



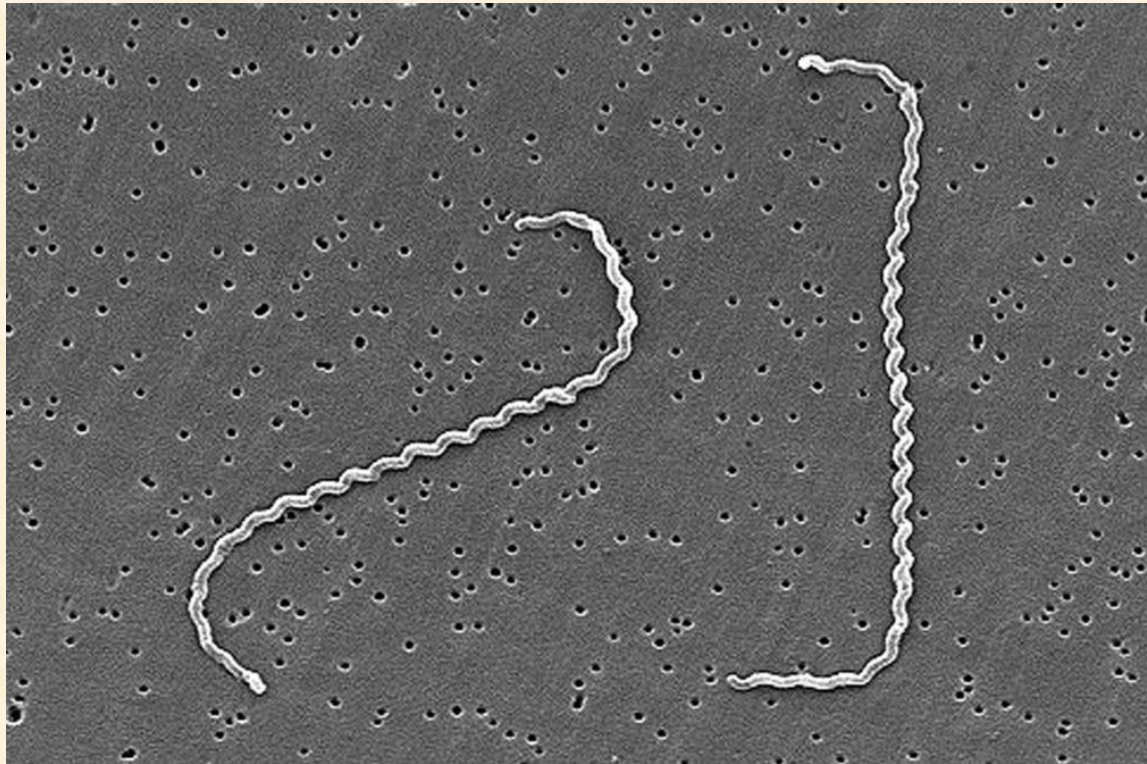
Dr. Davood Kalantar-Neyestanaki

Department of Microbiology and Virology

School of Medicine

Kerman University of Medical Sciences

SPIROCHETES



Taxonomy



Family: *Spirochaetaceae*

Genus: *Treponema* and *Borrelia*

Family: *Leptospiraceae*

Genus: *Leptospira*

Treponema Associated Human Diseases



Genus	Species	Subspecies	Disease
Treponema	<i>T. pallidum</i>	<i>pallidum</i>	Syphilis
	<i>T. pallidum</i>	<i>endemicum</i>	Endemic syphilis (bejel)
	<i>T. pallidum</i>	<i>pertenue</i>	Yaws
	<i>T. carateum</i>		Pinta

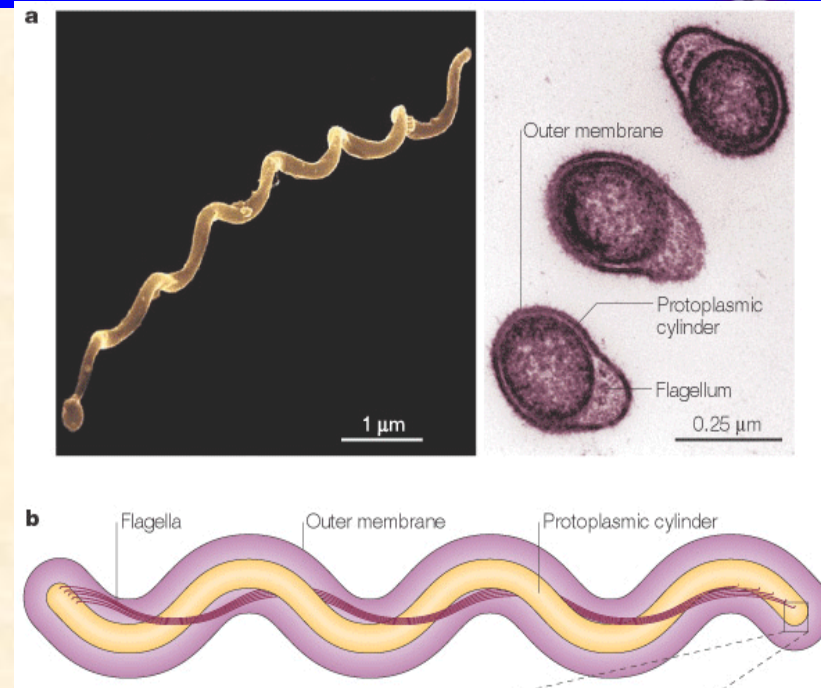
Syphilis: venereal diseases, **sexually transmitted disease (STD)**

Bejel, Yaws, Pinta: nonvenereal diseases

Physiology (*T. pallidum* and related pathogenic treponemes)



- ✓ **Thin, tightly coiled spirochetes**
- ✓ **Highly motile**
- ✓ **Three periplasmic flagella**
- ✓ **Do not grow :cell-free cultures**
- ✓ **Limited growth has been achieved: in cultured rabbit epithelial cells**
- ✓ **Replication is slow (Doubling time :30 hours)**





- ❖ Reason for this failure to grow *T. pallidum* in vitro:
- ✓ Lack of tricarboxylic acid cycle
- ✓ They are dependent on host cells: all purines, pyrimidines, and most amino acids
- ✓ Microaerophilic or anaerobic (extremely sensitive to oxygen)
- ✓ There are no genes :catalase or superoxide dismutase

Pathogenesis and Immunity



- ✓ Syphilis: disease of blood vessels, perivascular areas
- ✓ Outer membrane proteins: promote adherence
- ✓ **Five hemolysins**: it is unclear if they mediate tissue damage
- ✓ **Hyaluronidase**: facilitates perivascular infiltration
- ✓ *T. pallidum* has been shown to bind **fibronectin**
- ✓ Coating of **fibronectin**: protects against phagocytosis
- ✓ Tissue destruction and lesions: consequence patient's immune response to infection (immune reactivity against *T. pallidum*)

Clinical course of syphilis



- ❖ **Primary phase**
- ❖ **Secondary phase**
- ❖ **Late phase**

Primary Syphilis



- ✓ Is characterized by painless ulcer (**Hard chancre: genital region**) at the site of penetration of the spirochete
- ✓ In most patients, regional lymphadenopathy: develops 1 to 2 weeks after the appearance of the chancre
- ✓ Abundant spirochetes: are present in the **Hard chancre**
- ✓ Lymphatic system and blood: disseminated throughout the patient
- ✓ This ulcer heals spontaneously within 2 months: gives the patient false sense of relief

Secondary Syphilis

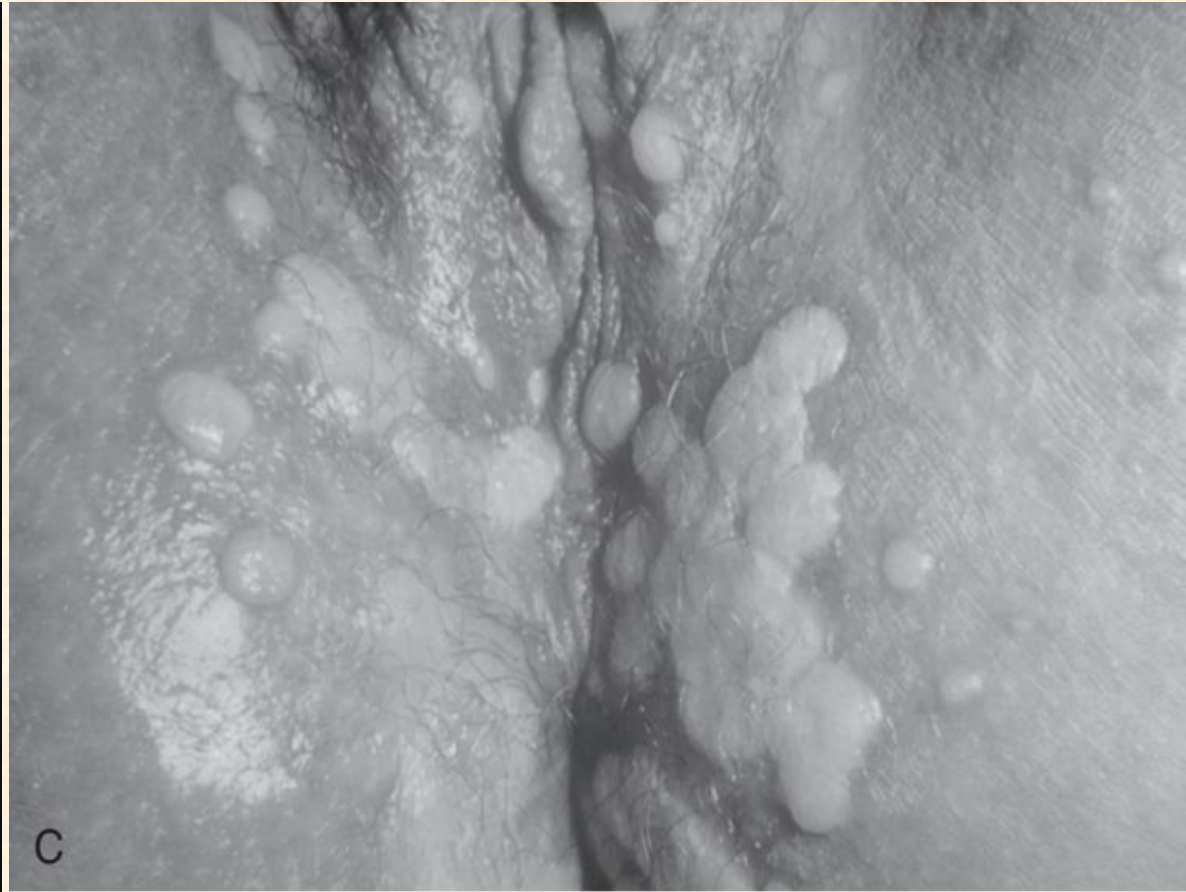


- ❖ **Disseminated disease marks the second stage**
- ✓ **In this stage patients experience**
- ✓ **Flulike syndrome, sore throat, headache, fever, muscle aches, lymphadenopathy, a generalized mucocutaneous rash**
- ✓ **The rash can be variable (macular, papular, pustular)**
- ✓ **Raised lesions, called **Condylomata lata (genital warts)**: a wart like growth on the moist skin folds**
- ✓ **As with the primary chancre these lesions:**
- ✓ **highly infectious**
- ✓ **The rash and symptoms: resolve spontaneously (within few weeks)**
- ✓ **Enters the latent (**clinically inactive stage**)**

Secondary Syphilis



Disseminated rash



Condylomata lata lesions

Tertiary (Late) Syphilis



- ❖ Is characterized by **diffuse, chronic inflammation**
- ✓ Can cause devastating destruction of virtually any organ or tissue
- ✓ Arteritis, dementia, blindness
- ✓ Granulomatous lesions (**gummas**): may be found in bone, skin, and other tissues
- ✓ Nomenclature of late syphilis: reflects the organs of primary involvement
- ✓ Neurosyphilis, cardiovascular syphilis
- ✓ Early stages: spirochetes are introduced into the central nervous system
- ✓ Neurosyphilis is not exclusively a late manifestation

Gumma of Face



Gummatous lesion that has ulcerated. (19)

Congenital Syphilis



- ✓ *T. pallidum* can cross the placenta
 - ✓ **Vertical transmission:** can occur at any time during pregnancy
 - ✓ lead to serious fetal disease (multiorgan malformations, or death of the fetus)
 - ✓ Teeth, bone malformation
 - ✓ Blindness, deafness
 - ✓ cardiovascular syphilis
- Are common in untreated infants who survive the initial phase of disease

Epidemiology



Humans: only natural host

Syphilis: is not highly contagious

Risk of contracting the disease after a single sexual contact: 30%

T. Pallidum

Extremely labile, unable to survive exposure to drying or disinfectants

cannot be spread through contact with

inanimate objects such as toilet seats

Syphilis occurs worldwide with no seasonal incidence



❖ Venereal syphilis transmitted by

1-sexual contact (common route of spread)

2- Congenitally (the second most common mode of infection)

3- Contaminated blood (by transfusion of fresh blood products from an infected person)

❖ Third most common sexually transmitted disease:

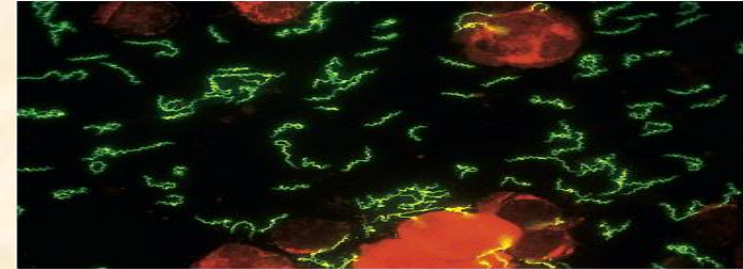
✓ after *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

✓ USA

Laboratory Diagnosis



- ✓ ***T. pallidum***: is too thin to be seen by light microscopy
- ✓ **Darkfield microscopy**
- ✓ **Special florescent stains must be used**
- ✓ **Diagnosis: primary, secondary, congenital syphilis can be made rapidly by:**
- ✓ **Darkfield examination of the exudate from skin lesions**
Material collected from oral and rectal lesions: not be examined
- ✓ **Limitations of darkfield microscopy**
- ✓ **Useful test for detecting: direct florescent antibody test**
- ✓ **Monoclonal antibody reagent: is available for pathogenic treponemes (so oral and rectal lesions can be examind)**



Laboratory Diagnosis



- ❖ *T. pallidum*: cannot be grown in any known culture media

- ❖ Nucleic Acid–Based Tests have been developed for

Detecting *T. pallidum* in:

1-genital lesions

2- infant blood

3- cerebrospinal fluid (CSF)

- ✓ But are only available in reference or research laboratories

Laboratory Diagnosis



Antibody Detection

Syphilis: is diagnosed in most patients on the basis of serologic tests

Serologic tests

1- Non specific (nontreponemal) tests used as:

screening tests

Because they are rapid to perform and inexpensive

2- Specific treponemal tests

Positive reactivity with nontreponemal test

Is confirmed with a treponemal test

Laboratory Diagnosis



❖ Nontreponemal tests

- ✓ measure immunoglobulin (Ig)G and IgM antibodies (also called reaginic antibodies)
- ✓ Antigen used for the nontreponemal tests: cardiolipin
- ✓ Derived from beef heart

❖ Two tests used most commonly (Nontreponemal tests)

1- Venereal Disease Research Laboratory (VDRL)

2- Rapid plasma reagin (RPR)

- ✓ Only VDRL test: should be used to test CSF (from patients with suspected neurosyphilis)

❖ ALL nontreponemal tests:

- ✓ Sensitivity: primary disease (70% to 85%), **Secondary disease (100%)**, late syphilis (70% to 75%)

Laboratory Diagnosis



❖ **Treponemal tests: use**

✓ ***T. pallidum*: as the antigen and detect specific anti-*T. pallidum* antibodies**

Treponemal tests results can be positive before the nontreponemal test results become positive in early syphilis

Laboratory Diagnosis



❖ Treponemal tests

- ✓ Florescent treponemal antibody–absorption (FTA-ABS) test

- ✓ Is an indirect florescent antibody test

T. pallidum immobilized on glass slides: as the antigen

- ✓ Slide: is overlayed with the patient's serum

- ✓ The fluorescein labeled antihuman antibodies: added to detect the presence of specific antibodies

Because these tests are technically difficult to interpret

Laboratory Diagnosis



- ❖ Most laboratories now use:
 - ✓ *T.pallidum* particle agglutination (TP-PA) test: (Treponemal tests)
 - ✓ Is a microtiter agglutination test
 - ✓ Gelatin particles sensitized with *T. pallidum* antigens
 - ✓ Are Mixed with dilutions of the patient's serum
 - ✓ antibodies are present, the particles agglutinate
- ❖ Successful treatment primary or secondary syphilis and late syphilis:
 - ✓ Leads to the reduced titers measured: VDRL and RPR tests
 - ✓ Thus these tests: can be used to monitor the effectiveness of therapy

Transient false-positive reactions



BOX 39-2

Conditions Associated with False-Positive Serologic Test Results

Nontreponemal Tests

Viral infection
Rheumatoid arthritis
Systemic lupus
erythematosus
Acute or chronic illness
Pregnancy
Recent immunization
Drug addiction
Leprosy
Malaria
Multiple blood transfusions

Treponemal Tests

Pyoderma
Rheumatoid arthritis
Systemic lupus
erythematosus
Psoriasis
Crural ulceration
Skin neoplasm
Drug addiction
Mycoses
Lyme disease
Acne vulgaris

Treatment, Prevention, and Control



choice for treating: Penicillin

early stages of syphilis: benzathine penicillin

congenital and late syphilis: penicillin G

allergic to penicillin: Doxycycline or azithromycin

protective vaccines: not available



***T. pallidum* subspecies endemicum**

❖ **Endemic syphilis (bejel)**

- ✓ **Diseases is spread person to person by the direct contact of early lesions**
- ✓ **Or use of contaminated eating utensils**
- ✓ **initial oral lesions: rarely observed**
- ✓ **secondary lesions include oral papules and mucosal patches**
- ✓ **late manifestations: Gummas of the skin, bones, and nasopharynx children**
- ✓ **Penicillin, tetracycline, and chloramphenicol: treat these diseases**



***T. pallidum* subspecies pertenue**

❖ Yaws

- ✓ A granulomatous disease
- ✓ Patients: have skin lesions
- ✓ Characteristic of yaws is widely distributed and painless
- ✓ Easily seen on dark field microscopy
- ✓ Spread among children by direct contact with infected skin lesions
- ✓ Penicillin, tetracycline, and chloramphenicol
- ✓ treat these diseases





T. pallidum subspecies *carateum*

❖ Pinta

- ✓ primarily affects the skin
- ✓ Small pruritic papules on the skin surface: 1- to 3-week incubation period
- ✓ Disseminated, recurrent, **hyperpigmented** lesions can develop over years
- ✓ Diagnoses of yaws and pinta :detection of spirochetes in skin lesions by darkfield microscopy
- ✓ Penicillin, tetracycline, and chloramphenicol: treat these diseases



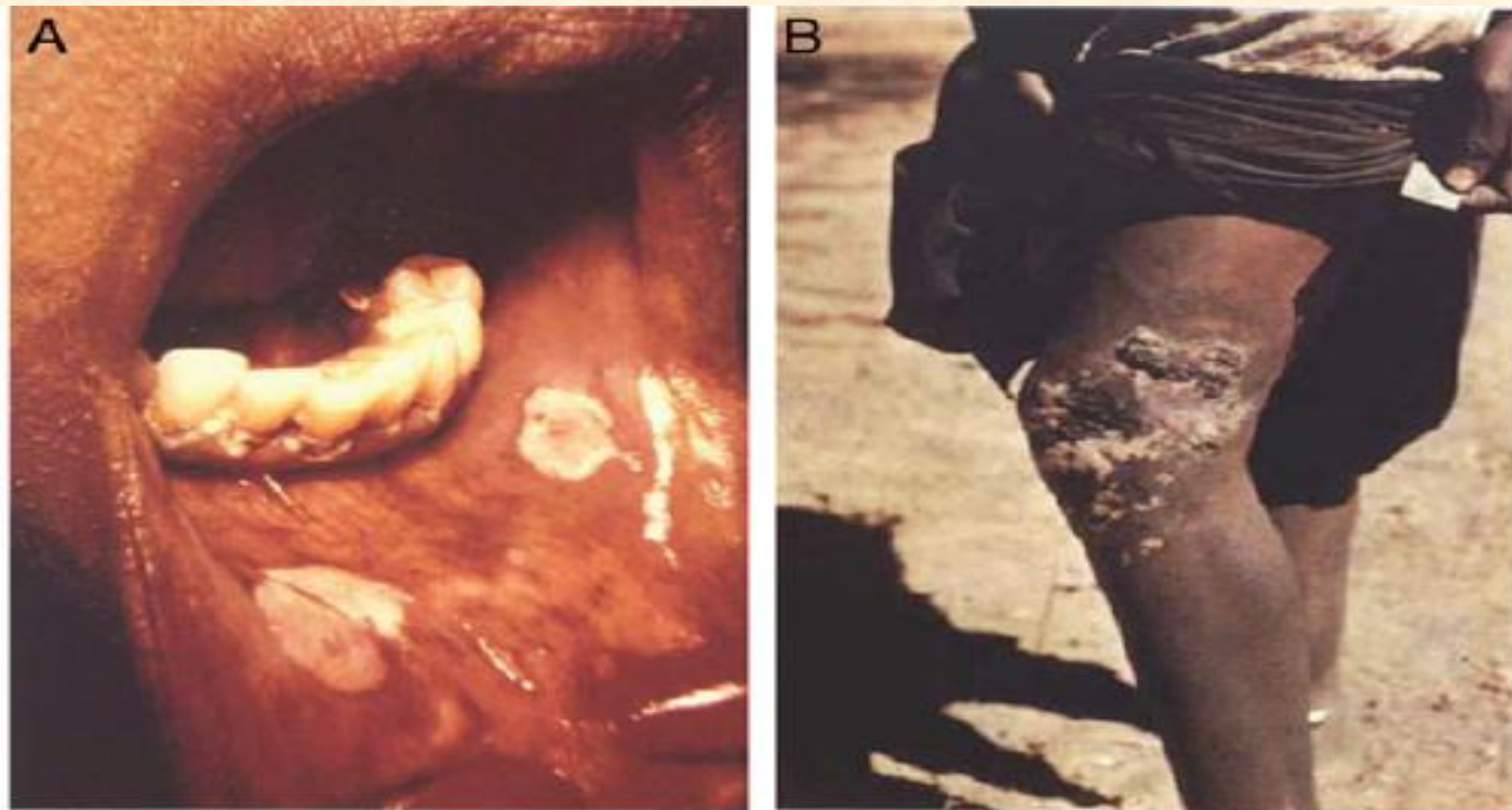


Figure 3. Lesions of bejel. (A) Oral lesion of primary bejel, (B) Chronic skin lesion of secondary bejel. Figure reproduced from Perine et al.² with permission of the publisher. This figure is available in black and white in print and in colour at Transactions online.



Figure 4. Lesions of pinta. (A) Erythematous plaque of early pinta, (B) Hyperpigmented skin lesion of late pinta. Figure reproduced from Perine et al.² with permission of the publisher. This figure is available in black and white in print and in colour at Transactions online.

BORRELIA



❖ Members of the genus *Borrelia* :two important human diseases

1- Lyme disease

2- Relapsing fever

- ✓ Lyme disease: tick-borne disease with protean manifestations
- ✓ dermatologic, rheumatologic, neurologic, and cardiac abnormalities
- ✓ Initially: *B. burgdorferi*
- ✓ 10 *Borrelia* species: Lyme disease in animals and humans
- ✓ *B. burgdorferi*, *B. garinii*, *B.afzelii*: human disease (Lyme disease)

Relapsing fever



Relapsing fever: febrile illness, characterized by recurrent episodes of fever and septicemia separated by afebrile periods

Periodic **febrile** and **afebrile** periods result from: **antigenic variation**

Two forms of the disease

1- Epidemic or louse-borne relapsing fever

Borrelia recurrentis

person-to-person by the human body louse (*Pediculus humanus*).

2- Endemic relapsing fever

15 species of borreliae

Spread by : infected soft ticks (genus *Ornithodoros*)

Physiology and Structure (Borrelia)



Members of the genus *Borrelia* :stain poorly with the Gram stain reagents

considered neither gram positive nor gram-negative

Even though they have outer membrane similar to gram-negative bacteria

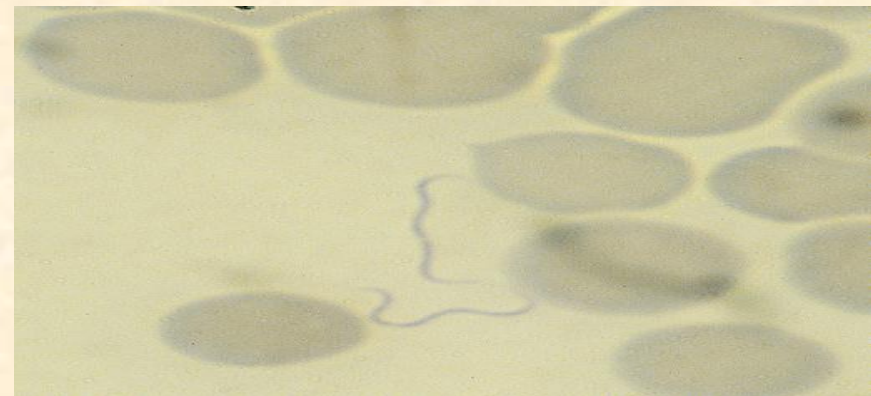
They stain well: **aniline dyes** (Giemsa or Wright stain)

Can be easily seen: light microscopy

When present in smears of peripheral blood from patient with **relapsing fever**

Microscopy: the test of choice for diagnosis of **relapsing fever**

Serology: test of choice for **Lyme disease**



Physiology and Structure (Borrelia)



periplasmic flagella: 7-20

twisting motility

Borrelia are microaerophilic and have

complex nutritional needs: N-acetylglucosamine, long-chain saturated and unsaturated fatty acids, glucose, and amino acids

Pathogenesis



Lyme disease: *Borrelia burgdorferi*

Transmitted to humans: the **bite** of the **hard Ixodes tick**

Hard ticks:

Ixodes scapularis, *Ixodes pacificus*, *Ixodes ricinus*

Hard ticks: major vectors of Lyme disease

B. burgdorferi: Transmitted by hard ticks from mice to humans

Major reservoirs for *B. burgdorferi*:

white-footed mouse

white-tailed deer

Pathogenesis



Louse borne epidemic relapsing fever: *B. recurrentis*

The vector: is the human body louse (*Pediculus humanus*).

Humans: only reservoir

Lice: become infected after feeding on an infected person

human infection occurs: when the lice are crushed during feeding

Lyme disease



Develops in 3 stages

1- Early localized disease:

small macule or papule: develops at the site of the tick bite and then

Enlarges to a lesion with a flat, red border and central clearing: **erythema migrans**

Erythema migrans: an expanding rash often seen in the early stage of Lyme disease



Erythema migrans



Lyme disease



2- Early disseminated disease:

hematogenous dissemination is characterized

systemic signs (severe fatigue, headache, fever, malaise)

arthritis

arthralgia

myalgia

erythematous skin lesions

cardiac and neurologic symptoms

Lyme disease



3- Late stage manifestations: include

Arthritis

Chronic skin involvement (**acrodermatitis chronica atrophicans**) is characterized **bluish-red** skin lesions (late, disseminated manifestations of Lyme)



Diagnosis of Lyme disease



Microscopy and culture of limited value

Nucleic acid amplification tests :available for Lyme disease

relatively insensitive

Serology is test of choice for Lyme disease

Relapsing Fever



clinical presentations of epidemic louse-borne and endemic tick-borne relapsing fever: same

Splenomegaly and hepatomegaly: common

A single relapse: epidemic louse-borne disease

10 relapses occur in endemic tick-borne disease

clinical course and outcome of epidemic relapsing fever: more severe than endemic disease

Mortality with endemic disease < 5%

Mortality with louse borne epidemic disease > 70%

Deaths: are caused by cardiac failure, hepatic necrosis

Culture



B. recurrentis

B. hermsii (a common cause of endemic relapsing fever in USA)

can be cultured in vitro on specialized media such as

BSK media: barbur stonner kelly medium

Treatment



Relapsing fever: tetracyclines or penicillins

Early localized or disseminated Lyme disease: amoxicillin, tetracycline, or cefuroxime

late manifestations (Lyme disease):intravenous penicillin or ceftriaxone

Vaccines are not available

LEPTOSPIRA



The genus: grouped by phenotypic properties, serologic relationships, pathogenicity

Pathogenic strains: placed in *Leptospira interrogans*

Nonpathogenic strains: placed in *Leptospira biflexa*

Thin, coiled spirochetes

Hook at one or both pointed ends

Motility: two periplasmic flagella

Obligate aerobes

Optimum growth: 28°C to 30 °C

Media supplemented: vitamins (B2, B12), long-chain fatty acids, and ammonium salts



Pathogenesis and Immunity



Leptospirosis (Symptomatic infections): develops in two stages

Initial phase: is a flulike symptoms with fever, muscle pain, chills, headache, vomiting, or diarrhea

Second phase is characterized more severe disease with sudden onset of headache

myalgia, chills, abdominal pain

Severe disease (icteric disease, or Weil disease): can progress to vascular collapse, thrombocytopenia, hemorrhage, and hepatic and renal dysfunction

Leptospire : can penetrate intact mucous membranes or skin through small cuts or abrasions

Pathogenesis



Leptospires infect two types of hosts: 1- reservoir hosts 2- incidental hosts

1- reservoir hosts

Most common reservoirs: rodents and other small mammals

Leptospires usually cause asymptomatic infections: reservoir host

spirochetes colonize the renal tubules

are shed in urine

Streams, rivers, standing water, and moist soil: can be contaminated with urine from infected animals

Pathogenesis



2- incidental hosts : farm animals, humans

Contaminated water or direct exposure to infected animals : can serve as a source for infection in incidental hosts

Most human infections: result from recreational exposure to contaminated water (e.g., lakes)

Occupational exposure to infected animals (farmers, veterinarians)

human infections: the warm months (when recreational exposure is greatest)

Person-to-person spread has not been documented

Laboratory Diagnosis



Microscopy

Leptospires are thin: not be seen by conventional light microscopy

Gram stain or **silver stain**: not reliable in detection of **Leptospire**s

Fluorescein-labeled antibody: used to stain leptospire

But are not available in most clinical laboratories

Culture

Leptospires can be cultured on specially formulated media

Fletcher, EMJH [Ellinghausen-McCullough-Johnson-Harris], **Tween 80-albumin**

grow slowly

incubation at 28° C to 30° C for as long as 4 months

Nucleic Acid–Based Tests: more sensitive than culture (not widely available at this time)

Laboratory Diagnosis



Antibody Detection

Reference method for all serologic tests: **microscopic agglutination test (MAT)**

MAT :measures ability of the patient's serum to agglutinate **live leptospire**s

serial dilutions of the patient's serum

mixed with the test antigens

Then examined microscopically for agglutination.

Reference laboratories

Treatment, Prevention, and Control



Patients treated: either intravenously administered penicillin or doxycycline

Doxycycline, but not penicillin: persons exposed to infected animals or water contaminated with urine

Difficult to eradicate leptospirosis: widespread in wild and domestic animals