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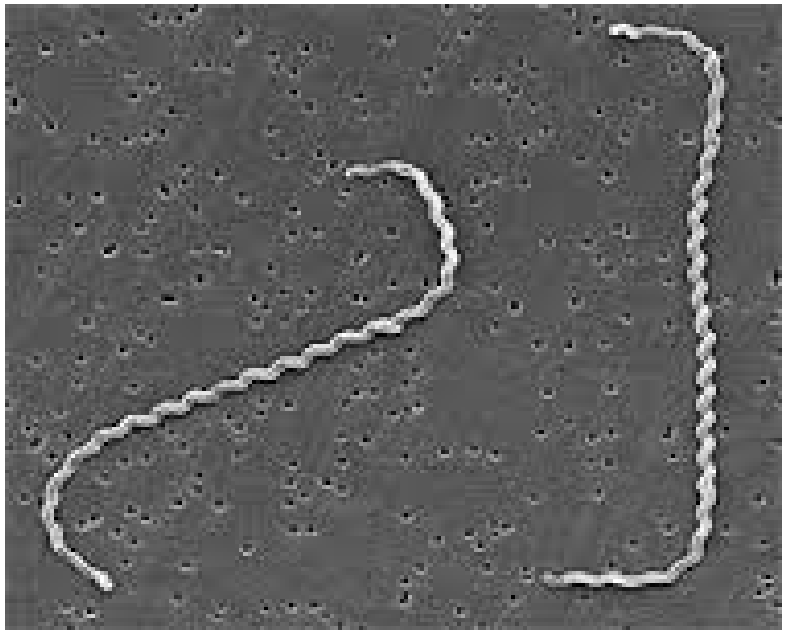
Order: Spirochaetales

contains two families:

- **F. Leptospiraceae which contains the genus leptospira**

- **F. Spirochaetaceae which contains 3 genera,**
- **Borerelia,**
- **Treponema**
- **Brachyspira.**

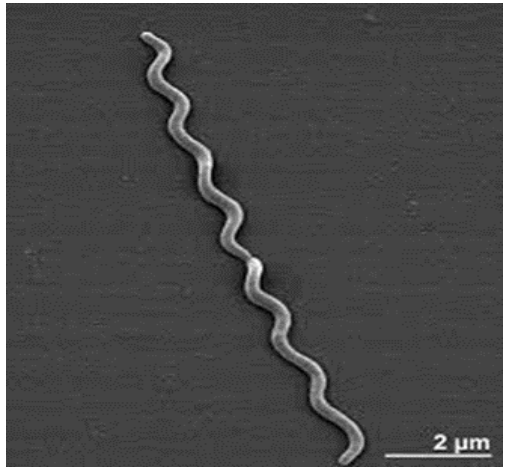


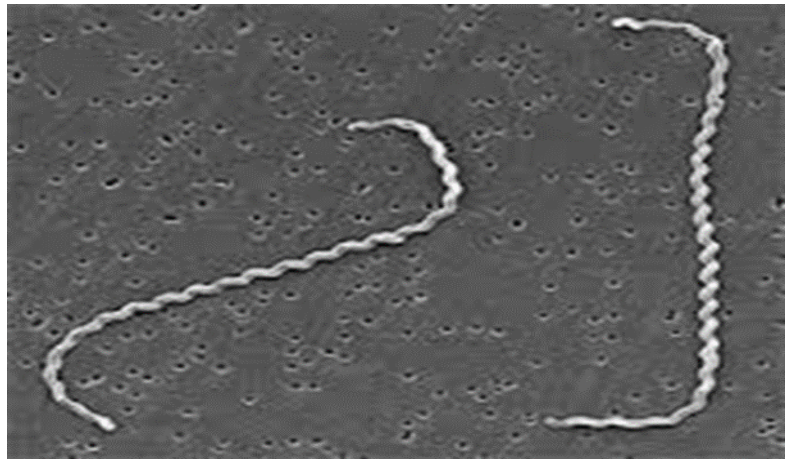
General Characteristics

- It comprises spiral or helical bacteria
- They are thin, flexible, spiral and coiled bacteria (3-500 µm length)
- Corkscrew-like that characterized by primary and secondary coils with hook-shaped ends

- It is cytochemically Gram-negative

- Highly motile by means of endoflagella. Motility occurs by rapid contraction of their spiral bodies along their long axis and movement is in one direction (Spirochaeteal motility).





Spirochaetes motility

General Characteristics

They could be examined either:

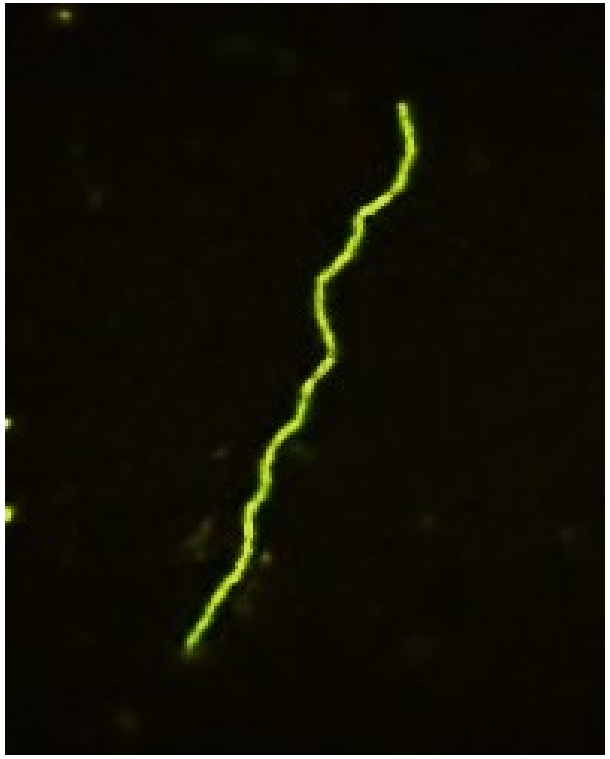
- ✓ **Unstained**; using dark ground microscope to detect motility,
- ✓ Or **stained** using Silver impregnation, Levaditi's stain, Fontanae stain (appears dark brown or black) or Fluorescent stain. While in case of avian spirochaetes; Giemsa, Leishman's stains are used.

❑ Spirochaetes are difficult to be cultivated on ordinary or artificial media and requires:

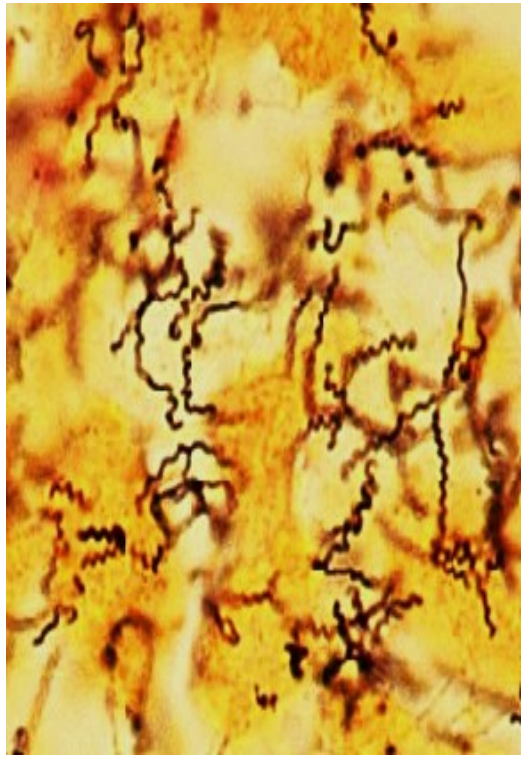
- ✓ Susceptible laboratory animal.
- ✓ Embryonated chicken egg.
- ✓ Complex specialized media (highly enriched media containing rabbit serum 10%, other body fluids and tissue fragments of liver)

Stains to detect **Spirochaetes**

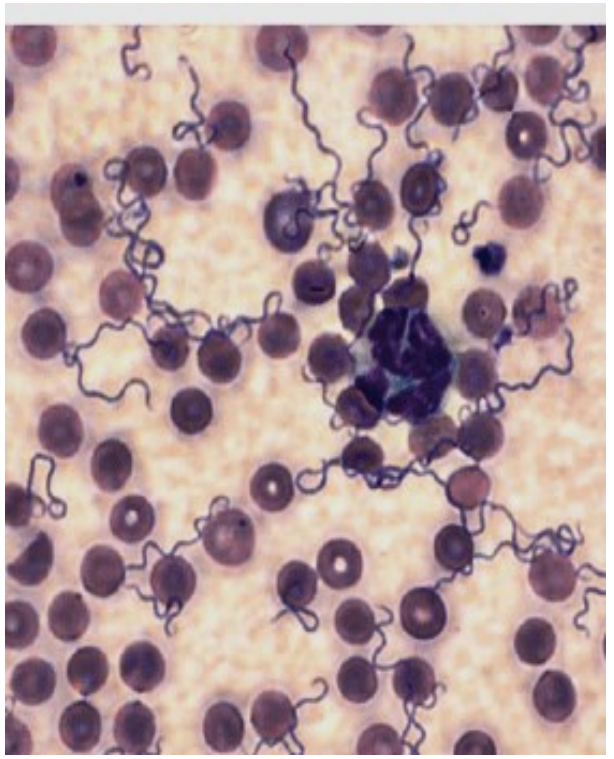
Dark ground microscope



Fluorescent stain

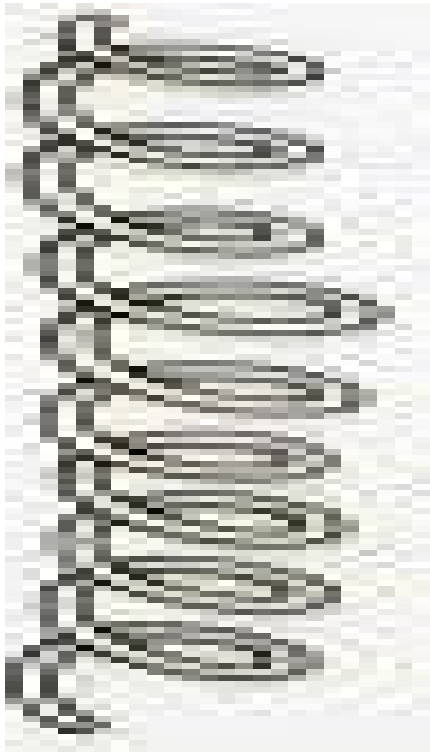


Fontanae silver impregnation stain (appears dark brown)



Giemsa-stained blood smear

1- Genus Leptospira



- Most Leptospira are aquatic free living Spirochaetes that present in river, sea, lake and sewage water.
- Motile helical bacteria; thin, spiral, coiled and corkscrew-like with hook-shaped ends, 0.1× 6-12µm in length, non sporulated, non-capsulated.
- They do not stain well with conventional bacteriological dyes (it is cytochemically Gram-negative) and are usually visualized in wet preparations using dark-field microscopy.
- Silver impregnation and immunological staining techniques are used to demonstrate leptospira in tissues.
- They are highly flexible, highly motile (Spirochaeteal motility) and pass through retained bacterial filters.
- **Leptospira serotyping:** are differentiated serologically into 2 species; ***L. interrogans;*** containing pathogenic Leptospira causing leptospirosis in man and animals, and ***L. biflexa*** containing saprophytes.
- Currently more than 250 serovars in 23 serogroups are defined.

Leptospirosis in domestic animals caused by serovars of *Leptospira interrogans*

Serovars	Hosts	Clinical diseases
L. Canicola	- Dogs, Man	- Acute nephritis in pups, chronic renal disease in adults - Canicola fever in man or canine fever (Stuttgart disease) in dogs - Stray dogs are carriers
L. Ictherohaemorrhagiae	- Cattle, sheep	- Acute septicaemic disease in calves and lambs. - Anaemia, haemoglobinurea and abortion in pregnant animal with jaundice
	Humans, dogs	- Acute hepatitis with Jaundice (Weil's disease or Infectious jaundice) - Rodents as rats act as important reservoir (urine)
L. Grippotyphosa	- Man, Cattle, dogs	- Septicaemia in young calves, death, abortion in pregnant - Mud or Swamp fever in man; anaemia, jaundice, black urine - Rodents as rats act as important reservoir
L. Pomona	- Cattle, sheep	- Acute haemolytic disease in calves and lambs, abortion.
	- Horses	- Abortion, periodic ophthalmia.

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

Laboratory diagnosis of Leptospirosis.

Collection of samples:

- Blood Samples during fever, at 1st week (7-10 days) after infection in the stage of leptospiraemia.
- Urine samples during leptospirurea.
- In dead animals: kidney and liver. Blood is taken for serological techniques.
- In aborted animal: uterine discharge, placenta, fetal membranes.
- All samples must be sent immediately to the lab within minimum of delay.

Diagnosis:

A. Direct Methods:

1. Microscopical examination:

a) Dark-field microscope.
b) Tissue stained sections.
c) Fluorescent Microscope.
d) Giemsa-staining.

1. Dark-field microscope:

- ✓ Put one drop of fresh urine, blood or uterine discharge on clean slide.
- ✓ Then cover with cover slip and examine with high power and dark-field microscope.
- ✓ Typical highly flexible bacteria corkscrew like with primary and secondary coils and double hooks. This technique is relatively insensitive.

2. Tissue stained sections:

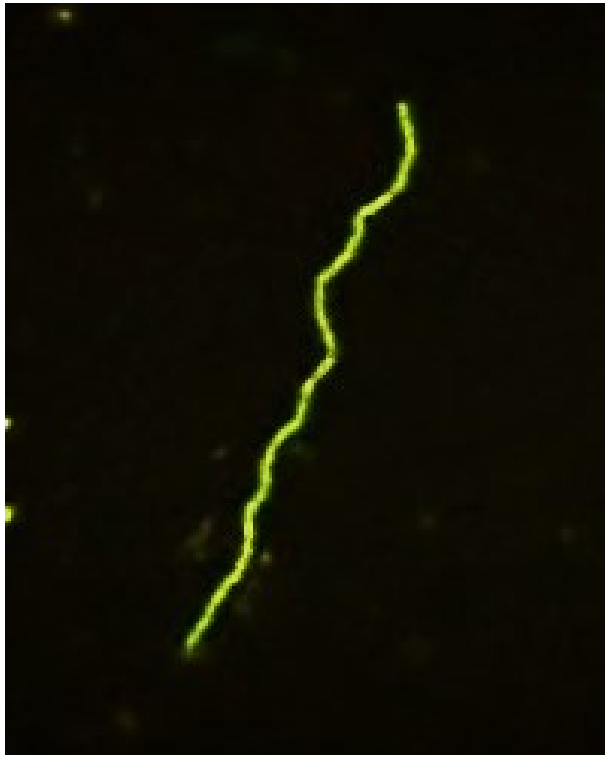
- Part from liver or kidney or fetal membrane is impregnated in 1% silver nitrate (silver impregnation technique) or Fontana stain for 3-5 min.
- Then washed with water then make impression smear between 2 slides and examine microscopically.
- In positive cases, the organism appears dark brown or black colour inside the tissue.

3. Fluorescent Microscope:

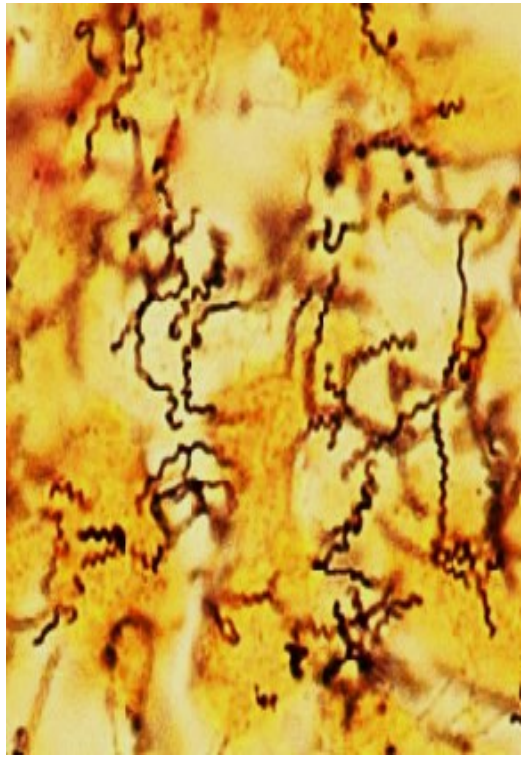
- ❖ Impression smear stained with fluorescin labeled with anti-*L. icterohaemorrhagiae* when examined with the fluorescent microscope show the characteristic morphology with yellowish green fluorescence.

Stains to detect **Spirochaetes**

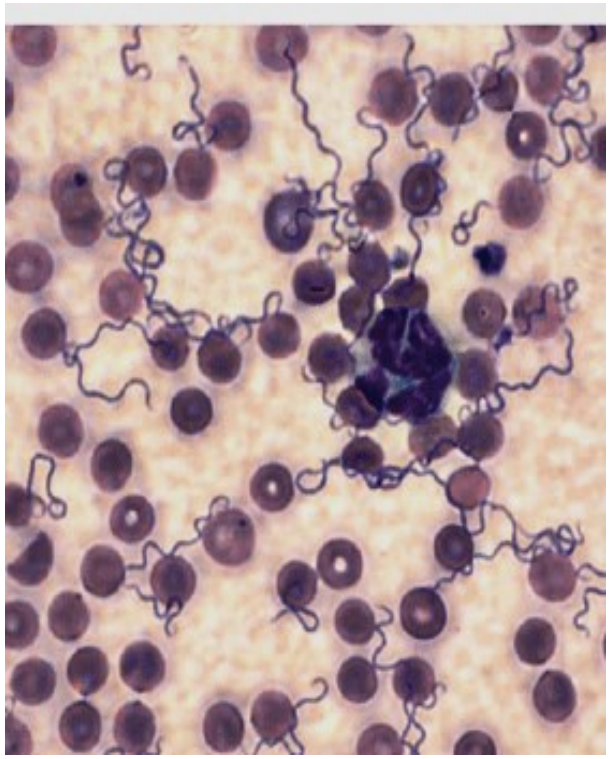
Dark ground microscope



Fluorescent stain



Fontanae silver impregnation stain (appears dark brown)



Giemsa-stained blood smear

2. Isolation by cultivation:

Leptospira does not grow on ordinary media or blood agar but requires complex enriched media with addition of 7-10% rabbit serum with traces of haemoglobin in addition to folic acid, tween 80 and albumin, (complex enrichment).

✓ **Incubation is aerobically at 37C (best at 35C) for 2 weeks.**

- **Liquid media:** e.g. **Stuart's medium & EMJH (Ellinghausen, McCullough, Johnson and Harris)**
- Growth appear 10-15 days (may reach one month) in the form of faint turbidity and form precipitation in old culture. A drop was examined with dark-field microscope corkscrew or by cultivation on semisolid media.
- **Semisolid media:** e.g. **Fletcher's medium**.
- inoculate sample and incubate for 2 weeks. Growth appears in the form of opaque visible ring about 1 cm under surface of media.
- **Solid media:** e.g. **Cox medium**
- It is used for purification of contaminant culture.

3. Animal inoculation:

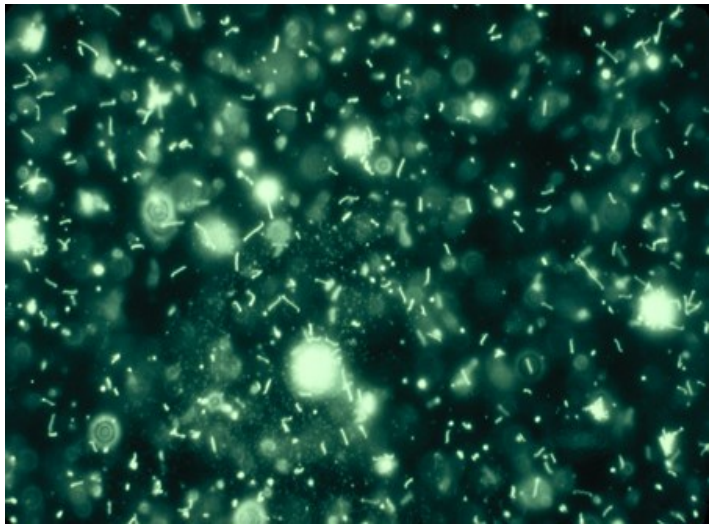
Suspension from suspected blood or urine is injected i/p or i/v into young G. pig (for *L.* Icterohaemorrhagiae) or young hamster (for *L.* Canicola). Death occurs within 2 weeks.

Main symptoms are fever, anaemia, jaundice and haemoglobinurea. The microorganism appears in blood during leptospiraemia and in urine during leptospirurea by examination of blood or urine with dark-field microscope.

✓ Leptospira may be inoculated also in embryonated chicken egg (ECE).

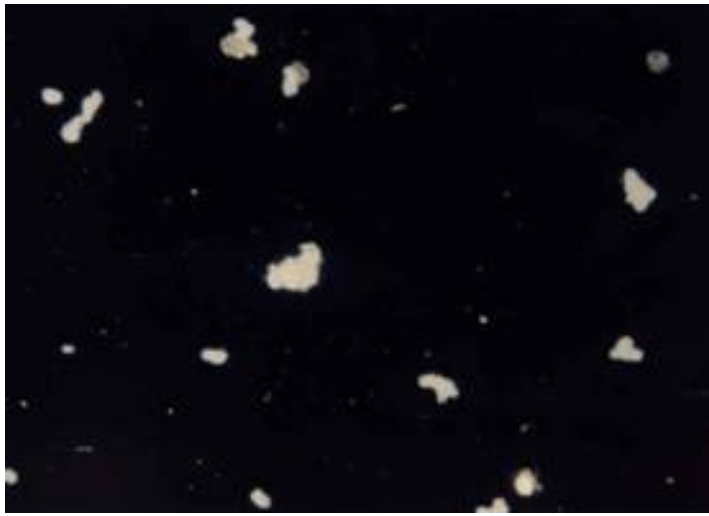
4. Molecular methods:

PCR can be used for detection of organism DNA in urine and tissues.



B. Indirect Methods:

1. **Microscopic agglutination test (MAT):** useful for diagnosing acute infection
   - ❑ On clean slide one put a drop of standardized Leptospira Ag and a drop of the tested serum then mix and examine under microscope to detect agglutination.
   - ❑ If positive, serial dilution, and apply tube agglutination for detection of antibody titer.
   - ❑ Titers in excess of 1:400 or a four-fold rise in the titer in paired samples are diagnostically significant when accompanied by clinical signs consistent with leptospirosis.



2. Macroscopic agglutination test:

One drop of blood is added to one drop of known formalized suspended antigen then mix. Agglutination is detected with eye but it is not accurate.

Control of leptospirosis.

- Eradication of rodents (wild rat) that carrier for *L.* icterohaemorrhagiae *& L.* Grippotyphosa.
- Eradication of stray dogs (carrier for *L. canicola*)
- Serological surveys to detect carriers.
- Treatment of diseased animal with penicillin or tetracycline injection.
- **Vaccination**: By killed vaccines; Formalized whole culture vaccine or Mixed killed Leptospira bacterin.

2- Genus Borrelia

- ✓ It is pathogenic for man, animal and bird.
- ✓ Borreliae have a similar helical shape but they are thicker and wider than other Spirochaetes with fewer open coils.
- ✓ Easily stained by aniline dyes but difficult to be cultivated on culture media.

B. burgdorferi, the cause of Lyme disease in animals and humans
B. anserina, the cause of avian borreliosis (Fowl Spirochaetosis).

Fowl Spirochaetosis:

This is an acute fatal septicaemic disease of fowl and water birds, characterized by fever, marked anaemia, weight loss and high mortality rate resulting in significant economic loss in flocks. Paralysis may develop as the disease progress.
P.M examination shows spleen enlargement.

Soft ticks of genus Argas frequently transmit the disease.

Laboratory diagnosis of Leptospirosis.

❖ It depends on presence of microorganism in blood (septicemia). Blood samples are collected. Diagnosis can be confirmed by:

1. Demonstration of the spirochaetes in unstained buffy coat smears using dark-field microscopy.
2. **Staining**: Giemsa-stained smears or Silver impregnation techniques show tangle form.
3. **The organism is usually isolated by:**

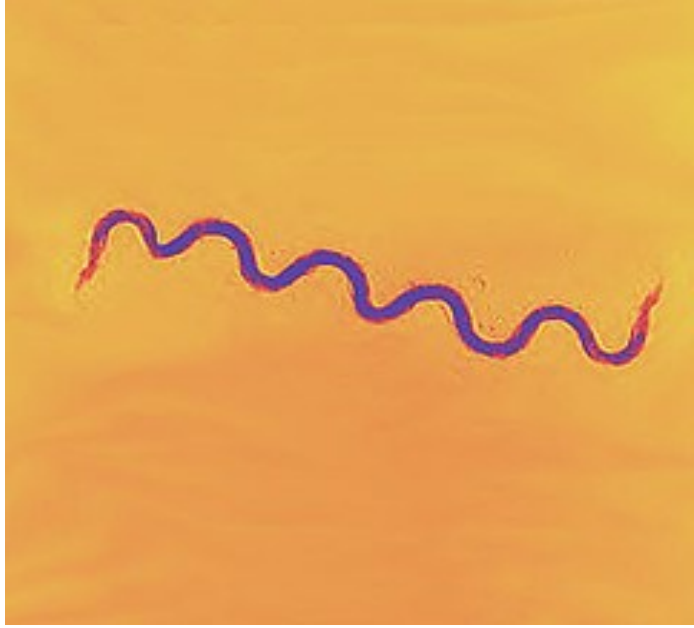
✓ Inoculation in embryonated chicken eggs where the microorganism could be seen in embryonic fluid few days post inoculation.

✓ **Complex media**: It appears after 4-5 days then disappears as occur degeneration and disintegration of it into granules and requires subculture.

Control of Borreliae

- Eradication of blood suckling insects (Soft ticks).
- Treatment of diseased animal with Chloramphenicol or Kanamycin injection.
- **Vaccination**: By Embryonated egg vaccine and Heat killed oil adjuvant vaccine.
- Recovered bird from natural immunity takes immunity from 8 months to a year.

3- Genus Treponema



- It is a true venereal disease in man and rabbit and caused mainly by:
- ***T. palladium*: (the cause syphilis in man).**
- ***T. cuniculi* :(the cause of syphilis in rabbit; while non-pathogenic to man.**

- **Syphilis** is characterized by nodules and ulcers on external genetalia, eye, nose, lips and secondary may reach lung and digestive system but lethal.
- Both male and female are carriers.

Laboratory diagnosis.

Samples: discharges oozing from ulcers.

Microscopical examination:

✓ **Dark ground microscope:**

Treponema appears as delicate with regular narrow coils, tapering or filamentous end about 4-16µm length. They are motile by rapid contraction of their spiral bodies along long axis.

✓ **Fluorescent microscope.**

Serology: (Wassermann's test in man).