agents most have a diameter between 20 and 300 nm (i.e., cannot be seen with optical microscope).
- Transmission: travelling within a fluid, such as mucus droplets from a sneeze.
- Replication <u>only inside the living cells</u>.
- Infect all types of hosts (animals, plants, microorganisms, including bacteria and archaea)



Genomic diversity among viruses					
Property	Parameters				
	- DNA				
Nucleic acid	- RNA				
	- Both DNA and RNA (at different stages in the life cycle)				
	- Linear				
Shape	- Circular				
	- Segmented				
	- Single-stranded				
Strandedness	- Double-stranded				
	- Double-stranded with regions of single-strandedness				
	- Positive sense (+)				
Sense	- Negative sense (-)				
	- Ambisense (+/-)				

A virus has either a DNA or a RNA genome and is called a DNA virus or a RNA virus, respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes.

Encophalitic/	ں Viral	verview of infections			
- JC virus - Measles - LCM virus	Common cold - Rhinoviruses - Parainfluenza virus - Respiratory syncytial		— Eye - Her - Ade - Cyt	 Eye infections Herpes simplex virus Adenovirus Cytomegalovirus 	
- Rabies	Gingivostomatitis—	- Mu	otitis umps rus	 Pneumonia Influenza virus, Types A and B 	
- Adenovirus	- Herpes simplex type	e 1		- Parainfluenza	
- Epstein-Barr vir	rus	ALLA I		virus	
 Cytomegaloviru 	IS	APR /		- Respiratory	
Cardiovascular		EAN ready		- Adenovirus	
- Coxsackie B vi	rus	TO TON		- SARS coronavirus	
Honotitio		ALL ALL ALLA			
- Hepatitis virus		No the		— Myelitis	
types A. B. C.	D. E			- Poliovirus	
o,	_,_			- HTLV-I	
Skin infections				Gastroontoritis	
- Varicella zoste	virus 6	12-1-		- Adenovirus	
- Smallpox	Wirds O	1 Start		- Rotavirus	
- Molluscum con	tagiosum	A STRAN		- Norovirus	
- Human papillo	mavirus Se	kually transmitted		- Astrovirus	
- Parvovirus B19) dis	eases		- Coronavirus	
- Rubella	- H	erpes simplex type 2	\ -) an ava atitia	
- Measles	- H	uman papillomavirus	— P	Coverations	
 Coxsackie A vi 	rus - H	IV	-	CONSACRIE D VIIUS	

Source: Häggström, Mikael (2014). "<u>Medical gallery of Mikael Häggström 2014</u>". WikiJournal of Medicine 1 (2). <u>DOI:10.15347/wjm/2014.008</u>. <u>ISSN 2002-4436</u>. <u>Public Domain</u>.





Author: Metju 12

VARICELLA

Varicella zoster virus



Source: https://study.com/academy/lesson/varicella-zoster-virussymptoms-treatment.html; author: Thomas Netsch

RUBELLA

Rubella virus (RuV)

Generalized rash on the abdomen. Source: CDC.com

Cataracts due to congenital rubella syndrome. Source: http://phil.cdc.gov/phil





MEASLES (Morbilli)

Causative agent: Measles virus (MeV)



A classic day-4 rash with measles.

Source: CDC/NIP/ Barbara Rice

Skin of a person after 3 days of measles infection. Source: CDC/Dr. Heinz F. Eichenwald

MEASLES (Morbilli)

Causative agent: Measles virus (MeV)



Kolpik's spots on the third pre-eruptive day. Source: CDC

Koplik's spots on the day of measles rash. Source: Wikipedia, <u>Dctrzl</u> - Own work

Deaths from measles per million persons in 2012



Source: WHO, Wikipedia



Rates of measles vaccination worldwide



Source: Wikipedia, BlankMap-World8.svg: AMK1211



Rotaviruses

(primary human infection by species A, B and C, most commonly by species A

> Electron micrograph of rovirus particles reacting with a monoclonal antibody specific for the viral capsid protein Vp6. *Author: Dr. Graham Beards. Source: Wikipedia.org*





Rotaviruses in the faeces of an infec-

ted child. *Author: Dr. Graham Beards. Source: Wikipedia.org*

INFLUENZA (the flu)

Influenza viruses



Influenza virus. Author: BruceBlaus, Source: Wikimedia Commons



Source: Medical gallery of Mikael Häggström 2014. WikiJournal of Medicine 1 (2). ISSN 2002-4436.



The different sites of infection (shown in red) of seasonal H1N1 versus avian H5N1. Author: TimVickers, Source: Wikimedia Commons

CORONAVIRUS DISEASE 2019 (COVID-19)

Other names:

- 2019-nCoV acute respiratory disease
- novel coronavirus pneumonia (2019-nCoV)

CORONAVIRUS DISEASE 2019 (COVID-19)

• infectious disease

• causative agent: severe acute respirátory syndrome coronavirus 2 (SARS-CoV-2) (formerly 2019 novel coronavirus 2019-nCo V)

• spread: between people via respiratory droplets

• time from exposure – to symptoms: generally 2-14 days

 limitation of spread: handwashing and other hygiene measures, wearing a surgical mask

CORONAVIRUS DISEASE 2019 (COVID-19)

 symptoms: the infection may initially present with few or no symptoms or develop into fever, coughing, shortness of breath, pain in the muscles, tiredness

- complications: pneumonia, ARDS, kidney failure
- diagnostic method: PCR testing, medical imaging
- treatment: symptomatic and supportive only no specific antivirals available

CORONAVIRUS DISEASE 2019 (COVID-19): SYMPTOMS





Source: WHO

CORONAVIRUS DISEASE 2019 (COVID-19): TRANSMISSION MODES

Scanning electron microscope

image of SARS-CoV-2 (yellow)

Transmission: \rightarrow direct \rightarrow contact - someone touches a surface \rightarrow aeroso contaminated with tainted respiratory droplets - inhalation of air contaminated with tainted respiratory droplets

Source: WHO

CORONAVIRUS DISEASE 2019 (COVID-19): LIMITATION OF SPREAD

- frequent washing of hands with soap and water
- not touching the eyes, nose, or mouth with unwashed hands
- practicing good respiratory hygiene

PREVENTION OF TRANSMISSION

- infected individuals stay at home except to get medical care
- call ahead before visiting a healthcare service
- wear a facemask (especially in public)
- cover couhgs and sneezes with a tissue
- regular wash hands with soap and water
- avoid sharing personal household items

CORONAVIRUS DISEASE 2019 (COVID-19): LIMITATION OF SPREAD

- frequent washing of hands with soap and water
- not touching the eyes, nose, or mouth with unwashed hands
- practicing good respiratory hygiene

PREVENTION OF TRANSMISSION IN HOSPITALS

recommendations see www.who.int

HEPATITIS

Hepatitis A virus (HAV) Hepatitis B virus (HBV) Hepatitis C virus (HCV) Hepatitis D virus (HDV) Hepatitis E virus (HEV)

TRANSMISSION

- fecal-oral
- by infectious fluids (blood, mucus)
- by infectious fluids (blood, mucus)
- by infectious fluids (blood, mucus)
- fecal-oral

Symptoms:

Yellow discoloration of the skin and whites of the the eyes, poor appetite, vomiting, tiredness, abdominal pain, or diarrhea.



Jaundiced eyes. Source: http://phil.cdc.gov/phil. Author: Thomas F. Sellers/Emory University Thomas F. Sellers/Emory University

POLIOMYELITIS

Highly contagious via the fecal-oral (intestinal source) and the oral-oral (oropharyngeal source) routes.



Poliovirus. TEM. Source: https://en.wikipedia.org/wiki/Poliomyelitis



Map of countries with polio cases in 2017. Source:https://en.wikipedia.org/wiki/Poliomyelitis



A girl with a deformity of her right leg due to polio Source: https://phil.cdc.gov



Man with atrophy and paralysis of the right leg and foot due to polio. Source: CDC/NIP/ Barbara Rice

HIV/AIDS

Causative agent: human immunodeficiency virus (HIV)



https://en.wikipedia.org/wiki/HIV/AIDS



Source: Medical gallery of Mikael Häggström 2014. WikiJournal of Medicine 1 (2). ISSN 2002-4436.

BACTERIA

Wide diversity in:

- sizes (typically 0.5–5.0 µm in length)
- shape (cocci, bacilli, vibrio, spirilla, spirochaetes)
- Unicellular

Lack of membrane-bound nucleus, mitochondria and membrane-bound organelles

Own metabolism



Source: http://www.printablediagram.com/bacteria

MORPHOLOGY AND ARRANGEMENT OF BACTERIA


GRAM	Gram staining procedure		Gram- <u>positive</u> cell wall		Gram- <u>negative</u> cell wall	
	Process of test	Appearance of Cells	Effect of Step	Effect on Cell Wall	Effect of Step	Effect on Cell Wall
	Step 1: Begin with heat fixed cells fixation		Step 1: Cell wall remains clear.		Step 1: Cell wall remains clear.	
	Step 2: Flood slide with crystal violet dye for 1 min. Crystal violet	t t	Step 2: Peptidoglycan cell wall is flocded with crystal violet and appears purple.		Step 2: Cell wall is stained purple from the crystal violet dye.	
	Step 3: Add iodine solution for 1 min. iodine	t t	Step 3: A crystal violet – iodine complex is formed within the peptidoglycan cell wall trapping the purple stain.		Step 3: A crystal violet- iodine complex is formed but does not adhere to the cell wall due to the thin layer of peptidoglycan.	
	Step 4: Wash slide with alcohol for 20sec. alcohol		Step 4: The crystal violet – iodine complex is trapped with the peptidoglycan cell wall and doesn't wash out.		Step 4: The crystal violet – iodine structure is washed out of the thin peptidoglycan layer.	
	Step 5: Counter stain with safranin. safranin		Step 5: As the peptidoglycan cell wall remains stained purple the red safranin has no effect.		Step 5: The red safranin stains the washed gram negative cells.	

Overview of **Bacterial infections**

Bacterial meningitis

- Streptococcus pneumoniae
- Neisseria meningitidis
- Haemophilus influenzae
- Streptococcus agalactiae
- Listeria monocytogenes

Otitis media

- Streptococcus pneumoniae

Pneumonia

Community-acquired:

- Streptococcus pneumoniae
- Haemophilus influenzae
- Staphylococcus aureus Atypical:
- Mycoplasma pneumoniae
- Chlamydia pneumoniae
- Legionella pneumophila Tuberculosis
- Mycobacterium tuberculosis

Skin infections

- Staphylococcus aureus
- Streptococcus pyogenes
- Pseudomonas aeruginosa

Eye infections

- Staphylococcus aureus
- Neisseria gonorrhoeae
- Chlamydia trachomatis

Sinusitis

- Streptococcus pneumoniae
- Haemophilus influenzae

Upper respiratory tract infection

- Streptococcus pyogenes
- Haemophilus influenzae

Gastritis

Helicobacter pylori

Food poisoning

- Campylobacter jejuni
- Salmonella
- Shigella
- Clostridium
- Staphylococcus aureus
- Escherichia coli

Urinary tract infections

- Escherichia coli
- Other Enterobacteriaceae
- Staphylococcus
- saprophyticus
- Pseudomonas aeruginosa

Source: Medical gallery of Mikael Häggström 2014. WikiJournal of Medicine 1 (2). ISSN 2002-4436.

Sexually transmitted diseases

- Chlamydia trachomatis

- Neisseria gonorrhoeae

- Treponema pallidum

- Ureaplasma urealyticum

- Haemophilus ducreyi

TONSILLITIS

group A streptococcus (G+)

Streptococcal phyryngitis.

Source: https://commons.wikimedia.org/wiki/, author: James Heilman, MD





Source: Blausen.com staff (2014). Medical gallery of Blasen Medical 2014. WikiJournal of Medicine 1 (2).

PURULENT DISEASES

Staphylococcus aureus







SCARLET FEVER (SCARLATINA)

Source: a group A *streptococcus* (Group A strep) infection (G+)

Signs and symptoms: sore throt, fever, headaches, swollen lymph nodes, and a characteristic (red) rash – sandpaper; the tongue may be red and bumpy. Mostly affects children between five and 15 years of age.





Strawberry tongue seen in scarlet fever. Source: https://en.wikipedia.org/wiki/; foto von Martin Kronawitter, Kellberg

The rash of scarlet fever. Source: he original uploader was Etreya at English Wikipedia. Foto: Alicia Williams.

SCARLET FEVER (SCARLATINA)



Red cheeks and pale area around the mouth in scarlet fever.

Source: he original uploader was Etreya at English Wikipedia. Foto: Alicia Williams.

TUBERCULOSIS

Causative agent: *Mycobacterium tuberculosis*



M. tuberculosis in the tissue

- a small, aerobic, non bacillus
- an unusual, waxy coating on its cell surface primarily due to the presence of mycolid acid

- coating makes the cells impervious to Gram staining, and as a result, the bacteriae can appear either Gram-negative or Gram-positive

- acid-fast stains [e.g., Ziehl-Neelsen or fluorescent stain (e.g.auramine)] are used instead to identify the bacteria microscopically



Source: Medical gallery of Mikael Häggström 2014. WikiJournal of Medicine 1 (2). ISSN 2002-4436.



In 2007, the number of cases of TB per 100,000 people was highest in sub-Saharan Africa, and was also relatively high in Asia



Egytian mummy in the British Museum – tubercular decay has been found in the spine.

SYPHILIS (BEJEL, PINTA, YAWS, etc.)

Causative agent:

spirochate bacterium (with subspecies) *Treponema pallidum*

Under light microscopy, treponemes are visible only by using dark field illumination.





Model of a head of a person with tertiary (gummatous) syphilis that is located in Musée de I,Homme, Paris. Source: wikipedia.com

LEPROSIS (Hansen's disease (HD)

Mycobacterium leprae





A 24-year-old man with leprosy (1886)

LEPER COLONY

Spinalonga, Crete – on of the last colony in Europe closed in 1957. Source: https://en.wikipedia.org/wiki/



Zdroj: Agricultural Research Service, photo by Scott Bauer.



Zdroj: CDC

YEASTS, MOLD

Eukaryota.

Reproduce mostly asexually, and many do so by the asymmetric division process known as budding.

Do not form mycelia (only pseudomycelia – similar to single-celled colonies)

Yeast - fungi that can adopt a single-celled growth habit

Mold - microscopical fungus that grows in the form of multicellular filaments called hyphae





Candida albicans: hyphal outgrowth and other morphological characteristics. Source:wikipedia.org;Y tambe – Y tambe s file

Many yeasts are used in various technologies



A block of compressed fresh yeast. Source:wikipedia.org;Hellahulla

Some of them can cause severe human diseases.



CAUSATIVE AGENTS OF HUMAN DISEASES - EXAMPLES

YEATS, MOLD





Penicillium spp. and Aspergilus spp. in axenic cultures

Examples of human pathogens	DISEASE
YEASTS Candida	candidiasis (thrush) (Candida albicans), systemic candidiasis
Cryptococcus	in immunosuppresed hosts, fatal pulmonary infections, meningitis, sepsis
Pneumocystis	in immunosuppresed hosts, intersticial pneumonia
DIMORPHIC FUNGI	in immunosuppresed hosts, severe pulmonary infections
Penicillium	In the past, <i>Talaromyces marneffei</i> (syn. <i>Penicillium marneffei</i>) was considered non-pathogenic, however, it can cause disseminated penicillosis in AIDS pacients (rezervoirs bamboo rats)
Fusarium	attack food, contamination of clinical material
Dermatophytes	infections of hair and nails



Thrush in a child who had taken antibiotics. Source:wikipedia.com;author James Hilman, MD.

Candida albicans



Source: CDC, Photo Credit: Sol Silverman, Jr., D.D.S.



Skin candidiasis. Source:wikipedia.org; U.S. Department of Veterans Affairs



Nail candidiasis (onychomycosis). Source: wikipedia.org;Medquy at English Wikipedia

Trichophyton rubrum



Inflammation in the skin between fingers Source: Wikipedia

Pneumocystis jirovecii



Cysts in bronchoalveolar fluid (Toluidine)



PARASITES

- size from 2 µm to metres
- eukaryots
- own metabolism

PROTOZOA HELMINTHS ARTHROPODS Giardia spp. Source: The Public Health Library, James D. Gathany Anopheles gambiae CDC/Dr. Mae Melvin Wikipedia

Plasmodium spp.

Hookworms

CAUSATIVE AGENTS OF HUMAN DISEASES - EXAMPLES

PARASITES





Examples of human pathogens	Disease
plasmodia	malaria (Plasmodium falciparum, P. vivax, P. ovale, P.malariae)
amoebae	dysentery (Entamoeba histolytica)
leishmania	visceral and cutaneous leishmaniasis
toxoplasma	toxoplasmosis (Toxoplasma gondii)
roundworms	ascariosis (Ascaris lumbricoides) , larval toxocarosis (Toxocara canis, T. cati)
tapeworms	cestodoses, cysticercosis, hydatidosis, echinococcosis
schistosomes	schistosomosis (Schistosoma mansoni, S. haematobium, S. japonicum)
hookworms	ancylostomosis intestinal symptoms and anaemia
filaria	filariosis



• causative agent:

Plasmodium falciparum

- P. vivax
- P. malariae
- P. ovale
- P. knowlesi

• vector:



- endemic occurrence: 103 countries of tropics and subtropics
- the most important parasitic disease
 - •• 300 500 millions infected
 - >100 millions reported cases/year
 - •• 1.5 2.7 millions of fatal cases/year (mostly children under 5 years)



MALARIA: OCCURRENCE



Plasmodium SPECIES INFECTING MAN

P. falciparum • causative agent of the most severe malaria:



malaria tropica, malaria perniciosa

- in tropics and subtropics
- responsible for 80% of malaria cases
- does not form hypnozoites in liver

P. vivax

• malaria tertiana



 in tropics, subtropics and temperate zones; more frequent in Asia and South America

• forms hypnoozoites in the liver \rightarrow relapses

Plasmodium SPECIES INFECTING MAN

P. ovale • malaria tertiana • in tropics and subtropics, geographic distribution

Source CDC

Africa - Ghana, Nigeria, Tanzania, Uganda Asia - Vietnam South America - Northern region

• forms hypnozoites in the liver \rightarrow relapses

P. malariae

distribution



• malaria quartana

• in tropics and subtropics, geographic limited:

Africa: east coast, west coast Asia: malaysia, indonesia, sri lanka America: caribean region

• preference for old erythrocytes (in blood, long life-span (over 10 year) at low parasitaemia (recrudescence)

LABORATORY DIAGNOSIS

IMPORTANT: data on patient history (chemoprofylaxis - which type, when it was used), visit of endemic country (which, long of stay)

- detection of parasites, DNA (PCR),
- material: peripheral blood

GOLD STANDARD: blood smears enable determination of

- species
- parasitaemia





- Direct imunofluorescence
- Antigenemia (only *P. falciparum*) RIA, dot ELISA
- Imunochromatography (ParaSight, BectonDickinson)

LEISHMANIA & LEISHMANIOSES

Occurrence: tropical and subtropical countries

(except of Australia)

risk of infection: 350 milion people prevalence: 12 millions incidence: 1.5-2 millions per year

- Vectors: sanflies (*Phlebotomus*, *Lutzomyia*)
- Host: humans and animals





LEISHMANIA SPECIES:

- Mostly ZOONOTIC (approx. 100 animal species)
- Few ANTHROPONOTIC (e.g. visceral caused by L.donovani)

dogs



Source: http://www.vet.uga.edu, Noah's Arkive, University of Georgia.

rodents



Source: www.moscowzoo.ru/galeng/asp

Leishmania sp.



CUTANEOUS LEISHMANIOSIS





Old World: Phlebotomus



New World: Lutzomyia

size: 2-3 mm

- daylight-inactive (most active at dusk)
- silent flighting downstairs



Data source: WHO/CSR/EDC-UNAIDS Map production: Public Health Mapping Group Communicable Diseases (CDS) World Health Organization, October 2003


CLINICAL SYMPTOMS AND SIGNS

VISCERAL LEISHMANIOSIS skin phase asymptomatic

escape of infected macrophages from skin to blood and lymph silent spread to liver spleen, bone marrow and lymphatic glands
proliferation of parazites → increasing load of infected macrophages → reticuloendothelial hyperplasia → splenomegaly



L. donovani anthroponosis (kala-azar) *L.infantum* zoonosis (dog)

incubation period: 10 days up to 1 year

• fever, malaise, shivering or chils (cough, diarrhoea), weight loss, wasting, anorexia, splenomegaly

DIAGNOSIS

DIRECT detection of amastigotes in organ biopsies (spleen, liver, **sternum**, lymph nodes) – histology, cultivation in vitro

INDIRECT detection of antibodies

Entamoeba histolytica & AMOEBOSIS

- intestinal (diarrhoea, dysentery, colititis) amoebosis: - extraintestinal (liver abscess)
- transmission: **per os** (contaminated food, drinks, + anal-oral sex)
- **cosmopolitan** (mainly in developing countries) occurrence: hyperendemic: Mexico, Egypt, India, Vietnam
- approx. 50 millions of invasive infections with prevalence: 40-100 thousands of fatal cases per year



risk factors - indadequate disposal of human feces; poor epidemiology: personal hygiene; use of human feces as fertilizer for vegetables; freshening of vegatables and fruits by contaminated water

Entamoeba histolytica: LIFE CYCLE

source of infections: a carrier with no or minor intestinal disturbances, by the stool of which the parasitic cysts are released



AMOEBIC DYSENTERY



The colonic mucosa invaded by *E. histolytica* trophozoites

Source of pictures:: Atlas of Medical Parasitology, Authors M. Lisci MD and G. Gera M.D.

Sigmoidoscopy: *E.histolytica* colitis



Sigmoidoscopy may show typical ulcers

114 Source of picture:: Atlas of Medical Parasitology

EXTRAINTESTINAL AMOEBOSIS



 always secondary: follows after intestinal infection (the spread of amoebae by venous system)

• in 80% the lesions develop in upper right liver lobe

• fever (38-40 °C), serology show the presence of specific antibodies

Entamoeba histolytica: LABORATORY DIAGNOSIS

IMPORTANT: data of patient history (i.e. contact with infected individuals, visit of endemic country

• DIRECT EXAMINATION OF STOOL SPECIMENS

- a) dysenteric stool (fresh less than 2 h), not refrigerated, detection of trophozoite (forma magna)
- b) standard coprological techniques detection of cysts produced by forma minuta

CULTIVATION

• **DETECTION OF COPROANTIGEN** (antigen capture ELISA from stool samples)

• INDIRECT – EXAMINATION OF PATIENT SERUM

detection of specific antibodies during extraintestinal form of the disease

HYDATID DISEASE (ECHINOCOCCOSIS)

1. Form: <u>CYSTIC</u> (CE)

AGENT: larval stages of *Echinococcus granulosus*



The eggs are infective for intermediate host immediately after their release into the surroundings.

Distribution: worldwide

The highest infection risks:

- certain areas of China
- North and East Africa
- South America (Uruguay)



Approximate Geographic Distribution of Hydatid Disease in Humans (Parasites and Parasitological Resources)

No risk: Iceland, Greenlad

E. granulosus: LARVAL STAGES





- slow growth (1-5 mm/year)
- life-span:

man.....even 53 year

CLINICAL SYMPTOMS AND SIGNS

- character of symptoms: dependence on cyst localisation
- early phase of the infection: asymptomatic manifestation delayed (several years)

most common: hepatic and pulmonary forms

hepatomegaly, bloody sputum in pulmonary form cerebral form - neurological symptoms

cyst rupture - allergic symptoms, anaphylatic shock, death



CE: LIVER INFECTION



in the liver: up to the size of 5 cm in diameter: usually, no symptoms

CE: LUNG CYST



chronic caugh, hemoptysis, pneumothorax, pleuritis, etc.

HYDATID DISEASE (ECHINOCOCCOSIS)

2. Form: <u>ALVEOLAR</u> (AE)

AGENT: larval stages of *Echinococcus multilocularis*



Occurrence: Northern Hemisphere

E. multilocularis: LARVAL STAGE

• development in the liver (99%)

- extremely slow growth (mm per year)
 + in the centre necrosis
 - $\downarrow \downarrow \downarrow \downarrow$

INCUBATION PERIOD: VERY LONG

(5-30 years)



Foto: Prof. P.Kern, Stuttgart

with progresion of the infection: invasion of surrounding tissues - malignant tumour (metastases of the parasite) fatality up to 100 % when untreated

HYDATID DISEASE: DIAGNOSIS

- IMMAGING (CT, MRI, ultrasound)
- SEROLOGY
- CLINICAL SYMPTOMS AND SIGNS
- PATIENT HISTORY
- Histology ("fine needle biopsy")

all data need to be evaluated together



PAIR puncture - aspiration - injection - reaspiration



SCHISTOSOMIASIS (BILHARZIASIS)

THE MOST IMPORTANT HELMINTHIC DISEASE



- infected: 193 millions of people
- at risk of infections: 652 mil. osob
- susceptibility to the infection: no differences in host sex and its age
- •• 85% of infections Africa

 \rightarrow from which 20% (i.e., 33 millions) of pregnant women from Subsaharan area

Forms of the disease:

urogenital: Schistosoma haematobium

intestinal: S. mansoni, S. japonicum group





Foto: Libuše Kolářová, In: För M: Atlas lékařské parazitologi



SCHISTOSOMIASIS

The main pathological agent: <u>EGGS</u> = SOURCE OF INFLAMMATORY REACTION



life-span of adult flukes: at least 35 years



eggs: limited life-span (cca 1 moth)

when adult flukes are present, in the tissues viable and non-viable



intestinal

urogenital

SCHISTOSOMIASIS: CLINICAL SYMPTOMS AND SIGNS





hepatosplenomegaly



involvement of other organs lungs, heart (cor pulmonale), etc.

Source: Atlas of Medical Parasitology, Author: Professor A.E. Butterworth FRS, Scientific Director

FILARIASES

Wuchereria bancrofti Brugia malayi, B. timori

- disease: lymphatic filariasis, elephantiasis
- vectors: mosquitoes of the gen. Aedes, Anopheles, Culex, Mansonia



LIFE CYCLE



Wuchereria bancrofti

http://www.dpd.cdc.gov/dpdx

SYMPTOMS AND SIGNS

infections

The main patogenic agent: adults in lymph vessels in the groin, scrotum, axillae etc.

excretion of antigens and toxic metabolites thickening of the walls of the lymph vessels lymphangitis, lymphadenitis elephantiasis

+ allergic reaction, secondary bacterial



Source: Atlas of Medical parasitology, Authors: 1,3 Professor Wallace Peters, 2 SmithKlime Beecham and WHO

Congestion of chyle - containing lymph around kidney and bladder (occasional breakage of lymph vessels) \rightarrow chyluria, hematochyluria)

In *B. malayi* – manifestations mild, usually without chyluria; mild elephantiasis, often i lower limbs only

Tropical eosinophilia – chronic inflammatory lesions in the lung (due to microfilariae); chest pain, cough,

dyspnea.



- vector: horse or deer fly (g. Chrysops)
- patobiology: adults in subcutaneous tissue (freely migrating)
 - acute inflammation mechanical injuries, allergic reactions, toxic metabolites
 - •• edema in focus with infiltrated eosinophils

(Calabar swelling)

ocasional migration in the eye or inner organs – specific symptoms



LIFE CYCLE



http://www.dpd.cdc.gov/dpdx

Wuchereria, Brugia, Loa: DIAGNOSIS

• microscopical detection of microfilariae: necessary repeated examinations of peropheral blood (each 6 hour in one day)

serology: detection of antibodies, circulated antigens W. bancrofti, Brugia - microfilaria nocturna



L. loa - microfilaria diurna



Onchocerca volvulus

- disease:
- vector:
- pathobiology:

1. onchocerciasis or 2. river blindness blackflies (g. Simulium)

- 1. adults in subcutaneous nodules (onchocercom, non migrating nodules)
- 2. microfilariae in the eye



Source: Bayer Manual of Pest Control



LIFE CYCLE

a = Diagnostic Stage

Blackfly Stages Human Stages Blackfly (genus Simulium) takes a blood meal www. (L3 larvae enter bite wound) 0 9 Migrate to head Subcutaneous tissues and blackfly's proboscis Λ 8 L3 larvae 3 Adults in subcutaneous nodule 7 L1 larvae Blackfly takes 5 Adults produce unsheathed a blood meal microfilariae that typically are (ingests microfilariae) found in skin and in lymphatics 6 Microfilariae pentrate of connective tissues, but also occasionally in peripheral blackfly's midgut blood, urine, and sputum. and migrate to thoracic muscles a Infective Stage

Onchocerca volvulus

http://www.dpd.cdc.gov/dpdx

ONCHOPHTALMIA

(river blindness)

due to the inlammatory reaction around the microfilariae in the eye



Zdroj: Otis Historical Archives Nat'l Museum of Health & Medicine

DIAGNOSIS

"skin snip" test





incubation at 37°C





Source: Atlas of Medical parasitology, Author: Gustavo A. Gini

histology


Thank you for attention