Spirochaetales

PROF. KÁROLY NAGY
Taxonomy

- *Spirochaetales* ordo
  - Spirochaetae classis

- *Spirochaetaceae* familia
  - *Treponema* genus
  - *Borrelia* genus

- *Leptospiraceae* familia
  - *Leptospira* genus
<table>
<thead>
<tr>
<th></th>
<th>Leptospira spp.</th>
<th>Borrelia spp.</th>
<th>Treponema pallidum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size [mikron]</strong></td>
<td>0,1×6–20</td>
<td>0,2–0,5×3–30</td>
<td>0,2×5–15</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td>spiral formed (corkscrew shaped), flexible</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>many smooth-waved winding with two terminal hooks</td>
<td>irregular, rough windings</td>
<td>compact, regular windings</td>
</tr>
<tr>
<td><strong>Staining</strong></td>
<td>stained readily with aniline dyes and silver impregnation</td>
<td>immune fluorescent staining only</td>
<td></td>
</tr>
<tr>
<td><strong>Native prep</strong></td>
<td>well visible with dark-field microscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cellwall</strong></td>
<td>Gram-negative</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><strong>Membranes</strong></td>
<td>outer and inner (cytoplasmic) membranes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Capsule</strong></td>
<td>glucoseaminoglycane</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Flagellae</strong></td>
<td>endoflagellae anchored at the pole</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>vigorous and characteristic: rotation, flection, locomotion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Morphology (ELMI)

Treponema genus

Leptospira genus

Borrelia genus
Biological properties in common

- Reproduction: binary fission
- Generation time: relatively long: 17-20 h
- High sensitivity for environmental factors
  ⇒ transmission (!)
  - Narrow temperature optimum
  - Narrow pH optimum
  - Poor osmotic resistance
### Culture of spirochaetae

<table>
<thead>
<tr>
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<th>Leptospira spp.</th>
<th>Borrelia spp.</th>
<th>Treponema pallidum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic culture media</strong></td>
<td>Cultivable</td>
<td>Cultivable</td>
<td><strong>Non-cultivable</strong></td>
</tr>
<tr>
<td><strong>Nutrient requirements</strong></td>
<td>fastidious</td>
<td>fastidious</td>
<td>?</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>8–13 weeks</td>
<td>4 days–6 weeks</td>
<td>?</td>
</tr>
<tr>
<td><strong>Atmospheric requirements</strong></td>
<td>Aerobic</td>
<td>Microaerophilic</td>
<td>(Anaerobic)</td>
</tr>
<tr>
<td><strong>Temperature optimum [°C]</strong></td>
<td>28–30</td>
<td>30–33</td>
<td>(30)</td>
</tr>
<tr>
<td><strong>Cell or tissue culture</strong></td>
<td>Cultivable</td>
<td>Cultivable</td>
<td><strong>Non-cultivable</strong></td>
</tr>
<tr>
<td><strong>Animal inoculation</strong></td>
<td>Many lab animals</td>
<td>Many lab animals</td>
<td>Only in rabbit testis</td>
</tr>
</tbody>
</table>
Treponema pallidum
Historical background: Syphilis

- Colombus, America 1492
- Transfer of syphilis to Europe in October 1492
- Spreading across the entire continent within short time
- Famous infected people: Nietzsche, Schubert, Heine, Gauguin, Hoffman, Maupassant
Historical background: the pathogen

- Discovery of *T. pallidum*, 1905
  - Fritz Richard Schaudin
  - Erich Hoffmann
- First diagnostic test, 1906
  - August Paul von Wassermann
  - Wassermann-reaction (complement fixation)
- First effective treatment, 1907
  - Paul Ehrlich: salvarsan (arsephenamine)
Antigenic properties

- **Surface antigens:** mucopolysaccharides
  - Treponema specific hyaluronic acid layer
  - Host specific hyaluronic acid & chondroitin sulphate layer
  - At distal end mucopolysaccharidase enzyme activity
- No extracellular products
Virulence factors

- **Mucopolysaccharides**: adhesion
- **Mucopolysaccharidase**: adhesion, invasion
- **Motility**: invasion
- **Intracellular survival** in epithel, endothel cells, fibro-, granulocytes & macrophages
Immunity

- **Treponema specific antibodies**
  - IgM: Week 1–several weeks
  - IgG: Week 2–many years (also after healing)
  - No long term protective effects

- **Lipid specific, cross reacting antibodies**
  - Characteristic but not specific (!)
  - Directed against mitochondrial lipid antigens
  - Formed on tissue decomposition: *eg.* syphilis, collagen diseases, tumours, malaria, tbc, pregnancy
Natural history of immunity

- **Sy I**
  - Antibody, T-cell response
  - Certain level of protection: rare re-infection

- **Sy II**
  - High titre of antibodies, intensive T-cell response
  - Effective protection against re-infection
  - No protection against bacterial invasion

- **Latency**
  - Mainly T-cell response
  - Late latency: patient is non-infective

- **Sy III**
  - T-cell mediated immune pathological processes
  - Gumma, perivascular lymphocytic infiltration
Clinical characteristic: Syphilis I

- Primary lesion: hard ulcer (**chancre**) at portal of entry
- **Lymphadenopathy**
- Lymphangiitis syphilitica (penis: chorda syphilitica)
- **Oedema induratum** (bell-hammer penis)
Penile chancre
hard ulcers in the shaft of the penis

06 December 2013
Vulval chancre
Anal chancre
Chancre on the face
Clinical characteristics: Syphilis II

- Bacteriaemia, generalised lymphadenopathy, occasionally fever
- Diverse skin affections
  - Roseola (macula)
  - Papula, condyloma lata
  - Mucous plaques
  - Syphilitic leucoderma
  - Syphilitic alopecia
  - Malignant syphilis (HIV!): ulcerating nodes, fever, meningeal signs
Maculopapular skin lesions
Papulo-squamous lesions
Papulo-pustular lesions
Condyloma lata
Syphilitic alopecia
Clinical characteristic: latency

- Positive serological reactions

- Early latency
  - ≤ 1 year
  - Patient is infectious

- Late latency
  - > 1 year
  - Patient is non-infectious
Clinical characteristics: Syphilis III

- Granulomatous tissue reactions in different organs
- Skin: gumma
- Cardiovascular sy: syphilitic aortitis, aneurysma
- Neurosyphilis
  - Meningitis
  - Menigovascular syphilis
  - Progressive paralysis (psychiatric symptoms)
  - Tabes dorsalis (spinal cord lesions)
  - Gumma (space occupying process)
Ulcerative gumma
Aortic valve lesion
Congenital syphilis at birth

- No signs of adult syphilis stage I
- Systemic involvement
- Intrauterine atrophy, death
- CNS involvement
- Anaemia
- Non-immune hydrops
- Pneumonia alba
- Hepatosplenomegaly, jaundice
Hepatosplenomegaly at birth
Osteochondritis
Vesicular lesions, runny nose
Late congenital syphilis

- ≥ 2 years of age
- Hutchinson-triad
  - Keratitis parenchymatosa, blindness
  - Central deafness
  - Hutchinson-teeth (peg-shaped upper incisors)
- Short maxilla
- Saddle nose
- Gumma
- Bone deformities
- Mental retardation, convulsions
Hutchinson-teeth

Peg-shaped upper incisors
Short maxilla, saddle nose
Ulcerative gumma: perforation of the palate
Microbiological diagnosis of syphilis

- **Direct microscopic examination**
  - Sample: chancre discharge, wet lesions
  - Method: dark-field microscopy

- **Serology**
  - Non-specific (reagin) type antibodies
  - Specific (anti-treponemal) antibodies

- *(Culture: not possible)*
Dark-field microscopy 1,000×
Estimated new cases of syphilis (WHO, 2005)

Region of the Americas
- Total: 2,930,000
  - Female: 1,160,000
  - Male: 1,230,000

European Region
- Total: 303,000
  - Female: 151,000
  - Male: 152,000

Western Pacific Region
- Total: 1,052,000
  - Female: 518,000
  - Male: 534,000

Eastern Mediterranean Region
- Total: 587,000
  - Female: 287,300
  - Male: 300,000

South-East Asia Region
- Total: 2,847,000
  - Female: 1,449,000
  - Male: 1,399,000

African Region
- Total: 3,410,000
  - Female: 1,490,000
  - Male: 1,920,000

Total number of cases
- Total: 10,700,000
  - Female: 5,060,000
  - Male: 5,540,000
Syphilis serology

- **Non-specific** (reagin) antibodies
  - Antigen: cardiolipin, Reiter-protein
  - Wassermann, **VDRL** (control of therapy),
  - RPR (rapid plasma reagin)

- **Specific** (anti-treponemal) antibodies
  - Antigen: *T. pallidum*
  - **TPHA**: screening test (IgM+IgG)
  - **FTA-Abs**: verification (IgM, IgG)
  - TIT (Nelson)
  - Most recently: **ELISA**, immune blot (IgM, IgG), LIA
VDRL/RPR (rapide plasma reagin)

TPHA
Treponema Pallidum Haemagglutination test

precipitate

agglutination
Detection of T. Pallidum antibodies by

**Western-blot**
Treatment of syphilis

- **Drug of choice:** penicillin G
  - High dose
  - Long course of treatment
- **Alternative drugs**
  - Ceftriaxon, erythromycin, doxycyclin
- **Jarisch-Herxheimer reaction**
  - Toxic material from dead bacteria
  - Interleukines, cytokines
  - Fever (40 °C), enhanced exanthema, shock
  - Treatment: glucocorticoids
Prevention of syphilis

- **Individual level**
  - Safe sex, avoiding promiscuity
  - Avoiding occupational exposition: plastic (latex) gloves

- **Population level**
  - Effective education
  - Mass screening
  - Exploration & treatment of infected individuals
Streamlined posters: Swiss, 1990’s
<table>
<thead>
<tr>
<th>Species</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. burgdorferi sensu lato</td>
<td>Lyme-borreliosis</td>
</tr>
<tr>
<td>B. burgdorferi sensu stricto</td>
<td>Erythema chronicum migrans, Lyme-arthiritis</td>
</tr>
<tr>
<td>B. garinii</td>
<td>Polymeningoradiculitis (Bannwarth's disease)</td>
</tr>
<tr>
<td>B. afzelii</td>
<td>Acrodermatitis chronica atrophicans</td>
</tr>
<tr>
<td>B. recurrentis</td>
<td>Febris recurrentis (Relapsing fever)</td>
</tr>
<tr>
<td>B. duttonii</td>
<td></td>
</tr>
</tbody>
</table>
Borrelia burgdorferi sensu lato

B. burgdorferi 400×

ELMI
Occurrence

- Natural reservoir
  - Red deer
  - Wild rodents

- Arthropod vector & reservoir: ticks (*Ixodes*)
  - *I. scapularis* (Eu), *I. pacificus* (USA), *I. persulcatus* (Asia)
  - Prevalence: 30–50%
  - Vertical (transovarial) transmission
Pathogenesis

- Portal of entry, local reproduction of bacteria
- Spreading with blood stream
- Organ manifestation (skin, joints, heart, liver, spleen, CNS)
- Perivascular infiltrate with lymphocytes / plasma cells (like in immune complex vasculitis)
Clinical characteristics: Lyme-borreliosis

- Incubation period: 2 days–4 months–? years
- Skin
  - Erythema chronicum migrans (ECM)
  - Lymphadenosis benigna cutis (LBC)
  - Acrodermatitis chronica atrophicans (ACA)
- Arthritis
- Carditis
- Neuroborreliosis
  - Peripheral neuritis (bilateral n. VII, rarely n. VI palsy)
  - Serous meningitis
  - Bannwarth-syndrome
- Prognosis: excellent if treatment started early
Lyme-borreliosis: ECM

Erythema chronicum migrans
Lyme-borreliosis: ECM
Erythema chronicum migrans
Microbiological diagnosis

- **Serology**
  - Sample: serum and/or CSF
  - Routinely: ELISA (IgM, IgG)
  - Verification: Western-blot

- **Detection of bacterial DNA**
  - Sample: biopsy material, CSF, synovial fluid
  - Method: PCR with hybridisation (†)
  - (Culture: for research purposes only)
Treatment

- ECM, LBC, ACA, arthritis
  - Penicillin-V orally for 10 days
  - Doxycyclin orally for 30 days
- Carditis
  - Penicillin G high dose iv. for weeks
- Neuroborreliosis, arthritis
  - Ceftriaxon iv. (3. generation cephalosporin) for weeks
Prevention

- **Specific:** vaccination approved by USA FDA (Osp A): **Lymerix®**
- **Non-specific**
  - Prevention of tick bite
    - Use of repellents
    - Correct clothing
  - Correct and early removal of ticks
Correctly selected clothing

Removal of ticks
B. recurrentis, B. duttonii

- **Reservoir**: humans, wild rodents (asymptomatic infection)
- **Arthropod vector**
  - *B. recurrentis*: human body loose (human-human)
  - *B. duttonii*: soft ticks, *Ornithodoros spp.* (animal-human)
- **Pathogenesis**: antigen shifts
- **Disease association**: Febris recurrentis (Relapsing fever)
  - *B. recurrentis*: epidemic (war, poverty)
  - *B. duttonii*: endemic
Consequence of *B. recurrentis* antigen shifts
Microbiological diagnosis

- Dark field microscopy
- Blood film
- Culture
- Animal inoculation
- Identification with fluorescent antibodies

*B. recurrentis* in blood film 400×
Treatment & prevention

- **Antibiotics:** doxycyclin, erythromycin
  - *B. recurrentis:* usually single dose cures
  - *B. duttonii:* 10-day course

- **Prevention**
  - Specific: no vaccination available
  - Non-specific: personal hygiene, control of vectors
Leptospira genus

- **Old (phenotypical) classification**
  - Human and animal pathogenic: *L. interrogans*
    - Several sero-groups (23) & sero-variants (200)
  - Saprophytic, non-pathogenic: *L. biflexa*
    - Several sero-variants (63)

- **Novel (genotypical) classification**
  - Leptospira genus
  - Leptonema genus
*Leptospira interrogans sensu lato*

*Leptospira spp.* scanning ELMI
Occurrence

- Worldwide
- Natural reservoir: rodents, cattle, domestic swine, dogs
- Asymptomatic carriage in renal tubuli
- Natural water sources contaminated with animal urine
Leptospirosis: Are you at risk?
Transmission

- Zoonotic infection, human:
  - accidental host
  - Direct
    - Contact with urine of animals
  - Indirect
    - Contaminated natural water sources
    - Contaminated fomites
    - Occupational risk (farmers, miners), laboratory accidents

- Portal of entry: skin (intact?), mucous membranes, conjunctiva
Pathogenesis

- **Establishment**: portal of entry
- **Invasion**
  - Regional lymph nodes, reproduction
  - Blood stream
  - Organ manifestation, interstitial space
- **Tissue damage**
  - Week 1: no inflammatory reactions
  - Week 2: inflammation (specific antibodies appear)
Clinical characteristics

- Systemic infection with two phases
- Incubation: days 7–13
- Phase 1: fever & muscle pain
- Phase 2: organ damage
  - Meningitis, uveitis, exanthema
  - Pneumonitis
  - Renal tubular damage, immune complex glomerulonephritis
  - Liver damage
  - Liver & kidney involvement & jaundice & pulmonary haemorrhage & rapid progression: Weil’s disease
- Lethality: 1–10–40%
Renal damage in leptospirosis, autopsy

Icteric sclera (jaundice)
Microbiological diagnosis

- **Serology**
  - At week 1, after symptoms develop
  - Microscopic agglutination test: titre \( \geq 100 \) or paired sera 4× rise of titre
  - ELISA (IgM, IgG)

- **Culture**
  - Secondary significance only
  - Week 1: blood, CSF
  - Week 2: fresh urine
Treatment

- **Specific**
  - Antibiotics: *penicillin G, ampicillin & doxycyclin*
  - Effective if given in early stage (!)

- **Supportive**
Prevention

- **Individual level**
  - Avoiding contact with animal urine, frequent hand washing
  - Avoiding contaminated water
  - Vaccination: formaldehyde inactivated
  - Chemo-prophylaxis: doxycyclin

- **Population level**
  - Vaccination of domestic animals (*eg.* dogs)
  - Control of rodents
Pathogenesis

- **Adhesion** (mucopolysaccharides & -ase)
- Local reproduction of bacteria
- **Invasion** (mucopolysaccharidase & motility)
- Escape from immune system (intracellular survival)
- **Tissue damage**
  - Endarteritis obliterans, periarteritis → ischemic damage
  - Granuloma formation → space occupying process
<table>
<thead>
<tr>
<th></th>
<th><strong>ssp. pallidum</strong></th>
<th><strong>ssp. pertenue</strong></th>
<th><strong>T. carateum</strong></th>
<th><strong>ssp. endemicum</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td>worldwide</td>
<td>hot, humid climate</td>
<td>Middle &amp; South America</td>
<td>hot, dry climate</td>
</tr>
<tr>
<td>Tropical areas</td>
<td></td>
<td>tropical areas</td>
<td>Middle &amp; South America</td>
<td>deserts</td>
</tr>
<tr>
<td>Affected age</td>
<td>adults</td>
<td>children, adolescents</td>
<td>children</td>
<td>children &amp; adults</td>
</tr>
<tr>
<td>Transmission</td>
<td>STD</td>
<td>skin</td>
<td>mucous membran</td>
<td></td>
</tr>
<tr>
<td>Congenital form</td>
<td>exists</td>
<td>none</td>
<td>rarely</td>
<td></td>
</tr>
</tbody>
</table>

**Epidemiology of the four treponematoses**

- **ssp. pallidum**: Venereal syphilis
- **ssp. pertenue**: Yaws, frambözia, pian
- **T. carateum**: Pinta, carate
- **ssp. endemicum**: Endemic syphilis, bejel
First detailed description

- **Girolamo Fracastoro** (Hieronymus), 1530
- Educational poem
RIT – rabbit inoculation test

Inoculation of *T. Pallidum* into rabbit testicle
Simultaneous exchange of Sy and HIV infection

KE HIV POS 1997 (2000.02)

DL HIV POS 2000.04

RI SY POS 1996

2000.11

Syphilis LIA

HIV-1 pol LIPA

06 December 2013
Erythromycin Resistance in Treponema pallidum

- Stamm and Bergen amplified a 692-bp region in the 23S rRNA gene of T. pallidum and T. pertenue strains

- A point mutation (A2058G*) was present in both 23S rRNA genes of T. pallidum street strain 14, obtained from a patient who had failed therapy with erythromycin

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>23S rRNA sequence in region of nucleotide position 2058*</th>
<th>Erythromycin susceptibility</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. pallidum</td>
<td>Nichols</td>
<td>TAGTTAGACGGAAAGACCCCC</td>
<td>S</td>
<td>–</td>
</tr>
<tr>
<td>T. pallidum</td>
<td>Street strain 14</td>
<td>TAGTTAGACGGGAAGACCCCC</td>
<td>R</td>
<td>A → G</td>
</tr>
<tr>
<td>T. pertenue</td>
<td>Gauthier</td>
<td>TAGTTAGACGGAAGACCCCC</td>
<td>S</td>
<td>–</td>
</tr>
</tbody>
</table>

E. coli numbering

Posters: USA, 2nd World War

Hitler  VD  Hirohito
Posters, USA, 1950’s
Posters: Great Britain, 1950’s

- Left: "The 'easy' girl-friend spreads Syphilis and Gonorrhoea, which unless properly treated may result in blindness, insanity, paralysis, premature death. If you have run the risk, get skilled treatment at once. Treatment is free and confidential."

- Right: "Here comes the bride. A man suffering from Venereal Disease who infects his wife commits a vile crime against her and children yet unborn. Treatment is free and confidential."

06 December 2013
Posters: Great-Britain, 1950’s

She may be...
a bag of TROUBLE
SYPHILIS - GONORRHEA

-a shadow on his future

VD

Syphilis inherited from parents may lead to life-long suffering

clean living safeguards the children

Advice and treatment are free and confidential at any V.D. clinic
Streamlined posters: USA, 1970’s

“I thought a thing like that could never happen to me”

1,500,000 Americans have syphilis or gonorrhea and don’t know it

ASK YOUR DOCTOR OR CALL YOUR HEALTH DEPARTMENT FOR A CHECKUP
Historical background

- Obermeier (1873): *B. recurrentis*
- Burgdorfer (1982): *B. burgdorferi*

- Name of the genus: Borell (1867–1936)
Immunity

- Specific T-cell & antibody response
- No reliable, long term protection after infection
Virulence factors

- Hyaluronidase
- Motility
- Haemolysins
  \(L. \text{interrogans} \text{ serovar. grippotyphosa, pomona}\)
- Exotoxins (?)
- Endotoxins (?)
Immunity

- Specific T-cell & antibody response → significance is little known
  - Specific antibodies appear at the end of week 1 (acute serum)
  - Highest titre at weeks 2–3 (reconvalescent serum)
- Immune pathological processes at phase 2