

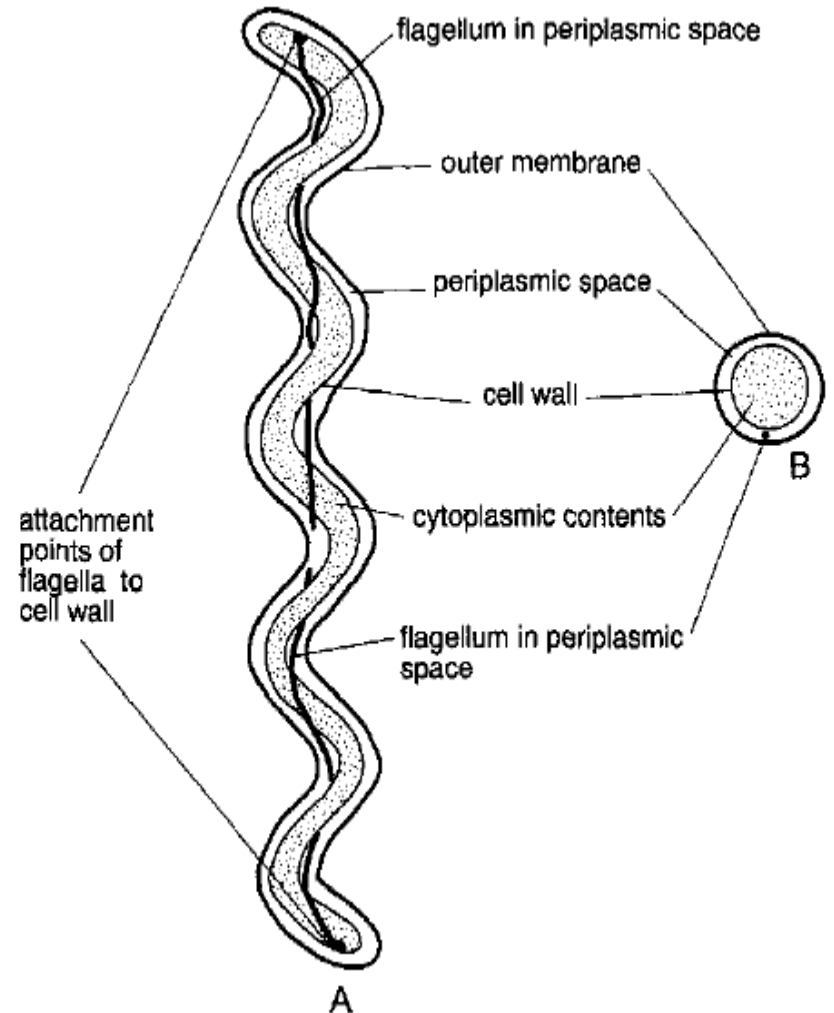
Spirochaetes

By

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Spirochaetes:

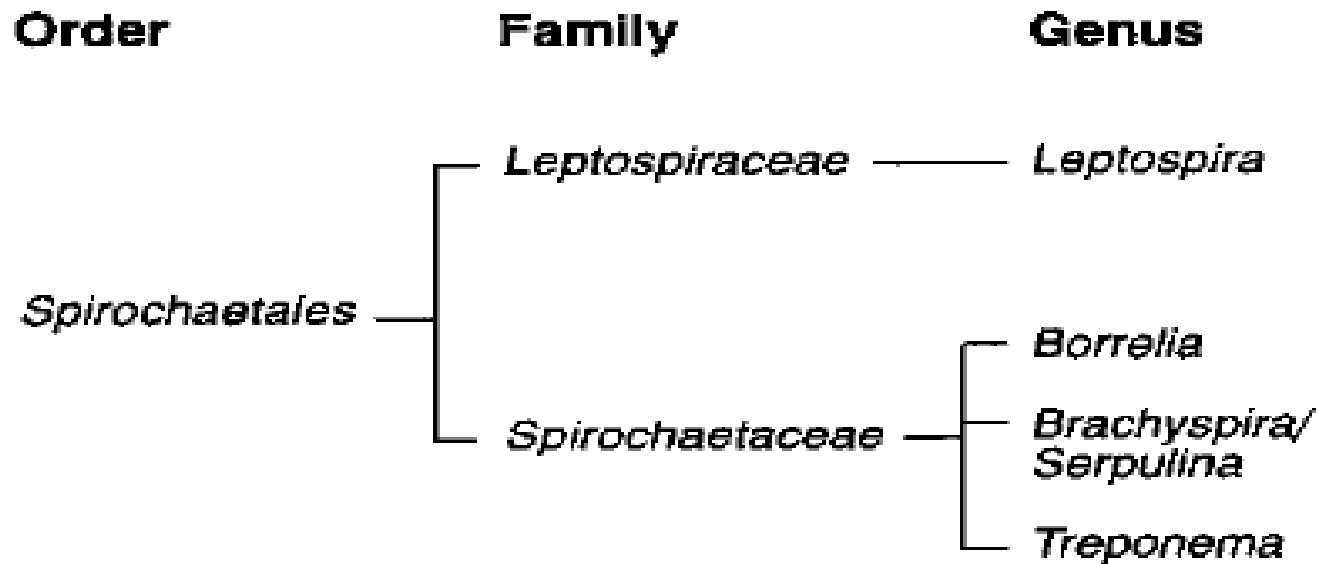
Order of microorganism comprises spiral or helical bacteria (spirochaetes) which share some unique morphological and functional features.



Taxonomy and classification

- **Order :Spirochaetales includes two families**
- **Family: Spirochaetaceae**
- **Family: Leptospiraceae**
- **Genus: Serpulina, Treponema, Borrelia (Spirochaetaceae) and Leptospira (Leptospiraceae)**
- **non-pathogenic spirochete**
- **pathogenic spirochete (Leptospira, Treponema, Borrelia)**

The order *Spirochaetales* contains two families, *Leptospiraceae* and *Spirochaetaceae*



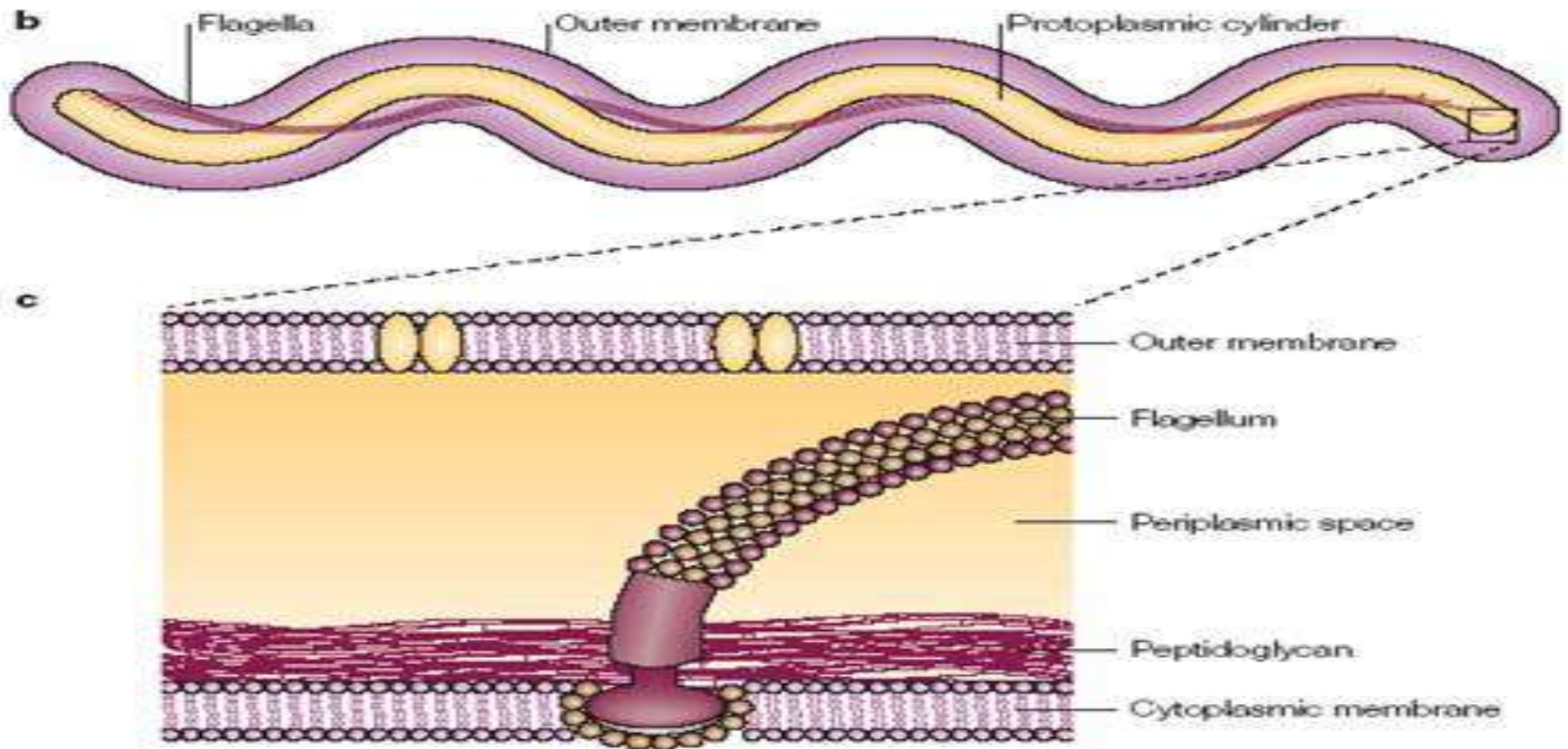
The pathogens in the family *Leptospiraceae* belong to the genus *Leptospira*.

The genera *Borrelia*, *Brachyspira* and *Treponema* in the family *Spirochaetaceae* contain significant animal and human pathogens. There are some non-pathogenic genera in each family.

General characteristics

- 1- Although Gram-negative, many stain poorly using conventional methods**
- 2- Motile organism, Spiral motile bacteria with endoflagella**
- 3- Tightly coiled bacteria typically slender and flexuous shape.
- 4- The cell made up of protoplasmic cylinder located in periplasm of cell, endoflagella and outer sheath.
- 5- Move by bending and rotating body according to the viscosity of the medium

- 6- The outer sheath most layer of spirochaete cell is multilayered membrane that completely surrounds the periplasmic cylinder
- 7- The cylinder consist of the nuclear material ,cytoplasmic membrane and the peptidoglycan portion of the cell wall



General characteristics

8- Pathogenic spirochaetes are difficult to culture; many require specialized media and some require liquid media.

9- Many produce zoonotic infections

10- Organisms in the group are classified on the basis of genetic relatedness.

Medical important

3 genera:

1. *Treponema*

2. *Leptospira*

3. *Borrelia*

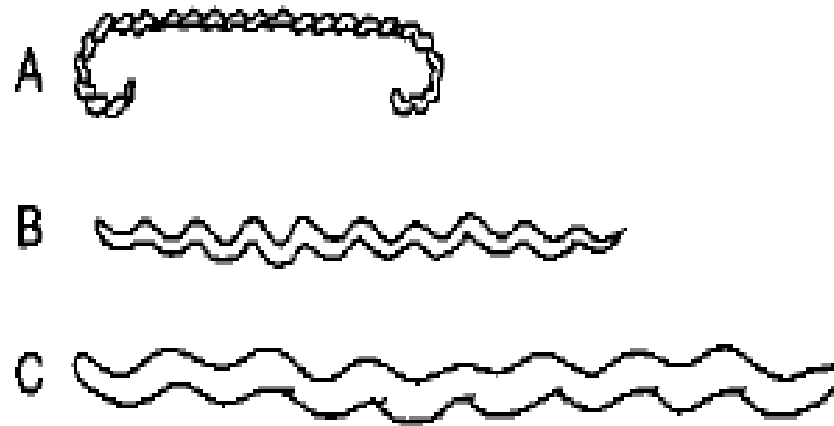


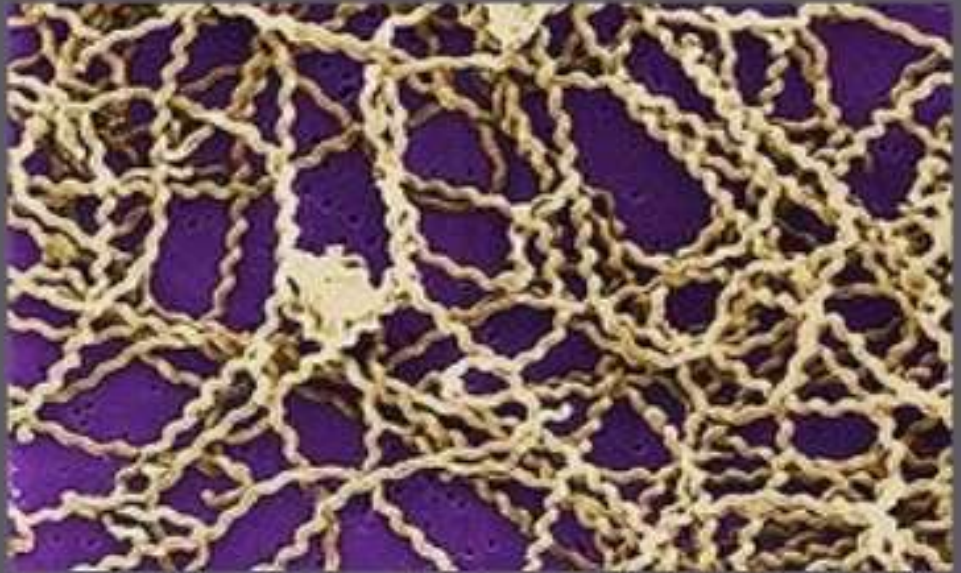
Figure 31.4 Spirochaetes of veterinary importance illustrating differences in size and shape: A, *Leptospira*; B, *Brachyspira*; C, *Borrelia*.

Motility of spirochetes

- Unusual mode of motility
- Endoflagellum located at one end and extends about two-third of the cell. Its rotate.
- Protoplasmic cylinder is rigid, whereas the outer sheath is flexible, therefore when both endoflagella rotates in the same direction while protoplasmic cylinder in opposite direction causing torsion of cell.

Leptospira

Scanning electron micrograph of a number of *Leptospira* sp. bacteria atop a 0.1 μm polycarbonate filter



Leptospira interrogans

Microscopy

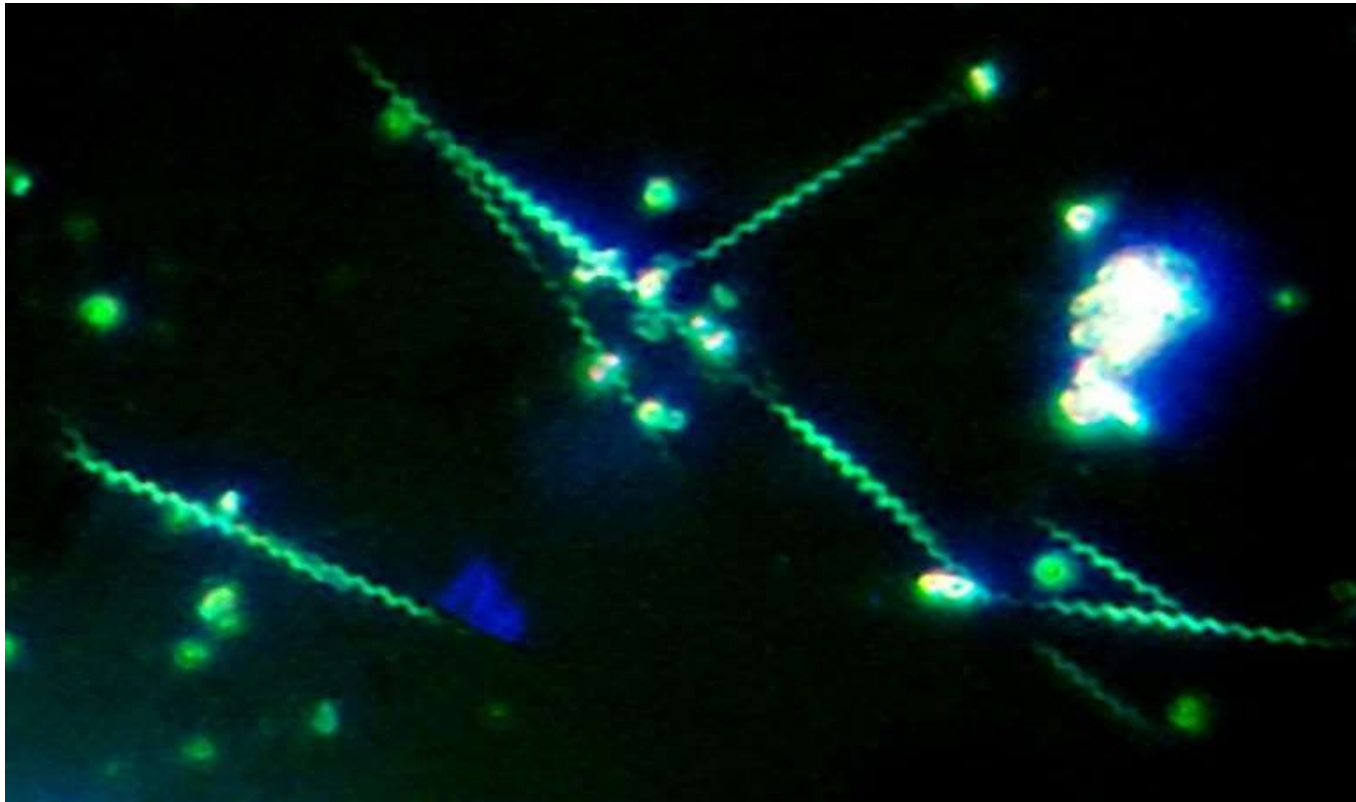
- Small 6-20 μm in size 0.1 μm diameter
- Actively motile spirochetes
- Screw-tight coils and hooked ends
- Easily stain only by impregnation techniques
- Best seen by dark-field illumination or phase contrast

***Leptospira* species**

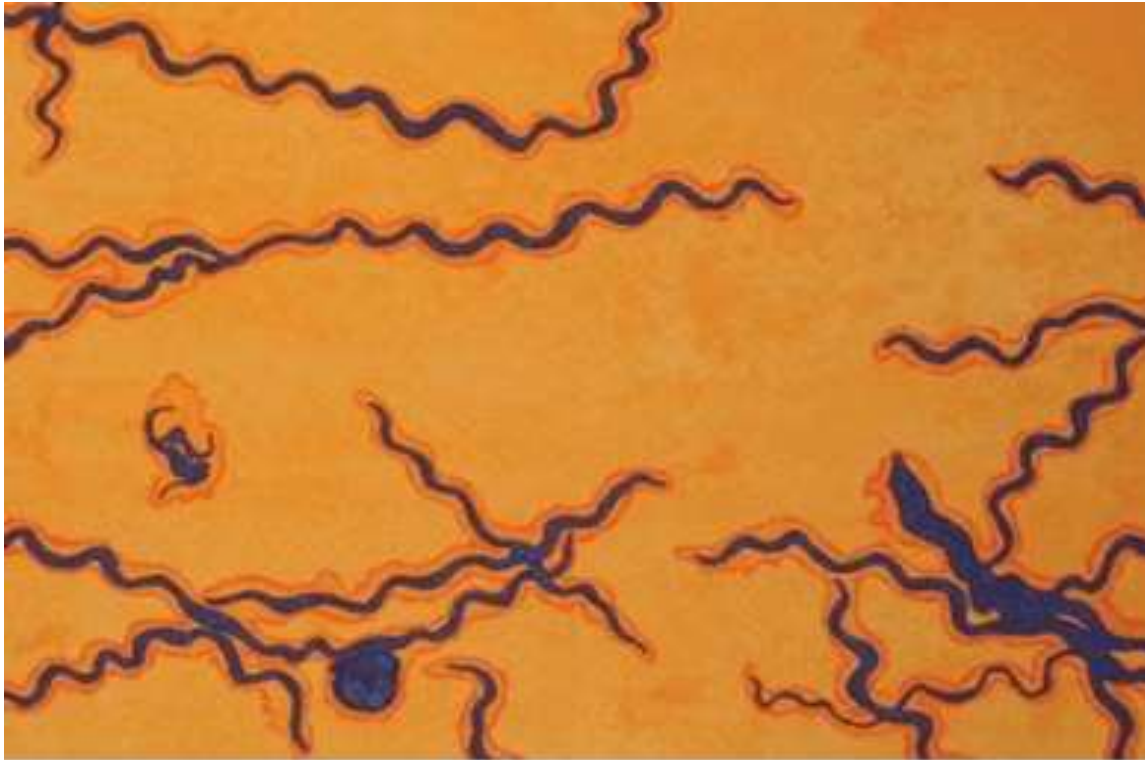
1- Members of this species (leptospire) are motile helical bacteria (0.1 x 6 to 12 μm) with hook-shaped ends.



2- Although cytochemically Gram-negative, they do not stain well with conventional bacteriological dyes and are usually visualized using dark-field microscopy.



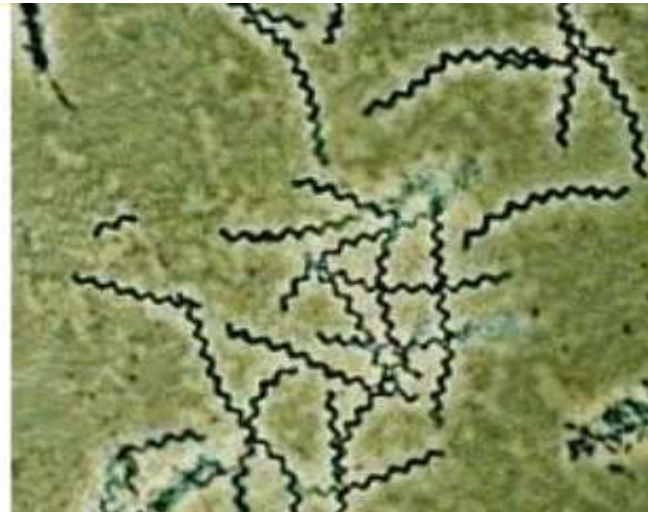
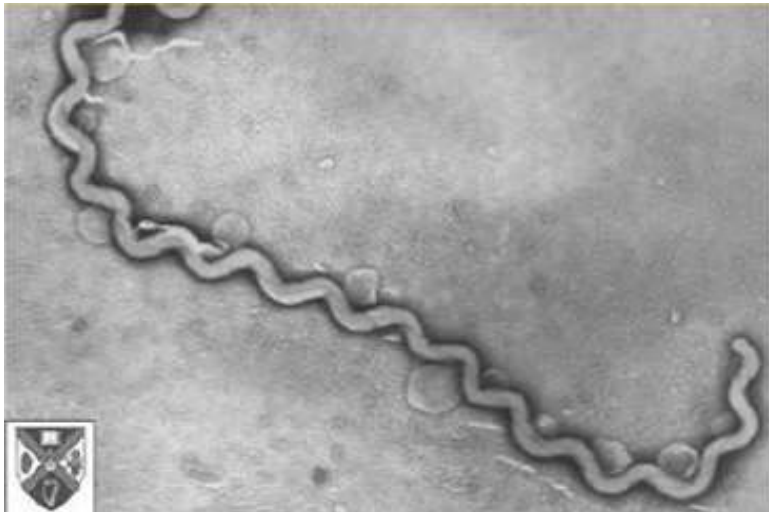
3- Silver impregnation and immunological staining techniques are used to demonstrate leptospire in tissues.



Thin helical corkscrew, dark field or special stains?

Culture

- Leptospire are strictly aerobes
- Can be cultured in a serum or albumin-Tween medium
- Late grow so resulting less efficient diagnostic
- Preferable serology test to identify several serogroups



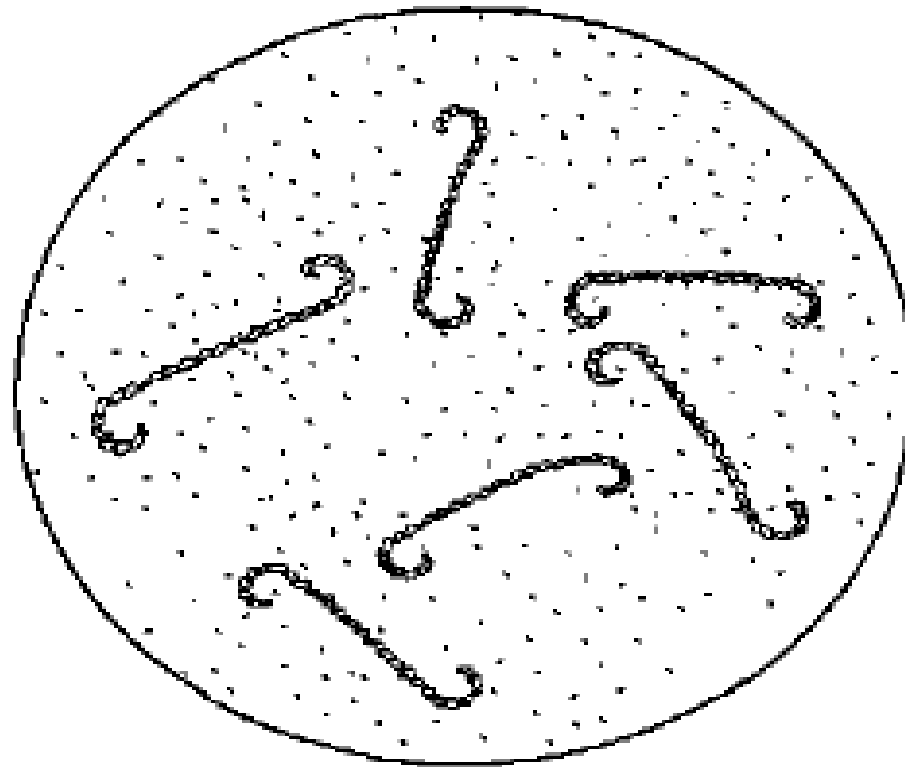


Figure 31.3 The appearance of leptospire when examined by dark-field microscopy. Their helical structure, which imparts a cord-like appearance, and their hooked ends differentiate these slender motile bacteria from most pathogenic microorganisms.

Normal Habitat

- Gram negative, obligate aerobe spirochete **Found in aquatic environments**
- Variety in domestic and wild animals such as rats, rodents, cattle, pigs and dogs. Humans are accidental host.
- **Produce systemic infections in many species**
- Largely secreted in urine **of affected species**
- Survive many weeks in soil and water, in alkaline and 28 – 32°C

Leptospira interrogans serovars

The genus *Leptospira* divided in to two species

pathogenic:

- *Leptospira interrogans* (parasitic)

non-pathogenic:

- *Leptospira biflexa* (saprophytic)

Leptospira species

--Shed in urine --Cultured in liquid media aerobically at 30°C

-Dark-field microscopy, silver staining and . immunofluorescence used for recognition

Infections

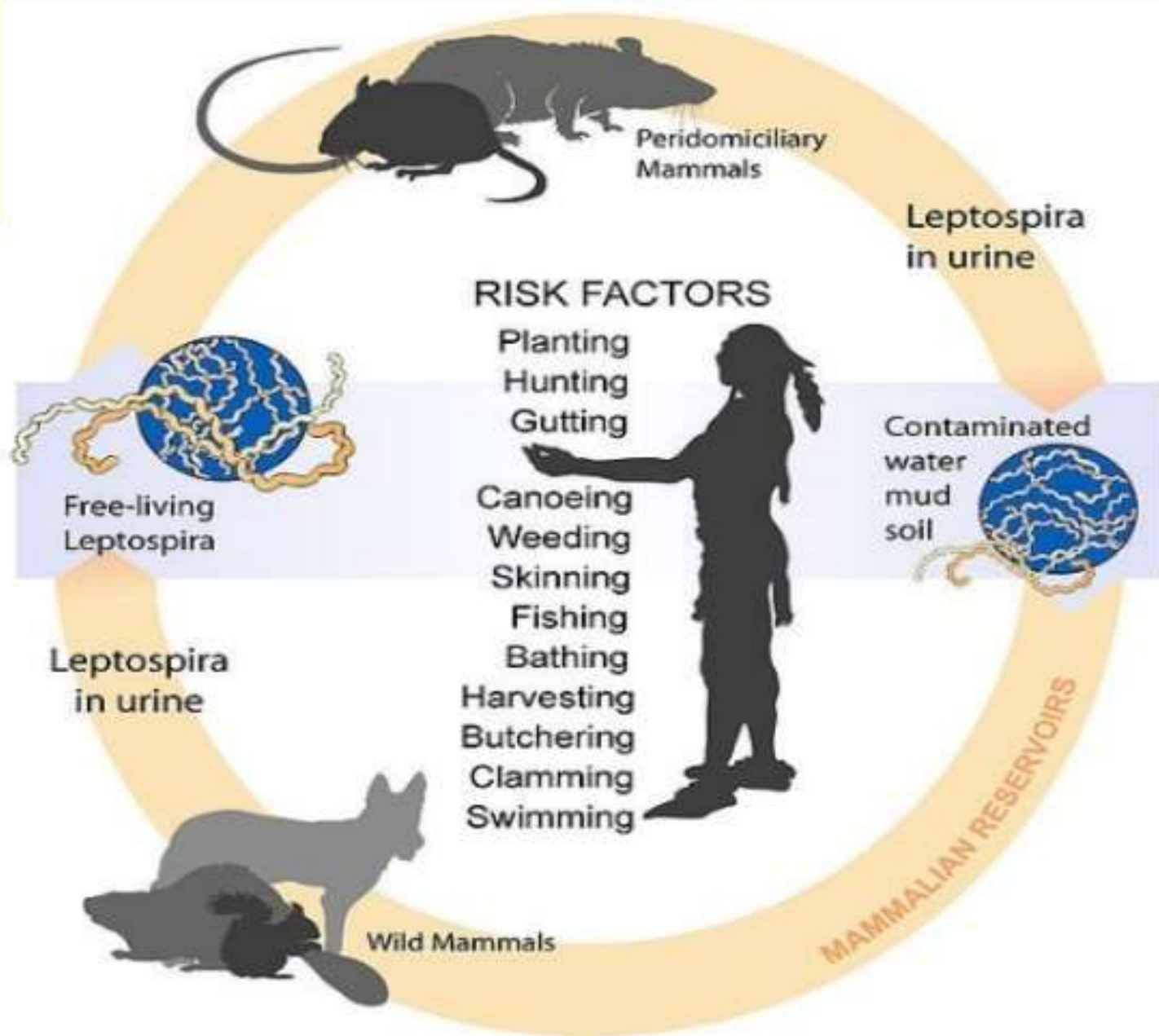
Leptospirosis, which can affect all domestic animals and humans, ranges in severity from mild infections of the urinary or genital systems to serious systemic disease.

Usual habitat

Leptospires can survive in ponds, rivers, surface waters, moist soil and mud when environmental temperatures are warm.

Pathogenic leptospires can persist in the renal tubules or in the genital tract of carrier animals.

Although indirect transmission can occur when environmental conditions are favorable, these fragile organisms are transmitted most effectively by direct contact.



Leptospirosis

Canine typhus or infectious jaundice

- The disease misdiagnosed with diseases cause hepatic or renal damage as:
 - Infectious hepatitis
 - Canine distemper

Epidemiology

1- Although leptospires are found worldwide, some serovars appear to have a limited geographical distribution. In addition, most serovars are associated with a particular host species, their **maintenance host**.

2- Disease is frequently mild or subclinical in these highly susceptible maintenance hosts and is often followed by prolonged excretion of leptospires in urine.

3- Maintenance hosts are the main source of environmental contamination and of natural transmission to other animal species which are termed **incidental hosts**.

4- Incidental host species usually exhibit low susceptibility to infection, develop severe disease and are inefficient transmitters of leptospire to other animals.

5- Genetic factors may account for differences observed in the severity of infection in different host species.

Pathogenesis

- 1- Leptospire**s invade tissues through moist, softened skin or through mucous membranes; motility may aid tissue invasion.
- 2- They spread** throughout the body via the bloodstream but, following the appearance of antibodies at about 10 days after infection, they are cleared from the circulation.
- 3- Some organisms** may evade the immune response and persist in the body, principally in the renal tubules but also in the uterus, eye or meninges.
- 4- There is evidence** that Leptospiral chemotaxis for haemoglobin may be involved in the initiation of infection (Yuri *et al.*, 1993).

Pathogenesis

5- Leptospires can evade phagocytosis in the bloodstream, possibly by inducing macrophage apoptosis (Merien et *al.*, 1997).

6- It has been suggested that, following attachment to host cells, the organisms gain entry by receptor-mediated endocytosis (Merien et al., 1997).

7- In susceptible animals damage to red cell membranes and to endothelial cells along with hepatocellular injury produces haemolytic anaemia, jaundice, haemoglobinuria and haemorrhage, associated with acute leptospirosis.

Diagnostic procedures

Diagnosis of leptospirosis in maintenance hosts usually:

1- Requires screening of a defined population.

2- Clinical signs, together with a history suggestive of exposure to contaminated urine, may suggest acute leptospirosis.

3- Organisms may be detected in fresh urine by dark-field microscopy, but this technique is relatively insensitive.

4- Leptospires may be isolated **from the blood during the first seven to ten days of infection and from urine approximately two weeks after initial infection** either by culture in liquid medium or by animal inoculation.

Slow-growing serovars such as hardjo may require incubation for six months in liquid media at 30°C. Commonly, EMJH (Ellinghausen, McCullough, Johnson and Harris) medium based on 1% bovine serum albumin and Tween 80, is used for isolation

5- Isolates should be identified using DNA profiles and serology.

6- Fluorescent antibody procedures are often used for the demonstration of leptospire in tissues. Suitable tissues include kidney, liver and lung.

7- DNA hybridization, PCR,

8- The standard serological reference test, the microscopic agglutination test

9-A number of ELISA tests, developed in certain countries, are based on the predominant serovars

Public health aspects

Leptospirosis is an occupational disease of abattoir workers, dairy and pig farmers, veterinary surgeons and those engaged in manual work related to sewage and drainage.

Treponema

- *Treponema pallidum pallidum*, which causes syphilis
- *T. pallidum endemicum*, which causes bejel or endemic syphilis
- *T. pallidum carateum*, which causes pinta
- *T. pallidum pertenue*, which causes yaws

YAWS



PINTA



SYPHILIS

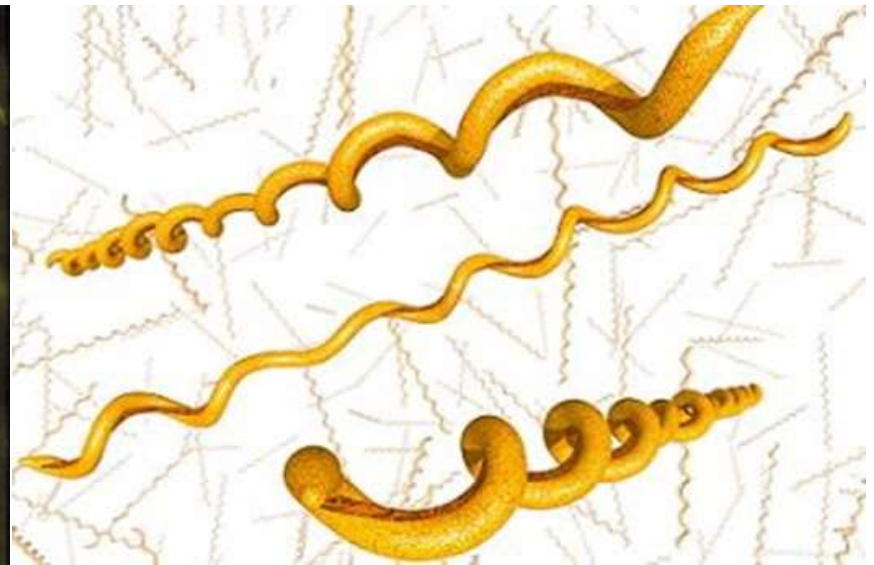


Borrelia



Borrelia burgdorferi
Borrelia recurrentis
Borrelia duttoni

Borrelia, which are longer and wider than other spirochaetes, have a similar helical shape. In addition to a linear chromosome, which is unique among bacteria, borreliae possess linear and circular plasmids.



Although these spirochaetes can cause disease in animals and humans, subclinical infections are also common. Borreliae are transmitted by arthropod vectors

Normal habitat

Borreliae are obligate parasites in a variety of vertebrate hosts. Although these organisms persist in the environment for short periods, they depend on vertebrate reservoir hosts and arthropod vectors for long-term survival

Microscopy

- Large 10-20 μm in size, 0.5 μm diameter
- Actively motile spirochaetes wt coils of varying size
- Agglutinate together
- Easily stain in Giemsa, Field's or other Romanowsky stain; prolonged Gram staining (Gram negative)
- Well seen by dark-field illumination

Culture

- *Borreliae* are aerobes
- Can be cultured in Kelly's medium

Differentiation of *Borrelia* species

Borreliae can be differentiated from other spirochaetes by their morphology, by the low guanine and cytosine content of their genomic DNA and by ecological, cultural and biochemical characteristics.

Pathogenesis

Transmission of *B. burgdorferi* sensu lato occurs when an infected tick feeds on a susceptible animal. Prior to feeding, the spirochaetes are restricted to the midgut of the ticks and, following ingestion of blood, they are found in the salivary glands. Following ingestion of blood by the tick, a change occurs in the expression of the outer surface protein (Osp) of the borreliae. This change in Osp expression appears to be essential for virulence .

Pathogenesis

After entering the bloodstream of a susceptible host, borreliae multiply and are disseminated throughout the body. Organisms may be demonstrated in joints, brain, nerves, eyes and heart.

Public health aspects

Lyme disease is an important tick-borne infection of humans. Infection is often acquired by walking in endemic areas during periods of tick activity. Dogs, cats and farm animals may act as transport hosts for infected ticks thereby exposing humans to the risk of infection.