SPIROCHAETES
Order: Spirochaetales

Family: *Spirochaetaceae*
Genus: *Treponema*
   *Borrelia*

Family: *Leptospiraceae*
Genus: *Leptospira*
Spirochaetales

- A multilayered outer membrane that surrounds the cylinder
- Periplasmic flagella which are attached to each end of the protoplasmic cylinder and extend toward the opposite end.
  - Are not typical flagella and are often called axial filaments
General Overview of Spirochaetales

- Gram-negative spirochetes
  - Spirochete from Greek for “coiled hair”
- Extremely thin and can be very long
- Tightly coiled helical cells with tapered ends
- Motile by periplasmic flagella (a.k.a., axial fibrils or endoflagella)
- Outer sheath encloses axial fibrils wrapped around protoplasmic cylinder
  - Axial fibrils originate from insertion pores at both poles of cell
  - May overlap at center of cell in Treponema and Borrelia, but not in Leptospira
  - Differereting numbers of endoflagella according to genus & species
Cross-section of *Borrelia burgdorferi*

**NOTE:** a.k.a., endoflagella, axial fibrils or axial filaments.

(Outer sheath)
# Spirochaetales Associated Human Diseases

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Disease</th>
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<td>Treponema</td>
<td><em>pallidum ssp. pallidum</em></td>
<td>Syphilis</td>
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<td><em>pallidum ssp. endemicum</em></td>
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<tr>
<td></td>
<td><em>pallidum ssp. pertenue carateum</em></td>
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<tr>
<td></td>
<td><strong>Borrelia</strong></td>
<td><strong>Lyme disease (borreliosis)</strong></td>
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<td></td>
<td><em>burgdorferi recurrentis</em></td>
<td><strong>Epidemic relapsing fever</strong></td>
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<tr>
<td></td>
<td>Many species</td>
<td><strong>Endemic relapsing fever</strong></td>
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<td></td>
<td><strong>Leptospira</strong></td>
<td><strong>Leptospirosis</strong></td>
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<tr>
<td></td>
<td><em>interrogans</em></td>
<td><em>(Weil’s Disease)</em></td>
</tr>
</tbody>
</table>
Treponema spp.
Nonvenereal Treponemal Diseases

✓ Bejel, Yaws & Pinta
✓ Tropical and subtropical regions
✓ Primarily in impoverished children
Treponema pallidum ssp. endemicum

➢ Bejel (a.k.a. endemic syphilis)
  • Initial lesions: nondescript oral lesions
  • Secondary lesions: oral papules and mucosal patches
  • Late: gummas (granulomas) of skin, bones & nasopharynx

➢ Transmitted person-to-person by contaminated eating utensils

➢ Tropical/subtropical areas (Africa, Asia & Australia)
Treponema pallidum ssp. pertenue
(May also see T. pertenue)

➢ **Yaws**: granulomatous disease
  • Early: skin lesions (see below)
  • Late: destructive lesions of skin, lymph nodes & bones

➢ Transmitted by direct contact with lesions containing abundant spirochetes

➢ **Tropical areas** (S. America, Central Africa, SE Asia)

Papillomatous Lesions of Yaws: painless nodules widely distributed over body with abundant contagious spirochetes.
T. Pertenue - Lesions
Treponema carateum

➢ **Pinta**: primarily restricted to skin
  • 1-3 week incubation period
  • Initial lesions: small pruritic papules
  • Secondary: enlarged plaques persist for months to years
  • Late: disseminated, recurrent hypopigmentation; depigmentation of skin lesions; scarring & disfigurement

➢ Transmitted by direct contact with skin lesions

➢ Tropical areas
  (Mexico, Central & South America)

Hypopigmented Skin Lesions of Pinta: depigmentation is commonly seen as a late sequel with all treponemal diseases
Treponema pallidum ssp. pallidum
Venereal Treponemal Disease

- Syphilis
- Primarily sexually transmitted disease (STD)
- May be transmitted congenitally
Darkfield Microscopy of Treponema pallidum
General Characteristics of *Treponema pallidum*

- Too thin to be seen with light microscopy in specimens stained with Gram stain or Giemsa stain
  - Motile spirochetes can be seen with darkfield microscopy
  - Staining with anti-treponemal antibodies labeled with fluorescent dyes
- Intracellular pathogen

- Do not survive well outside of host
  - Care must be taken with clinical specimens for laboratory culture or testing

- Cannot be grown in cell-free cultures in vitro
  - Koch’s Postulates have not been met
Koch's postulates

• The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
• The microorganism must be isolated from a diseased organism and grown in pure culture.
• The cultured microorganism should cause disease when introduced into a healthy organism.
• The microorganism must be re-isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
Epidemiology of *T. pallidum*

- Transmitted from direct sexual contact or from mother to fetus.
- Not highly contagious (~30% chance of acquiring disease after single exposure to infected partner) but transmission rate dependent upon stage of disease.
- Long incubation period during which time host is non-infectious.
  - Useful epidemiologically for contact tracing and administration of preventative therapy.
- Prostitution for drugs or for money to purchase drugs remains central epidemiologic aspect of transmission.
Pathogenesis of T. pallidum

➢ Tissue destruction and lesions are primarily a consequence of patient’s immune response
➢ Syphilis is a disease of blood vessels and of the perivascular areas
➢ In spite of a vigorous host immune response the organisms are capable of persisting for decades
  • Infection is neither fully controlled nor eradicated
  • In early stages, there is an inhibition of cell-mediated immunity
  • Inhibition of CMI abates in late stages of disease, hence late lesions tend to be localized
Virulence Factors of *T. pallidum*

- Outer membrane proteins promote adherence
- Hyaluronidase may facilitate perivascular infiltration
- Antiphagocytic coating of fibronectin
- Tissue destruction and lesions are primarily result of host’s immune response (immunopathology)
Pathogenesis of T. pallidum (cont.)

Primary Syphilis

- Primary disease process involves invasion of mucus membranes, rapid multiplication & wide dissemination through perivascular lymphatics and systemic circulation
  - Occurs prior to development of the primary lesion
- 10-90 days (usually 3-4 weeks) after initial contact the host mounts an inflammatory response at the site of inoculation resulting in the hallmark syphilitic lesion, called the chancre (usually painless)
  - Chancre changes from hard to ulcerative with profuse shedding of spirochetes
  - Swelling of capillary walls & regional lymph nodes w/ draining
  - Primary lesion heals spontaneously by fibrotic walling-off within two months, leading to false sense of relief
Primary Syphilis - Chancre
Primary Syphilis - Chancre

Fig. 171. *Primary Syphilis of the Lower Lip.* A chancre appearing on the lower lip has the same clinical appearance as one appearing on the genital mucosa. This lesion may simulate squamous cell carcinoma.
Pathogenesis of *T. pallidum* (cont.)

Secondary Syphilis

- Secondary disease 2-10 weeks after primary lesion
- Widely disseminated *mucocutaneous rash*
- Secondary lesions of the skin and mucus membranes are highly contagious
- Generalized immunological response
Generalized Mucocutaneous Rash of Secondary Syphilis
Following secondary disease, host enters latent period

- First 4 years = early latent
- Subsequent period = late latent

About 40% of late latent patients progress to late tertiary syphilitic disease
Pathogenesis of *T. pallidum* (cont.)

**Tertiary Syphilis**

- Tertiary syphilis characterized by localized granulomatous dermal lesions (gummas) in which few organisms are present
  - Granulomas reflect containment by the immunologic reaction of the host to chronic infection
- Late neurosyphilis develops in about 1/6 untreated cases, usually more than 5 years after initial infection
  - Central nervous system and spinal cord involvement
  - Dementia, seizures, wasting, etc.
- Cardiovascular involvement appears 10-40 years after initial infection with resulting myocardial insufficiency and death
Tertiary Syphilis Buboe of Neck
Tertiary Syphilis
Tertiary Syphilis - Gumma
Progression of Untreated Syphilis

Course of disease and blood tests

- Course of untreated disease (incidence—Oslo study)
- Positive blood
- Positive, doubtful, or negative

Late benign ➔ Gummas in skin and soft tissues

Tertiary Stage

Infection with Treponema pallidum
Pathogenesis of *T. pallidum* (cont.)

**Congenital Syphilis**

- Congenital syphilis results from transplacental infection
- *T. pallidum* septicemia in the developing fetus and widespread dissemination
- Abortion, neonatal mortality, and late mental or physical problems resulting from scars from the active disease and progression of the active disease state
Prevention & Treatment of Syphilis

- Penicillin remains drug of choice
  - WHO monitors treatment recommendations
  - 7-10 days continuously for early stage
  - At least 21 days continuously beyond the early stage
- Prevention with barrier methods (e.g., condoms)
- Prophylactic treatment of contacts identified through epidemiological tracing
### Diagnostic Tests for Syphilis

<table>
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<th>Diagnostic Test</th>
<th>Method or Examination</th>
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<td>Direct fluorescent antibody staining</td>
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<tr>
<td>Culture</td>
<td>Not available</td>
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<tr>
<td>Serology</td>
<td>Nontreponemal tests</td>
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<td></td>
<td>Venereal Disease Research Laboratory (VDRL)</td>
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<td></td>
<td>Rapid plasma reagin (RPR)</td>
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<tr>
<td></td>
<td>Treponemal tests</td>
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<tr>
<td></td>
<td>Fluorescent treponemal antibody absorption (FTA-ABS)</td>
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<tr>
<td></td>
<td>Microhemagglutination test for <em>Treponema pallidum</em> (MHA-TP)</td>
</tr>
</tbody>
</table>

**NOTE:** Treponemal antigen tests indicate experience with a treponemal infection, but cross-react with antigens other than *T. pallidum ssp. pallidum*. Since pinta and yaws are rare in USA, positive treponemal antigen tests are usually indicative of syphilitic infection.
Nontreponemal Reagin Tests

- **Non-specific or non-treponemal** serological test to detect *reagin*, utilized as screening test only.
  - Reagin is an antibody formed against cardiolipin.
  - Found in sera of patients with syphilis as well as other diseases.
  - This type of reagin not to be confused with same word originally used to describe IgE.
  - Non treponemal tests become positive 1 to 4 weeks after appearance of primary chancre.
  - In secondary stage may have false negative due to Prozone, in tertiary 25% are negative, after successful treatment will become nonreactive after 1 to 2 years.
Venereal Disease Research Laboratory - VDRL

- Flocculation test, antigen consists of very fine particles that precipitate out in the presence of reagin.
- Utilizes an antigen which consists of **cardiolipin, cholesterol and lecithin**.
  - Antigen very technique dependent.
  - Must be made up fresh daily.
- **Serum must be heated to 56 C for 30 minutes** to remove anti-complementary activity which may cause false positive, if serum is not tested **within 4 hours** must be **reheated for 10 minutes**.
- Calibrated syringe utilized to dispense antigen **must deliver 60 drops/mL +/- 2 drops**.
Rapid Plasma Reagin Test - RPR

• General screening test, can be adapted to automation.

• **CANNOT** be performed on CSF.

• Antigen
  – VDRL cardiolipin antigen is **modified with choline chloride** to make it more stable
  – attached to charcoal particles to allow macroscopic reading
  – antigen comes prepared and is very stable.

• **Serum or plasma** may be used for testing, serum is **not** heated.
Specific Treponemal Tests

• Performed to confirm a positive non-specific reagin test.
• Treponema Pallidum Immobilization
• Treponema pallidum hemagglutination
• Fluorescent treponemal antibody absorption test
• ELISA
**Treponema Pallidum Immobilization - TPI**

- An antibody present in the serum of a syphilitic patient, in the presence of complement, causes the immobilization of actively motile *Treponema pallidum* obtained from testes of a rabbit infected with syphilis.

- Cumbersome and expensive, no longer used in US and many places.
Treponema pallidum hemagglutination (TPHA)

• Adapted to microtechniques (MHA-TP)
• Tanned sheep RBCs are coated with T. pallidum antigen from Nichol’s strain.
• Agglutination of the RBCs is a positive result.
Treponema pallidum Hemagglutination (TPHA)

- Based on the agglutination of colored gelatin particle carriers sensitized with *T. pallidum* antigen.
- Patient sera incubated with sensitized particles in microtiter wells and unsensitized gelatin particles in control wells.
- Patient sera containing specific antibodies will react only with the antigen to form a smooth mat of agglutinated particles.
- A compact button formed by the settling of the non-agglutinated particles in the microtiter wells containing sensitized particles indicates lack of specific antibody in patient sera (-).
- If agglutination is seen with both sensitized and unsensitized particles, nonspecific agglutination is indicated.
## Sensitivity & Specificity of Serologic Tests for Syphilis

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<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
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<tr>
<td><strong>Nontreponemal</strong></td>
<td></td>
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<tr>
<td>VDRL</td>
<td>78 (74–87)</td>
<td>100</td>
</tr>
<tr>
<td>RPR</td>
<td>86 (77–100)</td>
<td>100</td>
</tr>
<tr>
<td><strong>Treponemal</strong></td>
<td></td>
<td></td>
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<tr>
<td>FTA-ABS</td>
<td>84 (70–100)</td>
<td>100</td>
</tr>
<tr>
<td>MHA-TP</td>
<td>76 (69–90)</td>
<td>100</td>
</tr>
<tr>
<td>Nontreponemal Tests</td>
<td>Treponemal Tests</td>
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<td>-------------------------------------------</td>
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<tr>
<td>Viral infection</td>
<td>Pyoderma</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>Skin neoplasm</td>
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<tr>
<td>Systemic lupus erythematosus</td>
<td>Acne vulgaris</td>
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<tr>
<td>Acute or chronic illness</td>
<td>Mycoses</td>
<td></td>
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<tr>
<td>Pregnancy</td>
<td>Crural ulceration</td>
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<tr>
<td>Recent immunization</td>
<td>Rheumatoid arthritis</td>
<td></td>
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<tr>
<td>Drug addiction</td>
<td>Psoriasis</td>
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<tr>
<td>Leprosy</td>
<td>Systemic lupus erythematosus</td>
<td></td>
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<tr>
<td>Malaria</td>
<td>Pregnancy</td>
<td></td>
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<tr>
<td></td>
<td>Drug addiction</td>
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<tr>
<td></td>
<td>Herpes genitalis</td>
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</tbody>
</table>
Effect of Treatment for Syphilis on Rapid Plasma Reagin Test Reactivity
Borrelia spp.
Giemsa Stain of Borrelia recurrentis in Blood

Light Microscopy

Phase Contrast Microscopy
## Epidemiology of Borrelia Infections

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<th>Infection</th>
<th>Reservoir</th>
<th>Vector</th>
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<td>Relapsing fever</td>
<td>Humans</td>
<td>Body louse</td>
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<tr>
<td>Epidemic (louse-borne)</td>
<td></td>
<td>Pediculus humanus</td>
</tr>
<tr>
<td>Relapsing fever</td>
<td>Rodents, soft-shelled ticks</td>
<td>Soft-shelled tick</td>
</tr>
<tr>
<td>Endemic (tick-borne)</td>
<td></td>
<td>Ornithodoros spp.</td>
</tr>
<tr>
<td>Lyme disease</td>
<td>Rodents, deer, domestic pets,</td>
<td>Hard-shelled tick</td>
</tr>
<tr>
<td></td>
<td>hard-shelled ticks</td>
<td>Lxodes spp.</td>
</tr>
</tbody>
</table>

- **Borrelia recurrentis**
- **Borrelia spp.**
- **Borrelia burgdorferi**
Borrelia recurrentis & other Borrelia spp.
Epidemiology of Relapsing Fever

- Associated with poverty, crowding, and warfare
- Arthropod vectors
  - Louse-borne borreliosis = Epidemic Relapsing Fever
    ✓ Transmitted person-to-person by human body lice (vectors) from infected human reservoir
    ✓ Infect host only when louse is injured, e.g., during scratching
    ✓ Therefore, a single louse can only infect a single person
    ✓ Lice leave host that develops a fever and seek normal temperature host
  - Tick-borne borreliosis = Endemic Relapsing Fever
    ✓ Sporadic cases
    ✓ Transmitted by soft body ticks (vectors) from small mammal reservoir
    ✓ Ticks can multiply and infect new human hosts
Pathogenesis of Relapsing Fever

➢ Relapsing fever (a.k.a., tick fever, borreliosis, famine fever)
  • Acute infection with 2-14 day (~ 6 day) incubation period
  • Followed by recurring febrile episodes
  • Constant spirochaetemia that worsens during febrile stages

➢ Epidemic Relapsing Fever = Louse-borne borreliosis
  • *Borrelia recurrentis*

➢ Endemic Relapsing Fever = Tick-borne borreliosis
  • *Borrelia spp.*
Clinical Progression of Relapsing Fever

- September 23: Visited cave, Denton County, Texas
- Rigor, headache, delirium, lethargy, nonproductive cough, arthralgia, myalgia
- Drenching sweat, asthenia
- Morbilliform rash
- Drenching sweat, asthenia
- October 1
- Rigor, headache, vomiting, myalgia, near stupor
- Blood smear +
- Mouse inoculation +
- Therapy: Tetracycline 10 days
- Proteus OXK 0
Borrelia burgdorferi
Pathogenesis of Lyme Borreliosis

Lyme disease characterized by three stages:

i. Initially a unique skin lesion (erythema chronicum migrans (ECM)) with general malaise
   ✓ ECM not seen in all infected hosts
   ✓ ECM often described as bullseye rash
   ✓ Lesions periodically reoccur

ii. Subsequent stage seen in 5-15% of patients with neurological or cardiac involvement

iii. Third stage involves migrating episodes of non-destructive, but painful arthritis

Acute illness treated with phenoxyethylpenicillin or tetracycline
Erythema chronicum migrans of Lyme Borreliosis

Bullseye rash
Diagnosis of Lyme Borreliosis

Clinical Case Definition
Either of the following:
  Erythema migrans (≥5 cm in diameter)
  At least one late manifestation (i.e., musculoskeletal, nervous system, or cardiovascular involvement) and laboratory confirmation of infection

Laboratory Criteria for Diagnosis
At least one of the following:
  Isolation of *Borrelia burgdorferi*
  Demonstration of diagnostic levels of IgM or IgG antibodies to the spirochetes
  Significant increase in antibody titer between acute and convalescent serum samples
Bacteria and Syndromes that Cause Cross-Reactions with Lyme Borreliosis Serological Tests

- Treponema pallidum
- Oral spirochetes
- Other *Borrelia* species
- Juvenile rheumatoid arthritis
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Infectious mononucleosis
- Subacute bacterial endocarditis
Epidemiology of Lyme Borreliosis

- Lyme disease was recognized as a syndrome in 1975 with outbreak in Lyme, Connecticut
- Transmitted by hard body tick (*Ixodes* spp.) vectors
  - Nymph stage are usually more aggressive feeders
  - Nymph stage generally too small to discern with unaided eye
  - For these reasons, nymph stage transmits more pathogens
- White-footed deer mice and other rodents, deer, domesticated pets and hard-shelled ticks are most common reservoirs
Leptospira interrogans
Silver Stain of Leptospira interrogans serotype icterohaemorrhagiae

- Obligate aerobes
- Characteristic hooked ends (like a question mark, thus the species epithet – *interrogans*)
Leptospirosis Clinical Syndromes

➢ Mild virus-like syndrome
➢ (Anicteric leptospirosis) Systemic with aseptic meningitis
➢ (Icteric leptospirosis) Overwhelming disease (Weil’s disease)
   ✓ Vascular collapse
   ✓ Thrombocytopenia
   ✓ Hemorrhage
   ✓ Hepatic and renal dysfunction

NOTE: Icteric refers to jaundice (yellowing of skin and mucus membranes by deposition of bile) and liver involvement
Pathogenesis of Icteric Leptospirosis

- Leptospirosis, also called Weil’s disease in humans
- Direct invasion and replication in tissues
- Characterized by an acute febrile jaundice & immune complex glomerulonephritis
- Incubation period usually 10-12 days with flu-like illness usually progressing through two clinical stages:
  - i. Leptospiremia develops rapidly after infection (usually lasts about 7 days) without local lesion
  - ii. Infects the kidneys and organisms are shed in the urine (leptospirosis) with renal failure and death not uncommon
- Hepatic injury & meningeal irritation is common
Clinical Progression of Icteric (Weil’s Disease) and Anicteric Leptospirosis

<table>
<thead>
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<th>Anicteric leptospirosis</th>
<th>Icteric leptospirosis (Weil’s syndrome)</th>
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<tr>
<td><strong>Fever</strong></td>
<td>First stage 3-7 days</td>
<td>First stage 3-7 days</td>
</tr>
<tr>
<td></td>
<td>(Septicemic)</td>
<td>(Septicemic)</td>
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<tr>
<td></td>
<td>Second stage 0 days-1</td>
<td>Second stage 10-30 days</td>
</tr>
<tr>
<td></td>
<td>month (Immune)</td>
<td>(Immune)</td>
</tr>
<tr>
<td><strong>Important clinical findings</strong></td>
<td>Myalgia, headache, abdominal pain, vomiting, conjunctival suffusion, fever</td>
<td>Meningitis, uveitis, rash, fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jaundice, hemorrhage, renal failure myocarditis</td>
</tr>
<tr>
<td><strong>Leptospires present</strong></td>
<td>Blood</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>CSF</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>Urine</td>
</tr>
</tbody>
</table>
Epidemiology of Leptospirosis

- Mainly a zoonotic disease
  - Transmitted to humans from a variety of wild and domesticated animal hosts
    - In USA most common reservoirs rodents (rats), dogs, farm animals and wild animals
- Transmitted through breaks in the skin or intact mucus membranes
- Indirect contact (soil, water, feed) with infected urine from an animal with leptospiruria
- Occupational disease of animal handling
## Comparison of Diagnostic Tests for Leptospirosis

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<th>Test Accuracy</th>
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<td>Darkfield examination</td>
<td>Insensitive, nonspecific</td>
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<tr>
<td></td>
<td>Silver stain</td>
<td>Insensitive, nonspecific</td>
</tr>
<tr>
<td></td>
<td>Direct fluorescent antibody</td>
<td>Insensitive, specific</td>
</tr>
<tr>
<td>Culture</td>
<td>Blood</td>
<td>Positive during first 10 days</td>
</tr>
<tr>
<td></td>
<td>Cerebrospinal fluid</td>
<td>Positive during first 10 days</td>
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<tr>
<td></td>
<td>Urine</td>
<td>Positive after first week</td>
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<tr>
<td>Nucleic acid probes</td>
<td>Direct hybridization</td>
<td>Insensitive, specific</td>
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<tr>
<td></td>
<td>Amplification (e.g., polymerase chain reaction)</td>
<td>Sensitive, specific</td>
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<tr>
<td>Serology</td>
<td>Indirect hemagglutination, slide agglutination,</td>
<td>Insensitive, nonspecific</td>
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<tr>
<td></td>
<td>enzyme-linked immunosorbent assay</td>
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<td>Microscopic agglutination test</td>
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<td>Sensitive, specific, reference laboratory test,</td>
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<tr>
<td></td>
<td></td>
<td>serovar specific</td>
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