

Department Of Biotechnology

SPIROCHETES

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INTRODUCTION

- Elongated, motile, flexible bacteria twisted spirally along the long axis – spirochetes
- Speira – coil, chaite – hair
- Endoflagella – polar flagella – along the helical protoplasmic cylinder – outer membrane and cell wall

SPIROCHETES

- Two families:
 - Spirochaetaceae – genera – *Spirochaeta*, *Cristispira*, *Treponema* and *Borrelia*
 - Leptospiraceae – genus – *Leptospira*

TREPONEMA

- Trepos – turn, nema – thread
- Short, slender spirochetes, fine spirals and pointed or rounded ends
- Disease in humans
 - Venereal syphilis (*T. pallidum*)
 - Endemic syphilis (*T. pallidum*, *T. endemicum*)
 - Yaws (*T. pertenue*)
 - Pinta (*T. carateum*)

T. pallidum

MORPHOLOGY

- Thin delicate spirochete with tapering ends
- Ten regular spirals, sharp and angular
- Actively motile, rotation along the long axis, backward and forward movement
- Flexion of the whole body

T. PALLIDUM

- Dark ground microscope
- Phase contrast microscope
- Stains
 - Silver impregnation
 - Fontana's method
 - Levaditi's method

T. PALLIDUM

CULTIVATION

- *Reiter strain* – non-pathogenic; used as antigen in group-specific treponemal tests
- *Nichol's strain* – serial intratesticular passage in rabbits

ANTIGENIC STRUCTURE

- Three types of antibodies
 - Reagin antibody – standard or non-specific tests for syphilis
 - Group antigen
 - Species-specific antigen

SYPHILIS

- Venereal syphilis – sexual contact – maximum infectivity – first two years
- Incubation period – 10–90 days
- Clinical manifestation – three stages
 - Primary
 - Secondary
 - Tertiary

PRIMARY SYPHILIS

- Chancre – painless, relatively avascular, circumscribed, indurated, superficially ulcerated lesion
- Hunterian chancre, hard chancre
- Regional lymph nodes swollen, discrete, rubbery, non-tender
- Spirochetes spread from site of entry – bloodstream and lymph
- Chancre heals – thin scar

SECONDARY SYPHILIS

- 1–3 months after primary lesion heals
- Roseolar or papular skin rashes, mucous patches in oropharynx and condylomata at mucocutaneous junctions
- Latent syphilis – serological tests

TERTIARY SYPHILIS

- Cardiovascular lesions – aneurysms, chronic granulomata, meningovascular lesions
- Late syphilis – neurological manifestations: tabes dorsalis, general paralysis of the insane

CONGENITAL SYPHILIS

- Transplacental
- Fourth month of gestation
- Abortion
- Still birth
- Live birth – stigmata of syphilis

LABORATORY DIAGNOSIS

- Specimen – highly infectious; margins gently scraped, serum that exudes
- Serum – serology

MICROSCOPY

- Wet film – direct microscopy – dark ground microscope
- Direct fluorescent antibody test
- Silver impregnation

SEROLOGICAL TESTS

- Standard tests for syphilis – reagin antibody tests – cardiolipin antigen
- VDRL tests
- Rapid plasma reagin (RPR)

SEROLOGICAL TESTS

- Group-specific treponemal tests – Reiter protein complement fixation test

SEROLOGICAL TESTS

- Specific *T. pallidum* tests
 - *Treponema pallidum* immobilisation (TPI)
 - Fluorescent treponemal antibody (FTA)
 - Fluorescent treponemal antibody–absorption (FTA–ABS)
 - *T. pallidum* hemagglutination assay (TPHA)
 - Enzyme immunoassay (EIA)

TREATMENT

- Penicillin – single injection 2.4 million units of benzathine penicillin G – early cases
- Late syphilis – repeated weekly for three weeks
- **Jarisch–Herxheimer reaction**

NON-VENEREAL TREPONEMES

- Endemic syphilis – *T. pallidum endemicum*
- Yaws – *T. pallidum pertenue*
- Pinta – *T. carateum*

BORRELIA

- Larger than spirochetes – 8–20 x 0.2–0.4 μm–wide open coils
- Pathogenic *Borrelia*
 - Relapsing fever (RF) – two forms:
 - Epidemic or louse–borne *B. recurrentis*
 - Endemic or tick–borne
 - Fusospirochetosis (Vincent’s angina) – *B. vincentii*
 - Lyme disease – tick–borne – *B. burgdorferi*

CULTURE

- Complex nutritional requirements
- Serum, blood or tissue-enriched media
- Noguchi's medium – ascitic fluid + rabbit kidney CAM of chick embryo
- Peritoneal cavity of rat + mice

PATHOGENICITY

- **Febrile bacteremia – 3–10 recurrences**
- **Dissemination – bloodstream, liver, spleen, gastrointestinal tract, kidney, meninges**

ANTIGENIC PROPERTIES

- Antigenic variation antigenic modulation (selection of new strains)
- High titres of lytic antibodies – CF antibodies
- Serotype specificity – variable membrane lipo protein (VMPS)
- Rearrangement of DNA of genes coding for VMPS – new genes
- Antigenic variation

CLINICAL FEATURES

- Incubation: 3–10 days
- Onset: insidious with chills; sharp rise in body temperature
- Numerous organisms – blood
- Afebrile period: 4–10 days
- Chills, fever, headache

LABORATORY DIAGNOSIS

- Smear: Dark field microscopy, thin or thick smears, Giemsa or Leishman stain
- Blood culture: *B. recurrentis*, culture difficult, low sensitivity
- Serology: not useful in diagnosis
- Animal inoculation: white mouse, 1–2 mL patient's blood, 2–4 days later tail vein, spirochete
- Treatment: erythromycin, tetracycline, penicillin

BORRELIA VINCENTII

- *B. vincentii* – normal commensal oropharynx
- Potential pathogen
- Morphology – large spirochete 5–15 μm x 0.5 μm
- Culture – media containing ascitic fluid, serum
- Pathogenicity – Vincent's angina (ulcerative oropharyngitis)
- Laboratory diagnosis – *B. vincentii* + fusiform bacteria – clinical term
- Gram-negative spirochete, cultivation difficult

BORRELIA BURGENDORFERI

- 1975 – unusual arthritis –Lyme, USA
- 1983 – *B. burgdorferi* – Burgdorfer
- Reservoir – Deer and small mammals – mice, rodent
- Humans – bite of Ixodid tick
- Incubation – 3–30 days
- Skin lesion at site of tick bite
- Lymph channels

BORRELIA BURGENDORFERI

Pathogenesis

- Bloodstream
- Week – months after initial episode – untreated → late manifestation – neurologic and cardiologic
- ↑ in serum IgM levels
- 60% develop arthritis – immune complexes are deposited in synovial tissue

BORRELIA BURGENDORFERI

Clinical syndrome

- Remissions and exacerbations
- Two stages of disease
 - Early stage – erythema migrans
 - Site of tick bite – macule/papule
 - Annular – necrotic centre, raised red border
 - Malaise, fatigue, headache, fever, chills, myalgia, lymphadenopathy

BORRELIA BURGENDORFERI

- Late stage – 80% untreated cases
- 1 week – 2 years

Two phases

- Neurologic and cardiological – meningitis encephalitis, peripheral neuropathy; cardiac dysfunction – heart block, pericarditis
- Arthritis, arthralgia – second phase

DIAGNOSIS

Clinical: erythema migrans

- Staining – febrile period – Wright or Giemsa, peripheral blood, CSF
- Culture – edge of lesion, blood, CSF – growth slow
- Serology – ELISA, IF – immunoblotting confirmatory
- PCR – *Borrelia* antigen – tissue or body fluid

LEPTOSPIRA

- Morphology
- Spiral organism– 5–20 $\mu\text{m} \times 0.1 \mu\text{m}$
- Closely set coils ,hooked ends
- Fluorescent antibody
- Silver impregnation
- Dark ground

CULTURAL CHARACTERISTICS

- Media – media enriched with rabbit serum.
- Korthof's , Stuart's, Fletcher's,.
- Embroyaned egg – CAM.
- Animal inoculation – intraperitoneally in guinea pig.