LEPTOSPIRA

Learning objectives

To know in detail about,

- Spirocheates, Weil's disease, sugarcane fever and stuttgart disease
- Disease syndrome caused by leptospira in domestic animals
- Media for leptospira
- Morphology and cultural characters of leptospira
- Dark field examination
- Microscopic agglutination test
- Diagnostic methods used to identify leptospira

SYSTEMATICS

Domain	Bacteria
Phylum	*Spirochaetes*
Class	*Spirochaetes*(Spira- coil, Chaite- hair)
Order	*Spirochaetales*
Family	*Spirochaetaceae*
Genus	*Borrelia*
Family	*Serpulinaceae*
Genus	*Serpulina*
Family	*Leptospiraceae*
Genus	*Leptospira*(Leptos-fine or thin) *Leptonema*

- The spirochaetes are slender, motile, unicellular, helically coiled, bacteria ranging from 0.1 -3µm in width.
- Based on pathogenicity, DNA composition, and inability to grow at 13°C the genus *Leptospira* has traditionally been divided into two groups
    - The pathogenic species - *L.interrogans*
    - Free-living non-pathogenic strains - *L.biflexa*
- The common *Leptospira interrogans* serovars are *L.interrogans* serovar Pomona, *L.interrogans* serovar Hardojobovis, *L.interrogans* serovar Icterohaemorrhagiae, *L.interrogans* serovar Canicola, *L.interrogans* serovar Grippotyphosa, *L.interrogans* serovar Bratislava, and *L.interrogans* serovar Tarassovi.

HISTORY AND HABITAT

History

- Weil described the first case of leptospirosis in man in 1886.
- The causative agent of Weil's disease was isolated by India in 1915 and named *L. icterohaemorrhagiae*.
- Subsequently a very large number of leptospires have been isolated from human patients and animals from different parts of the world.

Synonyms of leptospirosis

- Weil's disease, Seven-day fever, Japanese autumnal fever, Rice field fever, Sugar cane fever and Stuttgart disease (dogs).

Natural habitat

- Leptospirosis occurs worldwide. Leptospires are present in the proximal convoluted tubules of mammalian kidneys (Sometimes genital tract) and are excreted in urine, often for several months. Characteristically, a reservoir host shows minimal or no clinical signs.
- Several animals act as carrier. Rats are particularlyimportant and they carry most pathogenic serotypes.
- Animal carriers often excrete upto 100 million leptospirae per ml of urine.
- A warm moist environment with the presence of ground water with a neutral or slightly basic pH favours the survival of the *Leptospira* outside the host.
- In India the disease has been more frequently reported from southern states than rest of the country.

MORPHOLOGY

- Leptospires are actively motile, helically shaped, slender spirochaetes possessing large number of light and fine spirals.
- They are 0.2 –0.3 µm in d.m. and 6-20 µm in length.
  Characteristically, *Leptospira* have hooked ends and two periplasmic flagella (PF), also known as axial filament and endoflagella.
- Rotation of the PF results in the distinct spinning mobility of *Leptospira*.
- They stain weakly or not at all with both aniline and Romanowsky stains. They may be stained with giemsa stain.
- But they can be bestviewedunder dark field illumination microscope and can be stained by silver impregnation technique of Fontana. *Leptospira* divide by binary fissio

CULTURAL CHARACTERISTICS

- *Leptospira* are obligate aerobes that use long chain fatty acids or fatty alcohols rather than CHO and aminoacid as their energy and carbon sources.
- In addition to this it also requires vitamins B1, B12 and purines. Optimum temperature for growth is 25°C to 35°C. They are highly fastidious.
- They grow very well in media enriched with rabbit plasma (rabbit plasma contain high concentration of bound vitamin B_{12}).
- Several liquid and semisolid media areavailableKorthof's modified medium, Stuart's liquid medium, Fletcher's semi solid medium, EMJH (Ellinghausen, McCullough (1965), Johnson and Harris (1967), protein free medium (commomly used for preparation of vaccine) and Ellis medium (mainly for isolation of leptospires from the genital tract of cows).
- Among these, the EMJH medium contains bovine albumin (fraction V), Tween 80 and rabbit plasma and is most commonly used for isolation of *Leptospira*.
- The generation time in laboratory media is 12 –16 hrs. The cultures are incubated at 30°C for upto 8 weeks.
- In semisolid media, growth occurs characteristically a few millimeters below the surface.
- Addition of 5-flurouracil (100 mgm/ml) in medium is inhibitory for most of the

microorganism but not for leptospires.

- The colonies of *Leptospira* strains appear colorless and below the surface of the agar. They may not be visible until held against opaque light.
- The leptospires are identified on the basis of their typical morphology and motility under dark field microscopy.
- In fluid medium, the leptospires appear to rotate alternatively along their axis, moving backward and forward with no apparent polar differentiation.
- In semisolid media, flexing, boring and serpentine movements are seen.

RESISTANCE, ANTIGENS AND TOXINS

Resistance

- *Leptospira* are highly susceptible to heat, being killed in 10 mts at 50°C and in 10 sec at 60°C.
- They are also sensitive to acid and are destroyed by gastric juice in 30 mts. Bile destroys them rapidly.
- They are also readily destroyed by most antiseptics and disinfectants.
- They can survive for several days in alkaline water and only 12 –14 wks in sewage.
- Dogs may shed leptospires in their urine for 2 to 6 months, cattle for 3 months and rats for longer periods.
- *Leptospira* have remained viable for atleast 6 days in coagulated blood and also they remain viable in unfrozen kidneys for several days after the death of the animal.

Antigens and toxins

- A lipopolysaccharide antigen appears to be present in all members of the genus.
- Based on surface antigens, probably composed of protein-polysachharide complexes *L. interrogans* is divided into more than 190 different serovars, arranged into 25 major antigenically related serogroups. Animals and humans can be infected with wide variety of serovars.
- The serovars causing disease in animals vary between countries and sometimes between regions in the same country.
- Pathogenic leptospires produce haemolytic, lipolytic and cytotoxic substances.

PATHOGENESIS

- The accidental (incidental) hosts are infected by direct transmission through infected urine, placental or fetal tissues or indirectly through contact with a contaminated environment.
- Venereal transmission plays a major role in pigs. Vertical transmission from the mother to the foetus may also occur in cattle.
- Leptospires gain entry through mucous membrane (nasal, genital, ocular, intestinal) or through abraded or water-softened skin.
- After epithelial penetration there is haematogenous spread (Leptospires able to invade the blood stream more rapidly than other bacteria), with localization and proliferation in parenchymatous organs, particularly the liver, kidneys, spleen and sometimes meninges for upto 16 days.
- It causes damage to endothelium of small blood vessels, leading to extravasion of blood and secondary ischaemia result in damage to liver, kidney and adrenals.
- In the kidneys, the organism reaches and localize in the lumen of proximal convoluted tubules.
- Penetration and multiplication in the fetus leads to fetal death and resorption, abortion or weak-off spring. (In foetus, if infection occur at 3rd trimester, can produce specific antibodies and may overcome the infection).
- The leptospires tend to persist in sites such as renal tubules, eyes and uterus where antibody activity is minimal.
- *Leptospira* causes following disease syndromes in domestic animals.

Host	Disease syndrome

Cattle	• Subclinical with leptospiruria (Hardjo) • Milk drop syndrome (Hardjo) • Abortion and neonatal mortality (Pomona and Hardjo) • Infertlity (Hardjo) • Haemoglobinuria, jaundice and fever in calves (Pomona, Grippotyphosa and Icterohaemorrhagiae)
Pigs	• Subclinical with leptospiruria (Pomona) • Fever and non-suppurative mastitis (Pomona) • Infertility, abortion and still birth (Canicola, Pomona, Icterohaemorrhagiae) • Haemoglobinuria, jaundice and fever (Icterohaemorrhagiae)
Dogs	• Subclinical with leptospiruria (Pomona) • Acute haemorrhagic type characterized with fever, vomiting, prostration and death (Icterohaemorrhagiae) • Less acute icterus type (Canicola and Icterohaemorrhagiae) • Ureamic type (Canicola)
Horse	• Recurrent iridocyclitis/periodic opthalmia / moon blindness (Pomona) • Abortion at 6 month of gestation
Sheep	• Subclinical infection with leptospiruria (Hardjo)

PATHOGENECITY

Symptoms and lesions

- Leptospirosis can occur as
  - Acute severe disease (Septicaemia with endotoxaemia such as haemorrhages, hepatitis, nephritis and meningitis)
  - Subacute moderately severe disease (nephritis, agalactiae, hepatitis and meningitis)
  - Chronic disease (iridocyclitis, abortion, still birth, infertility)
  - Subclinical form (recognize only by the development of antibody)
  - Symptoms include fever, severe muscle pain, haemoglobinuria, jaundice, anemia, anuria, meningeal signs, gastro intestinal signs, skin rashes and photophobia.
  - Cattle: Acute form characterized with marked drop in milk production associated with pyrexia in chronic form- abortion, still birth or premature calves.
  - Pigs: Haemoglobinuria and jaundice in piglets, abortion in the last 3rd of pregnancy and stillbirth.
  - Dogs: The acute form of leptospirosis is known as Stuttgart disease.
  - It is characterized by fever, haemorrhage, anemia and jaundice.

DIAGNOSIS

Specimens

- PBS containing 1%BSA is used as transport medium
    - Heparinised blood for culture
    - Whole blood/serum for serological tests (blood must be collected during early febrile stage)
    - Mid-stream urine for dark filed examination
- Leptospires can disintegrate quite quickly in a urine sample, especially if it is acidic.
- So, if the urine cannot be examined within 20 mts, it should be neutralized with N/10 Hcl, or N/10 NaOH.
- To preserve the morphology of the leptospires for several days, 20 ml of midstream urine should be added immediately to 1.5ml of 10% formalin.
- By adding formalin, the leptospires will be killed but will retain their morphology for several days and can be examined by dark field microscopy.
- Kidneys for both darkfield microscopy and culture. Kidneys/or liver sections in 10% formalin for histopathology.
- Foetal kidney smears (cryostat sections or smears) for FAT. Foetal abomasal contents, cotyledons and uterine discharge for differential diagnosis.
- CSF and semen from bull can also be useful for demonstration of leptospires.

Direct microscopy

- Leptospires can be demonstrated in urine, other body fluids, and tissues by darkfield microscopy (DFM) and by FAT.
- To detect under DFM, one probably needs 10,000 to 20,000 leptospires/ml in the sample toviewatleast one *Leptospira* in the high power field.
- Urine is centrifuged to concentrate the leptospires. Unclotted blood is centrifuged at low speed to sediment the RBC after which the plasma can be removed and centrifuged at high speed.

Isolation in culture media

- After inoculating the suitable media incubate at 30°C for upto 8 weeks. A drop of the culture is examined by DFM.

Animal inoculation

- Guineapigs, hamsters and weaning gerbils can be inoculated i/p with 0.5 to 1 ml of neutralized urine, unclotted blood or a 10% tissue suspension in EMJH or 1% BSA.
- Cardiac blood is taken aseptically when a temperature rise is detected or at 5,8,10 and 14 days post infection.
- Media are inoculated with 2-3 drops of the freshly collected blood.

By serology

- Macroscopic agglutination test: it is a screening test and uses dead antigen (lack of specificity).
- Microscopic agglutination test (MAT): it uses live leptospires as antigen and is highly sensitive and serovar specific.
- CFTand ELISA are also useful for detection of leptospiral antibodies in serum.
- To identify the serovar, MAT, restriction endonucleae, DNA analysis and monoclonal antibodies are useful.

TREATMENT AND CONTROL

- Immunity appears to be mainly humoral in that the organisms are not intracellularand bacterins give protection for short duration.
    - Dogs: Bacterins usually contain serovars, Canicola and Icterohaemorrhagiae.
    - Cattle: Bacterins usually contain serovars Hardjo, Grippotyphosa, Canicolaand Icterohaemorrhagiae.
- Combined penicillin, streptomycin are highly effective. The tetracycline and macrolide antibiotics are also effective.