SPIROCHETES

BORRELIA

BORRELIA

- Structure similar to *Treponema and Leptospira* with minor differences
- **Size:** Larger, 10–30 µm in length and 0.2–0.5 µm breadth
- **Spirals:** less in number (3–10) with wider spirals (3 µm)
- **Endoflagella:** More in number (7–11), attached subterminally at the pole
- **Microscopy:** Borrelia is poorly Gram-stained
- Better viewed under dark ground microscope or by sliverimpregnation staining

Borrelia

- Most species occur as commensals on the buccal and genital mucosa
- Human Pathogens:
- *B. recurrentis* - epidemic relapsing fever
- *B. Burgdorferi* - Lyme disease
- *B. vincentii* - Vincent's angina in association with fusiform bacilli

RELAPSING FEVER

- Recurrent episodes of fever and nonspecific symptoms. Two types:

1. **Epidemic RF**: Caused by *B. recurrentis and* transmitted by louse
2. **Endemic RF**: Caused by *Borrelia species other* than *B. recurrentis such as B. duttoni, B. hermsii and B.turicatae.* Transmitted by tick

Pathogenesis

- **Mode of transmission**
- **Epidemic RF**

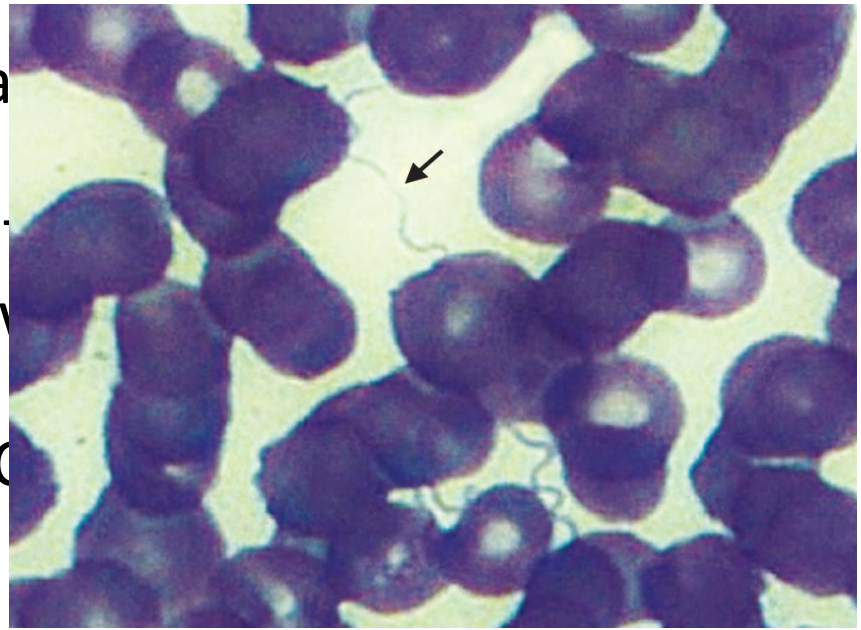
- Transmitted by human body louse(*Pediculus humanus)* – crushing

- **Endemic RF**: By bite of an infected tick (*Ornithodoros species)*
- **Frequent Antigenic variation** of borrelial surface antigens →repeated bacteremia and recurrent febrile episodes.

Clinical Manifestations

- Incubation period: 7–8 days.
- **Recurrent febrile episodes** (3–5 days) with intervening **afebrile periods (**7–9 days)
- **Non-specific symptoms** - alteration of sensorium, abdominal pain, vomiting and diarrhea
- **Hemorrhages:** Petechiae, epistaxis and blood-tinged sputum
- **Neurologic features** - meningitis, seizure, focal deficits, paraplegia and psychosis (in 10–30%)

- **Microscopy:**
  - Peripheral thick or thin smea
    by Wright- or Giemsa-stain
- Direct fluorescent antibody
- Dark ground microscope (lo
  sensitivity)
- Quantitative buffy coat (QBC
  analysis
- Poorly gram-negative



Laboratory Diagnosis

- **Culture:** from blood
- **Animal pathogenicity testing** - intraperitoneal inoculation into white mice
- **ELISA and IFA (indirect fluorescence assay)** - Fourfold rise of titer significant
- **GlpQ assay:** most reliable serological method. Immunoblot assay detecting antibody against the recombinant GlpQ antigen
- **Molecular methods:** Multiplex Real-time PCR targeting *16S rRNA and GlpQ genes*

Treatment Relapsing fever

- Doxycycline or erythromycin – DOC
- Single dose for epidemic RF, and 7–10 days course for endemic RF

LYME DISEASE

- **Agent**

- 3 genomospecies *Borrelia burgdorferi sensu lato (i.e. B. burgdorferi* in the general sense):

1. *Borrelia burgdorferi sensu stricto (B. burgdorferi in the* strict sense)
2. *Borrelia garinii*
3. *Borrelia afzelii.*

LYME DISEASE

- **Epidemiology**
- Reservoirs - Rodents and deer
- All three genomospecies found in Europe
- *B. Burgdorferi* - sole cause of Lyme disease in USA
- *B. garinii and B. afzelii* infections - Asia

Lyme Disease

- **Transmission**
- **Tick bite (*Ixodes ricinus*** complex)
- All three stages of tick (larval, nymphal, andadult stages) infective
- **Expression of surface proteins:**

- Outer surface protein A **(OspA)** in midgut of tick – important for survival
- **Protein OspC** that binds to a tick Salivary-gland protein **(Salp15)** - crucial for transmission

- **Stage 1: Early localized infection:**
- Incubation period 3–32 days
- Annular maculopapular lesion at site of the tick bite - **erythema migrans**
- Sites - thigh, groin, and axilla

Clinical Manifestations

- **Stage 2: Early disseminated infection:** Spreads hematogenously to many sites within days or weeks resulting in:

- Secondary annular skin lesions
- Musculoskeletal pain (arthralgia)
- Profound malaise and fatigue
- Neurological abnormalities (***Bannwarth's syndrome)***
- Cardiac involvement

Clinical Manifestations

- **Stage 3: Late persistent infection (Lyme arthritis):**
- Frank arthritis involving large joints (especially the knees)
- **Acrodermatitis chronica atrophicans -** *B.afzelii,*
- **Post-Lyme syndrome (Chronic):** Chronic fatigue symptoms and neurocognitive manifestations

Laboratory Diagnosis

- **Isolation of *B. Burgdorferi***
- Culture: skin lesions, blood or CSF - Barbour-Stoenner-Kelly medium
- Incubated at 34°C →dark field microscope weekly for two months
- **Molecular methods*:***
- PCR – Better for detection in joint fluid. But its sensitivity is poor for CSF, blood or urine samples
- PCR-RFLP

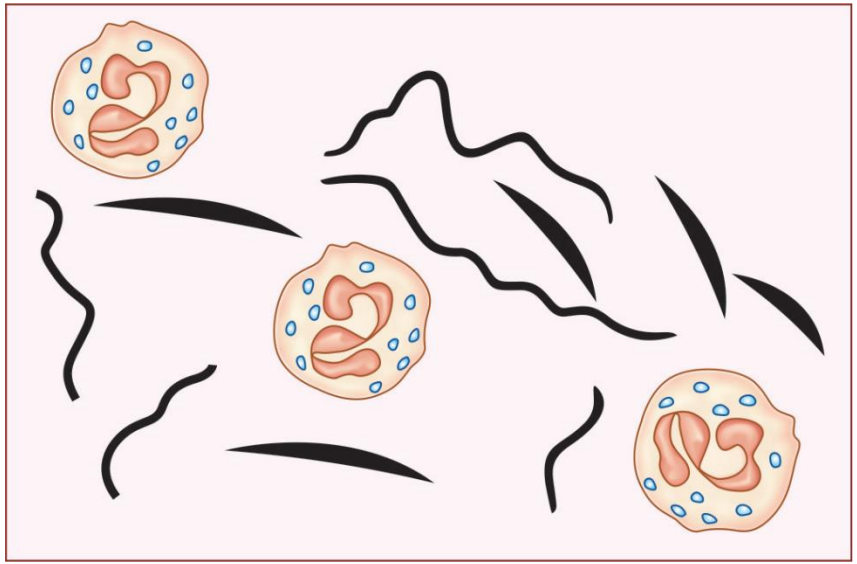
Serology (antibody detection)

- **The most common** method of diagnosis
- **ELISA and western blot**– detect IgM and IgG separately
- **Fourfold rise of antibody -** more significant
- **Two-test approach:** ELISA first → if found positive, confirmed by western blot
- **C6 peptide IgG ELISA -** second generation ELISA, uses VlsE lipoprotein antigen of *B. burgdorferi*
- **↑WBC count:** Joint fluid - elevated polymorphonuclear cells, CSF → lymphocytosis

Treatment Lyme disease

- **For all stages of Lyme disease** except CNS and CVS infection:
- Adults - Oral doxycycline - DOC
- Children - amoxicillin
- **Duration** of treatment is as follows:
- Localized skin infection (14 days)
- Early disseminated infection (21 days)
- Acrodermatitis (30 days)
- Arthritis (30–60 days)
- **For CNS or CVS infection**: Ceftriaxone for 14–28 days

- Acute ulcerative necrotising gingivostomatitis
- *Borrelia vincentii in association with Leptotrichia buccalis*
- An anerobic gram-negative bacillus, spindle-shaped with pointed ends



VINCENT'S ANGINA

- Both the agents are normal flora of mouth, potential pathogens in presence of malnutrition or viral infections
- Inflamed pharyngeal mucosa covered by greyish membrane, Peels off easily
- **Laboratory Diagnosis**

- Demonstration of spirochetes and fusiform bacilli in stained smears

- **Treatment Vincent's angina**

- Penicillin and metronidazole

LEPTOSPIRA - CLASSIFICATION

- **Phenotypic Classification**
- **Species: *Leptospira***

1. L. *interrogans (pathogenic for humans): It causes* leptospirosis or Weil's disease involving liver and kidney

2. *L. biflexa (saprophyte)*

- **Serovars and serogroups:**

- *L. interrogans - 26 serogroups* → >300 serovars

- Serogroup Icterohaemorrhagiae → serovars Icterohaemorrhagiae, Copenhageni, Lai, Naam and Mwogolo

LEPTOSPIRA INTERROGANS

- Size: 6–12 µm × 0.1 µm → pass through filters used to sterilize culture medium
- Tightly & regularly coiled, with characteristic hooked ends - *interrogans*— resembling interrogation mark
- Single endoflagellum attached at pole →highly motile exhibiting spinning and translational movements
- Dark ground or phase contrast microscope
- Silver impregnation method and immunofluorescence

Epidemiology

- **Zoonotic**
- **Source**: Rats, dogs, cattle and pigs
- **Mode of transmission:** Direct human-to-human transmission does not occur
- Indirect contact with water, moist soil and wet surfaces contaminated with animal urine
- Direct contact with urine and products of parturition, placenta of infected animals
- **Seasonality**: Rainy and post monsoon period

Epidemiology

- **Risk factors that promote transmission**
- Lower socioeconomic status, Urban and rural slum areas
- Rainfall and floods
- Occupational exposure: Agricultural workers, fishermen, sewer workers
- **3R's:** Three important epidemiological determinants - rodents, rainfall and rice field

Epidemiology

- **Incidence:** 0.1–1/100,000 per year in temperate climates to 10–100/100,000 in tropical countries
- **Global distribution**: Worldwide in distribution
- **In India:**

- Coastal districts of Andaman and Nicobar, Gujarat, Kerala, Maharashtra and Tamil Nadu

- The serovars predominantly present in India are L. andamana, L. pomona, L. grippotyphosa, L. hebdomadis, L. semoranga, L. javanica, L. autumnalis, L. canicola.

Pathogenesis

1. **First phase (septicemic phase):** Entry through mucosa/ abraded skin → bloodstream → disseminate brain, liver, lung, heart & kidney

- **Vascular damage:** capillaries, medium and large -sized vessels
- Penetration and invasion of tissues due to active motility and release of hyaluronidase

Pathogenesis

- 2. **Second phase (immune phase):**

- Antibodies develop, spirochetes disappear from the blood
- Antigen antibody complexes are deposited in various organs

- Renal colonization - excreted in urine

- **Clinical Manifestations**

- Incubation period 5–14 days

1. Mild anicteric febrile illness: (90%)

- Biphasic; septicemic phase → immune phase

2. **Weil's disease (Hepato-renal-hemorrhagic syndrome):** (10%)

- Severe form of icteric illness patients.

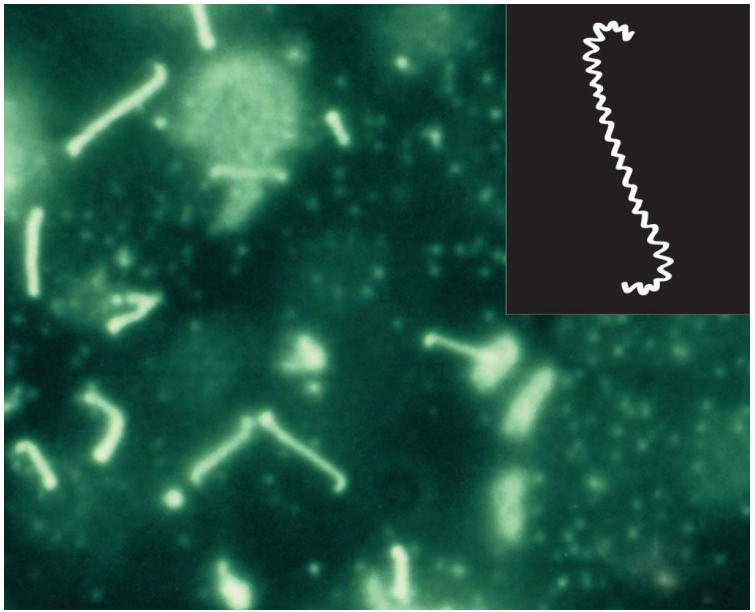
Leptospirosis

	Mild anicteric febrile illness	
	First stage 3-10 days (septicemic)	Second stage 10-30 days (immune)
Clinical Findings	Fever, Myalgia, Headache, Conjunctival, suffusion, Abdominal pain, Pharyngeal, erythema Without , Exudates, Vomiting	Meningitis, Uveitis, optic neuritis, chorioretinitis Rash, Fever Peripheral neuropathy
Isolation	From blood and CSF	From Urine
Serum IgM	Absent	Present
Antibiotics	Susceptible to antibiotics	Refractory to treatment

Leptospirosis

	Weil's disease	
	First stage 3-10 days (septicemic)	**Second stage** 10-30 days (immune)
Clinical Findings	High grade fever, Liver- Jaundice and raised liver enzymes Hemorrhages- Pulmonary hemorrhage, Petechiae & purpura, Conjunctival hemorrhage, Gastrointestinal hemorrhage Kidney- Raised serum urea and creatinine & Renal failure	
Isolation	Blood and CSF	Urine
Serum IgM	Absent	Present
Antibiotics	Susceptible to antibiotics	Refractory to treatment

- **Specimens:**
- First 10 days **-** CSF and blood urine
- Between 10 and 30 days - Urine
- **Microscopy:**
- **Wet films: D**ark ground or phase contrast microscope
- **Staining:** sliver impregnation stains - Fontana stain & modified Steiner technique



Isolation

- **Culture condition:** Obligate aerobe and slow growing
- Incubated at 30^{o}C for 4–6 weeks at pH 7.2–7.5
- Culture fluid examined under dark ground microscope periodically
- **EMJH medium (Ellinghausen, McCullough, Johnson, Harris)** - albumin fatty acid supplement added to the basal media containing 0.1% agar.
- **Dinger's ring** - Dense ring of growth under surface of the medium

Isolation

- **Korthof ’s medium with rabbit blood**
- **Fletcher’s semisolid medium**
- **Advantages**
- Isolation confirms the diagnosis
- To maintain the stock culture

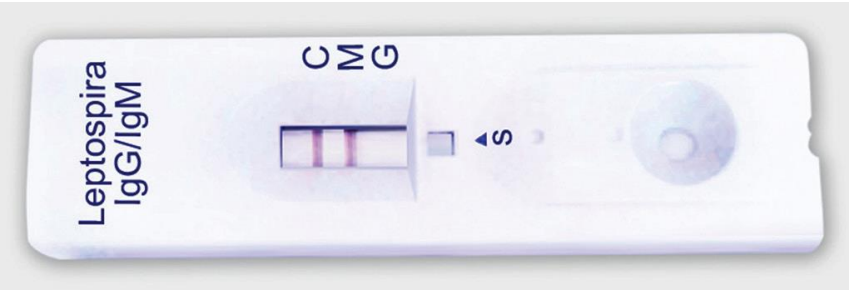
Laboratory Diagnosis

- **Disadvantages of Culture**
- Laborious, technically demanding and time-consuming
- False-positive - contamination of culture media with other organisms or saprophytic leptospires
- False-negative - prior use of antibiotics, or incubating in improper temperature and pH
- **Animal inoculation**: Hamsters (4–6 weeks old) & young guinea pigs → peritoneal fluid is examined for leptospires

Serology for antibody detection:

- **Genus-specific tests:** uses antigen prepared from nonpathogenic *L.biflexa Patoc 1 strain*
- Macroscopic slide agglutination test
- Microcapsule agglutination test (MCAT)
- Latex agglutination test
- ELISA: IgM and IgG detected separately
- Lepto dipstick assay: detects IgM

- Immunochromatographic test (ICT): It detects IgM and IgG antibodies separately



Serology for antibody detection:

❖ **Serovar-specific test:**

- **Microscopic agglutination test (MAT)** - detects antibodies against specific serovars of *L. Interrogans*
- Gold standard method and reference test
- **Cross agglutination and absorption test (CAAT)** – detects relatedness between the strains

Laboratory Diagnosis

- **Molecular methods: PCR**

- Useful in severe disease, before seroconversion occurs
- Target Genes -16S or 23S rRNA or IS1533 insertion sequence
- PCR is not serovar-specific
- PCR-RFLP or PFGE - to determine the genomo species
- **Faine's criteria:** WHO-approved guideline - based on clinical, epidemiological and laboratory findings
- Altered renal and liver function – nonspecific finding

Treatment Leptospirosis

- **Mild leptospirosis**
- Oral doxycycline (100 mg twice a day for 7 days)
- Alternative - Amoxicillin
- **Severe leptospirosis**
- Penicillin (1.5 million units IV, QID for 7 days)
- Alternatives - ceftriaxone or cefotaxime