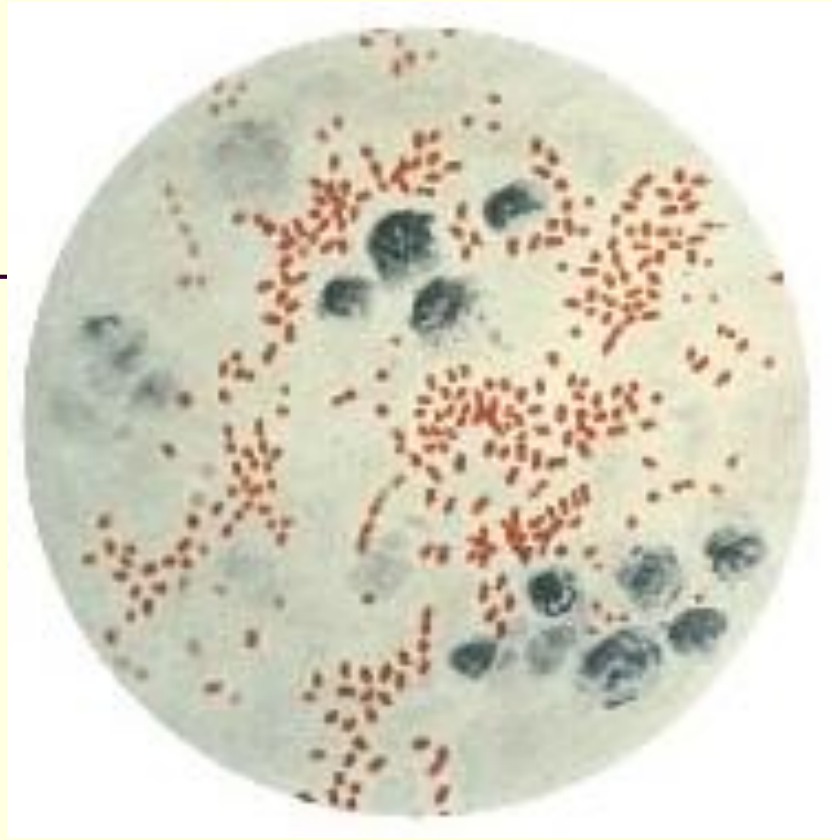

PLAGUE and TULARAEMIA
Gilles Le musit/
Royal library of Belgium

Plague



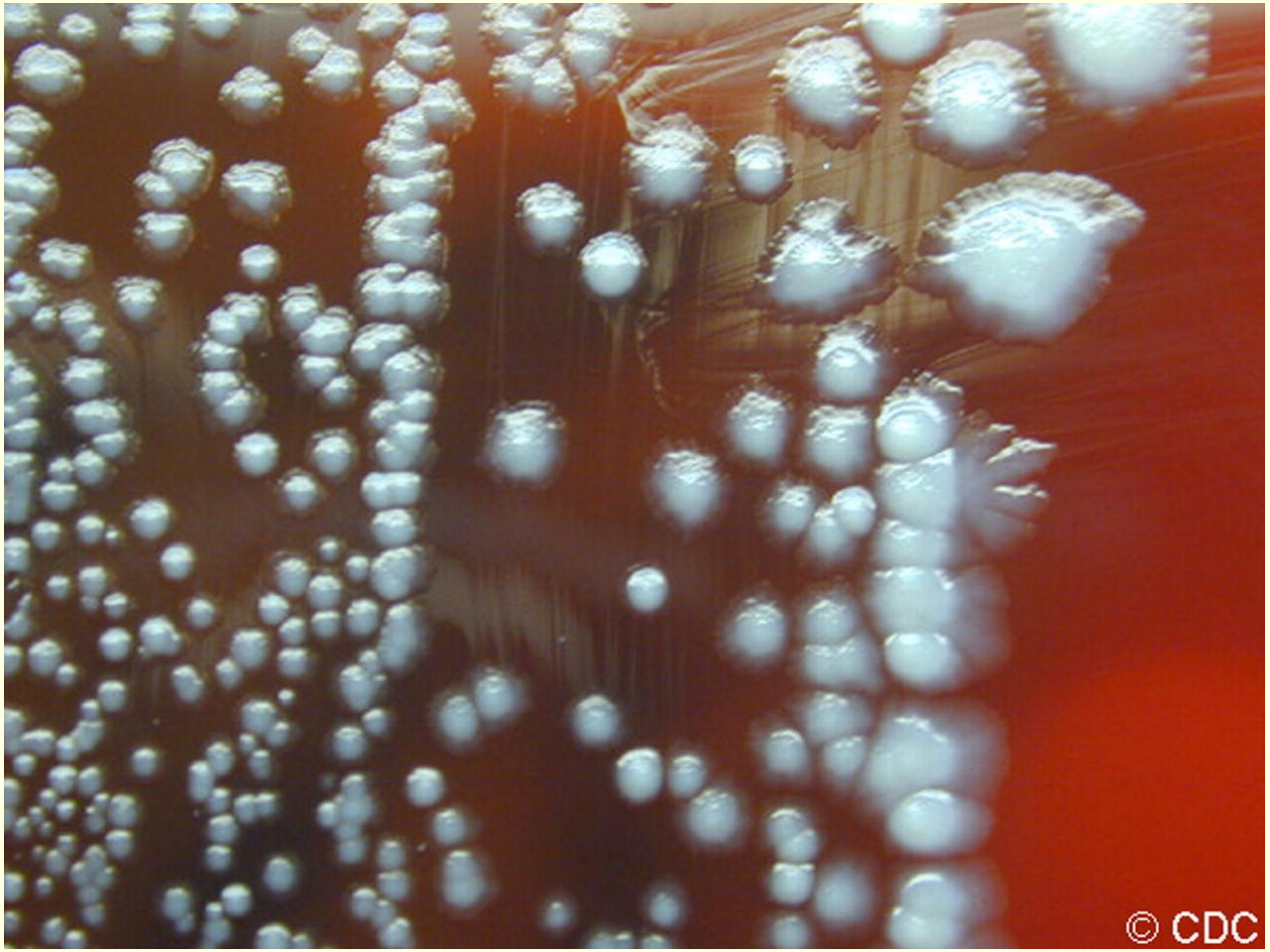
Family Enterobacteriaceae
Genera Yersinia
Species Yersinia pestis

Morphology and staining: it is short, plump, Gram-negative, 1.5 x 0.5 μ to 0.7 μ in size with rounded end and convex sides. Pleomorphism is common. It shows bipolar staining (safety pin appearance). It is non-motile, non-sporing surrounded by slime layer.

Cultural characteristic

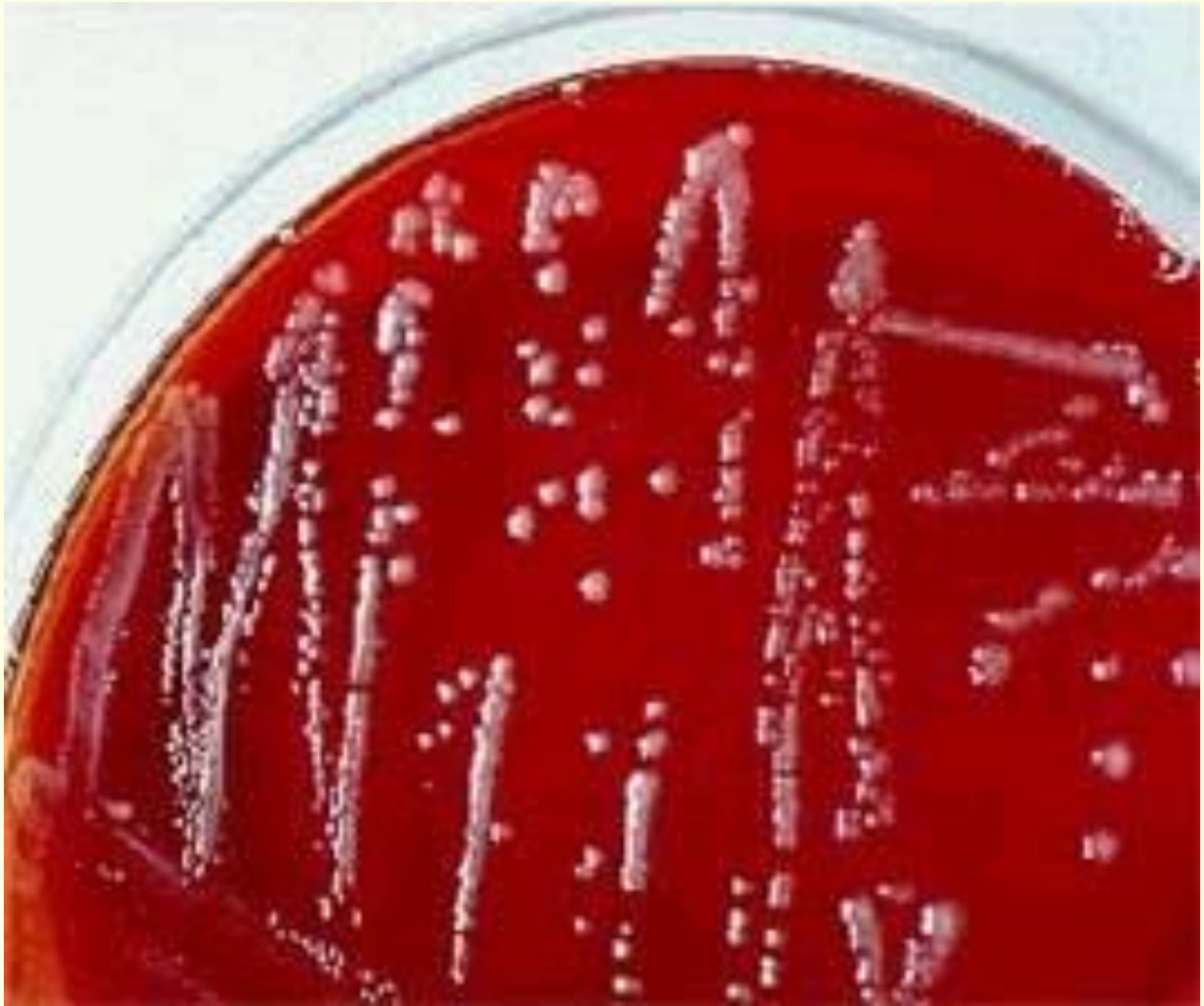
- It is aerobic and facultatively anaerobic. Optimum temperature for growth is 27^{o}C and optimum pH is 7.2. It can grow in 3% sodium chloride.
- Broth shows flocculent growth occurring at the bottom and along sides of the tube with little or now turbidity.
- Ghee broth shows growth which hangs down from the surface into the broth resembling stalactites.
- Nutrient agar: colonies are small and transparent becoming opaque on continued incubation.
- Blood agar: colonies are dark brown due to absorption of hemin pigment.
- MacConkey medium: colonies are colorless.

Yersinia pestis on sheep blood agar, 72 hours. ***Y pestis*** grows well on most standard laboratory media, after 48 to 72 hours, grey-white to slightly yellow opaque raised, irregular "fried egg" morphology; alternatively colonies may have a "hammered copper" shiny surface.



72-hour ***Y. pestis*** culture with characteristic “fried egg”. Morphology colonies have a raised, irregular "fried egg" morphology, which becomes more prominent as the culture ages.

48-hour ***Y. pestis*** culture with characteristic “hammered copper” morphology. Colonies also can be described as having a "hammered copper," shiny surface. There is little or no hemolysis of the sheep red blood cells.



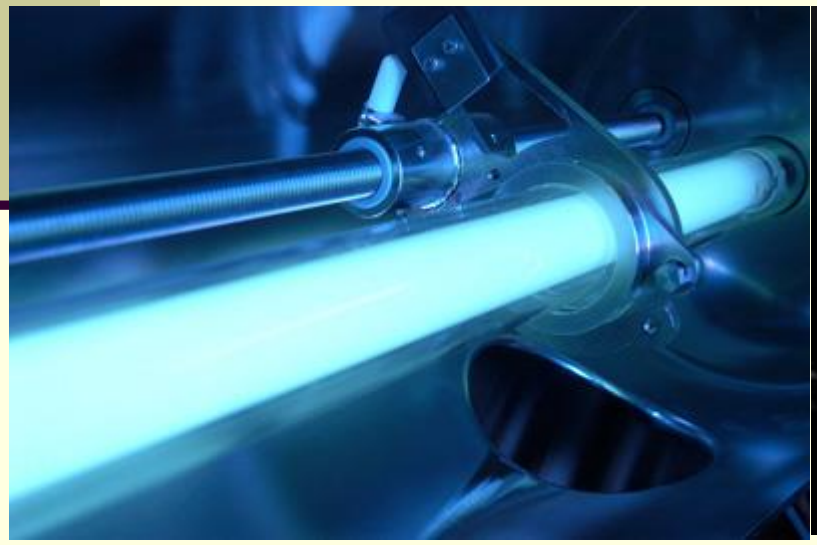
Biochemical activity

- Catalase test positive
- Salicin test positive
- Gelatin hydrolysis negative
- Milk is not hydrolysed
- Indol test negative

- Carbohydrate utilization is very little: Acid without gas is produced from glucose (not always)

Resistance:

Yersinia is destroyed by exposing to heat(55°C) , sunlight, drying and chemical disinfectants. It is destroyed by 0.5% phenol in 15 minutes. It can remain viable in soil or rodent burrows.



Antigenic structure

There are two types of antigens:

- Capsular or envelope antigen which is heat labile and is readily lost when the organism is growing in vitro or in the insect vector. It inhibits phagocytosis and is present in virulent strain.
- Somatic antigens V and W are associated with virulence. These antigens are highly toxic for the mouse and, to a lesser extent, for guinea pigs.

Virulence Factors

Toxins: It produces two classes of toxin:

- Endotoxin which is lipopolysaccharide.Endotoxin contributes to tissue damage.
- Second toxin is protein in nature and possesses properties of both exotoxin and endotoxin.
- Virulent strain of plague bacilli produces ***bacteriocin*** and it is ***coagulase*** positive also showing ***fibrinolytic activity***.
- Encapsulated organisms resist phagocytosis.

Epidemiology

Yersinia pestis is primarily a rat pathogen. Human infections are initially transmitted by rat fleas, or by direct exposure to infected tissues but later the disease may shift into the pneumonic form and continue by direct person- to-person spread by respiratory droplets. Also infections are transmitted by

Clinical Disease

The disease is characterized by fever, chills, headache, malaise, prostration, and leukocytosis that manifests in one or more of the following principal clinical forms:

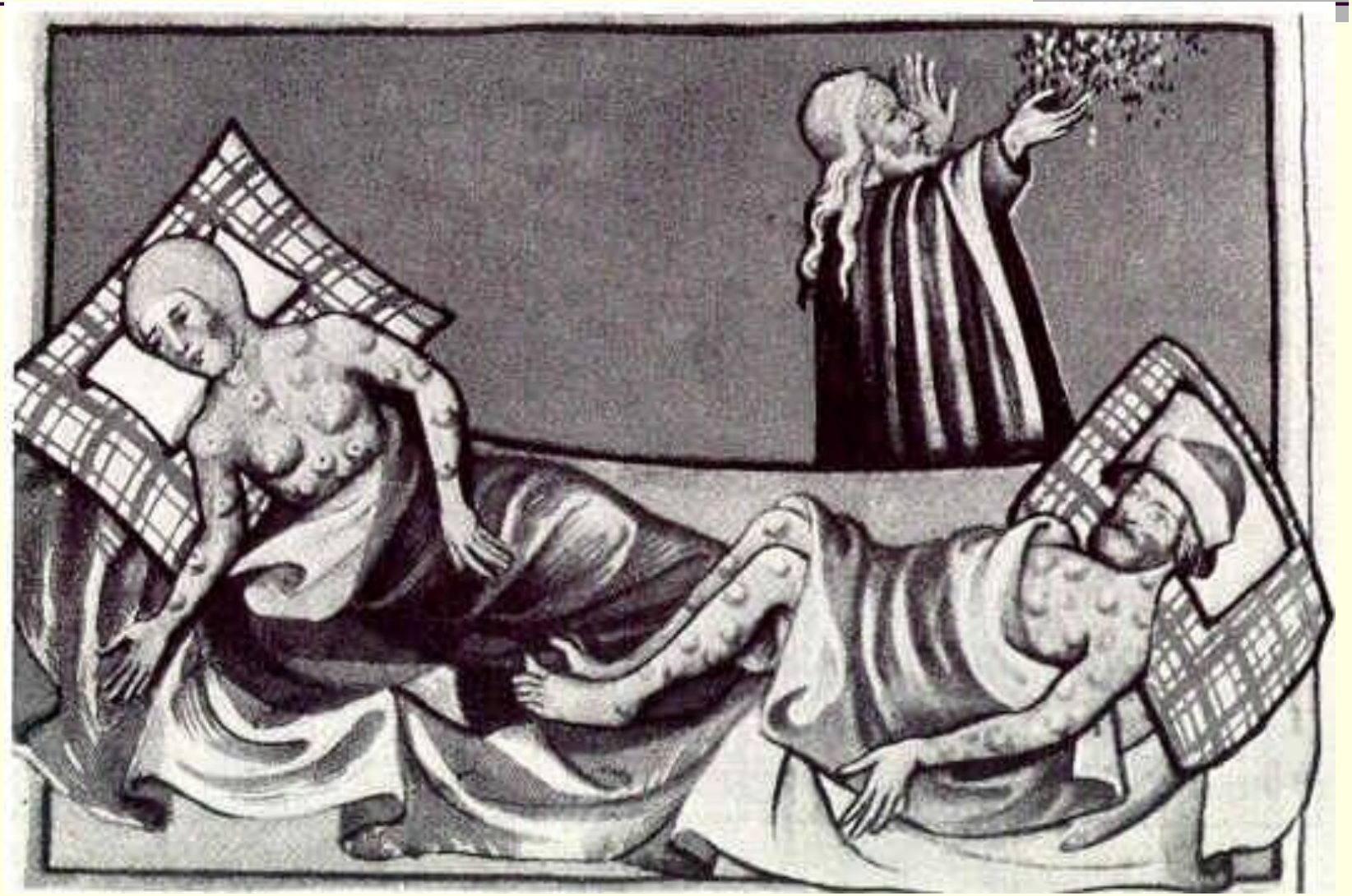
Regional lymphadenitis (bubonic plague)

Septicemia without an evident bubo ***(septicemic plague)***

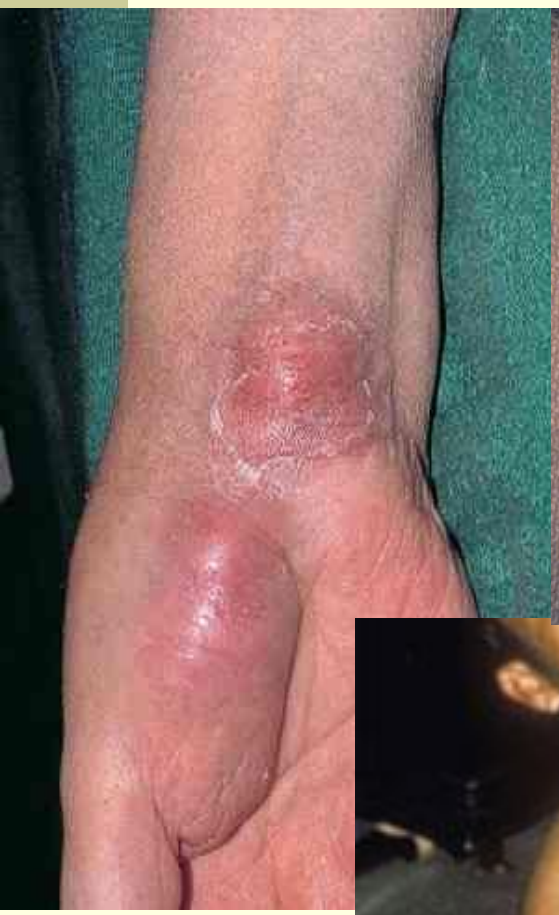
Plague pneumonia, resulting from hematogenous spread in bubonic or septicemic cases ***(secondary pneumonic plague)*** or inhalation of infectious droplets ***(primary pneumonic plague)***

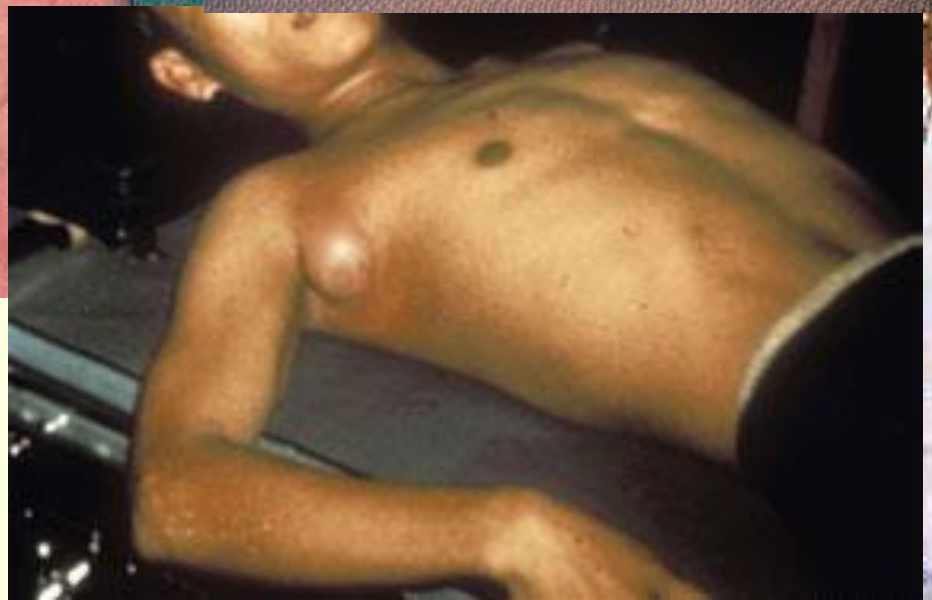
Pharyngitis and cervical lymphadenitis resulting from exposure to larger infectious droplets or ingestion of infected tissues ***(pharyngeal plague)***

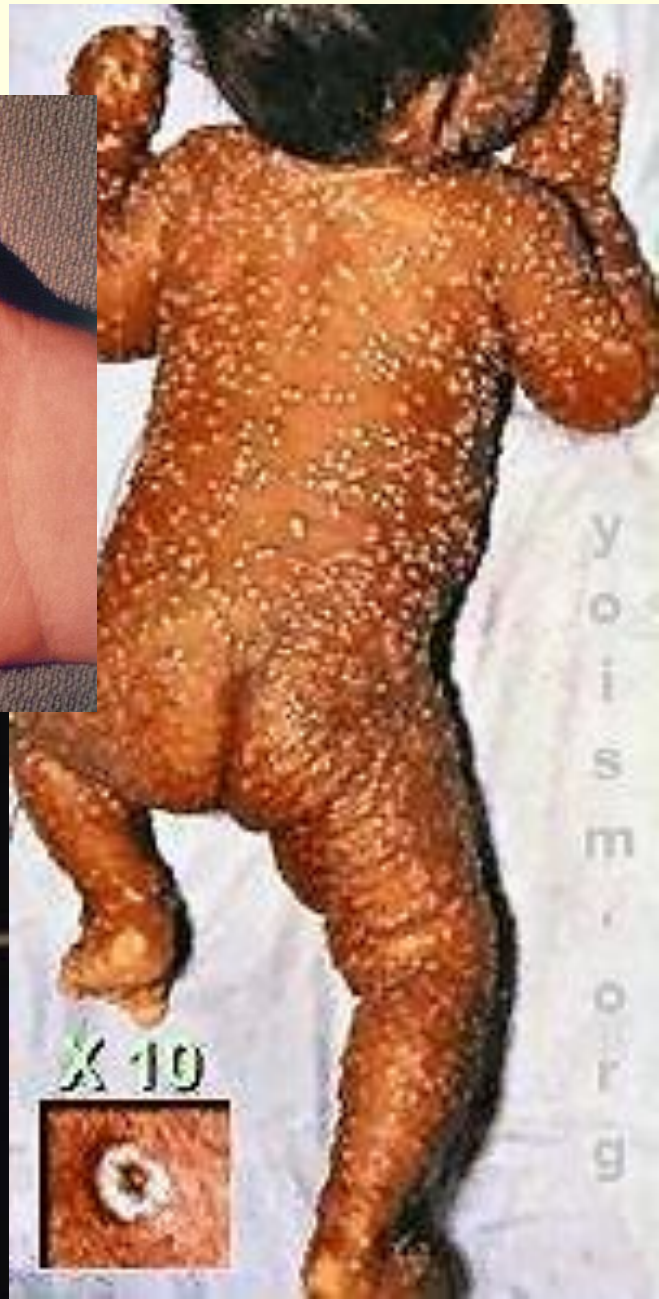
Bubonic plague



Example of Bubonic Plague







Bubonic plague

Two to five days after infection, patients experience a sudden fever, chills, seizures, and severe headaches, followed by the appearance of swellings or "buboes" in armpits, groin, and neck. The most commonly affected sites are the lymph glands near the site of the first infection. As the bacteria multiply in the glands, the lymph node becomes swollen. As the nodes collect fluid, they become extremely tender. Occasionally, the bacteria will cause an ulcer at the point of the first infection.

- **Buboes** — Smooth, oval, reddened, and very painful swellings in the armpits, groin, or neck that occur as a result of infection with the plague.

Septicemic plague

Bacteria that invade the bloodstream directly (without involving the lymph nodes) cause septicemic plague. (Bubonic plague also can progress to septicemic plague if not treated appropriately.) Septicemic plague that does not involve the lymph glands is particularly dangerous because it can be hard to diagnose the disease. The bacteria usually spread to other sites, including the liver, kidneys, spleen, lungs, and sometimes the eyes, or the lining of the brain. Symptoms include fever, chills, prostration, abdominal pain, shock, and bleeding into the skin and organs.

Pneumonic plague

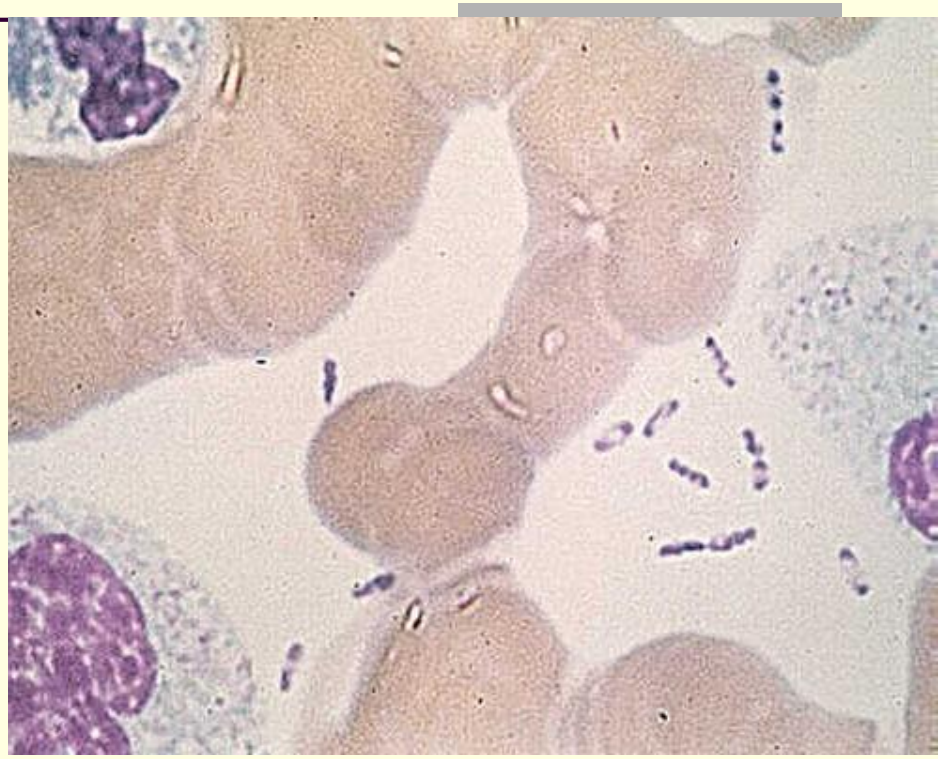
Pneumonic plague may occur as a direct infection (primary) or as a result of untreated bubonic or septicemic plague (secondary). Primary pneumonic plague is caused by inhaling infective drops from another person or animal with pneumonic plague. Symptoms, which appear within one to three days after infection, include a severe, overwhelming pneumonia, with shortness of breath, high fever, and blood in the phlegm. If untreated, half the patients will die; if blood poisoning occurs as an early complication, patients may die even before the buboes appear. Life-threatening complications of plague include shock, high fever, problems with blood clotting, and convulsions.

Pathogenesis

After entering the body, Y.pestis multiples in the local lymphnodes (in bubonic plague) and epithelial tissues. Acute inflammatory response is provoked. Characteristic hemorrhagic necrosis occurs initiating bacteremia and septicemia. As a result bubonic plague, occurs in about 10 to 20% cases. There may be involvement of lungs (pneumonic plague) secondarily. Fibrin thrombi may be extensive in blood vessels of lung, kidney and skin. Infection of serous membranes (pleura, pericardium and meninges) may occur. Endotoxic shock and disseminated intravascular coagulation may also take place.

Laboratory diagnosis

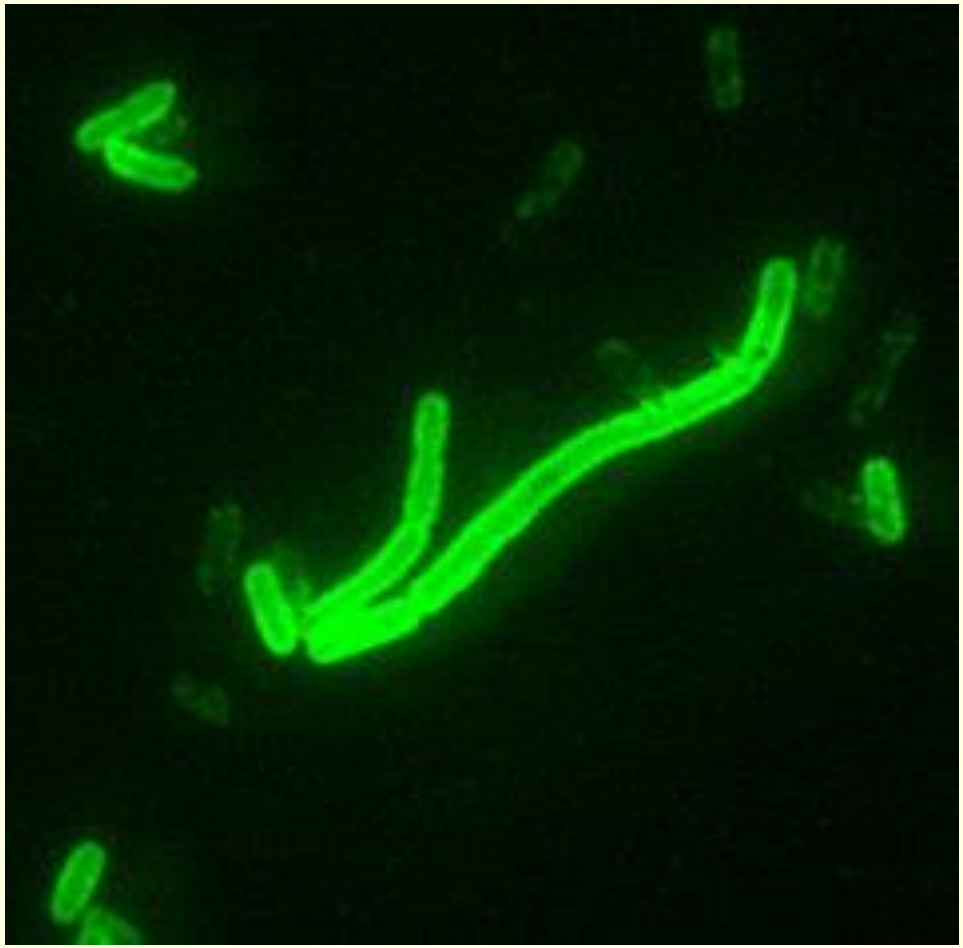
A **presumptive** diagnosis by identifying a Gram negative coccobacillus and safety-pin bipolar staining organisms in Gram-stained or Wright-Giemsa-stained or Wayson's stained smears is diagnostic. These could be found in peripheral blood, lymph node needle aspirate, sputum and other clinical specimens.



Yersinia pestis, Wayson's stain. Wayson's staining shows cocco-bacilli with bipolar staining.

Yersinia pestis under fluorescent staining

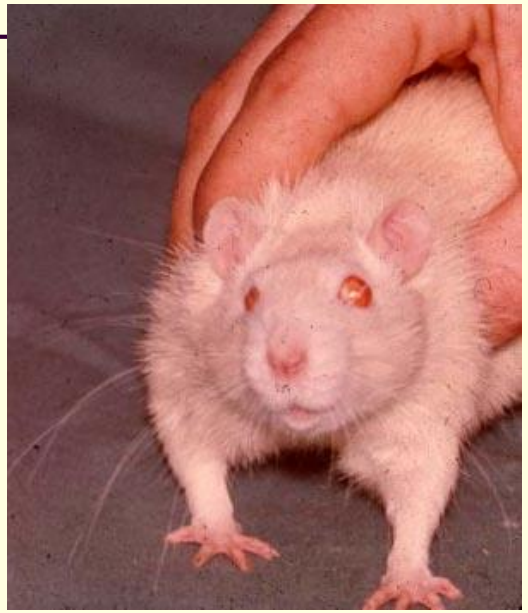
Immunofluorescence of the capsule of the bacteria is diagnostic.



Bacteriological investigations

- **Specimen:** Exudate from involved lymph nodes, sputum throat swab, CSF, and blood (septicemic cases).
- **Isolation and Identification** of *Y. pestis* from a clinical specimen. It is done on blood agar plate. Once isolated, *Y. pestis* can be **identified** by the biochemical tests, gram-staining, antigenic properties, serologic methods, bacteriophage lysis tests.

Biological investigations



Animal inoculation: Guinea pig or albino rats are infected subcutaneously with isolated culture of plague bacilli. Animal dies soon. An autopsy shows necrosis, edema with involvement of regional lymph nodes is spleen is enlarged and congested. In septicemia, bacilli may be demonstrated by smear from local lesionised nodes, splenic pulp and

Serological tests

- Serological methods like IHA using *Yersinia pestis* antigen-F1.F1 capsule antigen could be detected in serum samples by immunoassay. A four-fold increase in antibody titre is also diagnostic

- Polymerase chain reaction.

Treatment and prophylaxis

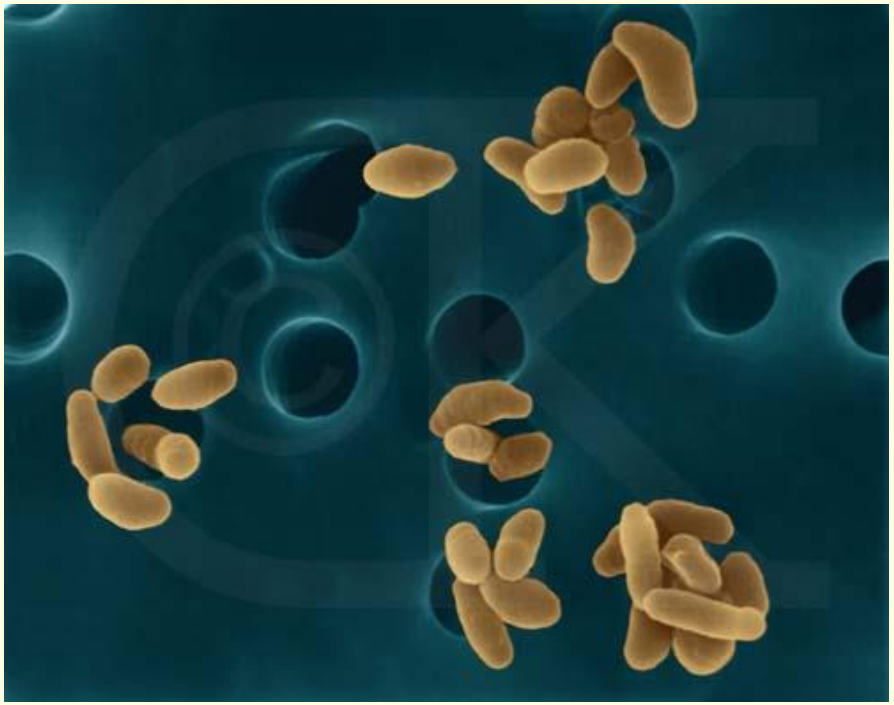
The preferred treatment is **streptomycin** administered as soon as possible. Alternatives include gentamicin, chloramphenicol, tetracycline, doxycycline, or trimethoprim/sulfamethoxazole.

Stable, **avirulent** mutants have been employed for vaccination against plague, (e.g. strain EV-76).
Heat killed or formalin **inactivated** suspension of a virulent bacteria and chemical fraction of bacilli may be used as vaccine.

Francisella tularensis

Species

Francisella tularensis



Morphology and staining: F.tularensis is small, capsulated, non-motile and Gram-negative pleomorphic coccobacillus.

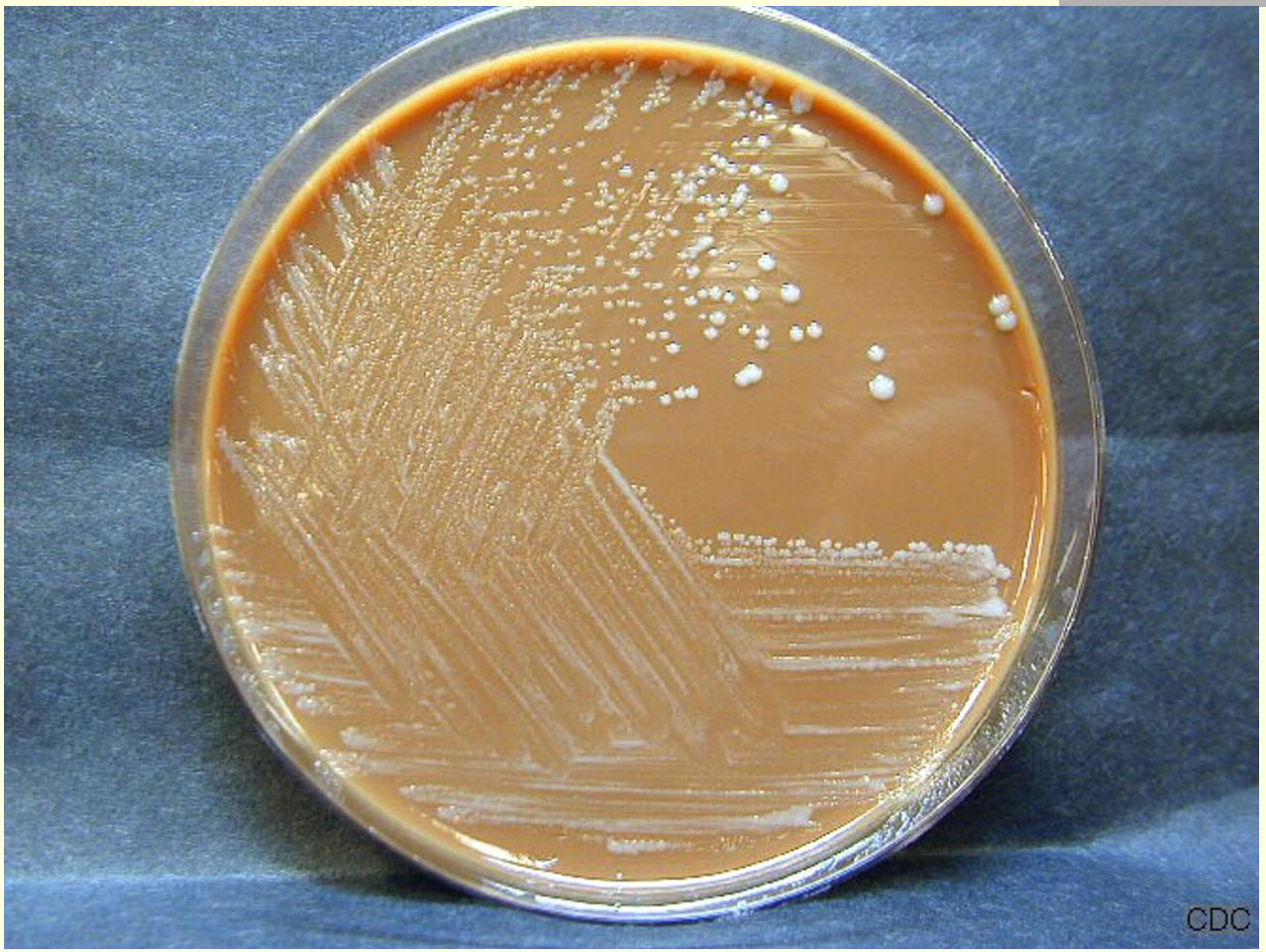
Cultural characteristic

Francisella tularensis is forms small translucent colonies on glucose blood agar or on Dorset egg slants. The organism grows readily in developing chicken embryos.

Tularemia. *F tularensis*, Colony Characteristics when grown on Cysteine Heart Agar, colonies 2-4 mm, smooth, entire, greenish-white, butyrous with opalescent sheen at 48-72hrs.

Tularemia. *Francisella tularensis* colony characteristics when grown on cystine-heart agar. Colonies are 2 to 4 mm, smooth, entire, greenish-white, and butyrous with an opalescent sheen at 48 to 72hrs.

Tularemia. *F tularensis*, Colony characteristics when grown on Chocolate Agar, Martin Lewis or Thayer-Martin medium include colony size of 1-3 mm, grey-white at 48-72hrs.



Classification, Antigenic Types and Resistance and Virulence Factors

Nutritionally and biochemically Francisella tularensis bears a close resemblance to the Brucellae, but it can be differentiated from members of this genus on the basis of DNA homology tests.

Francisella tularensis is suseptible to inactivation by mild heat (55°C for 10 minutes) and disinfectants.

Virulence Factors:

- Endotoxin
- Invasins: Fibrinolysin, etc.

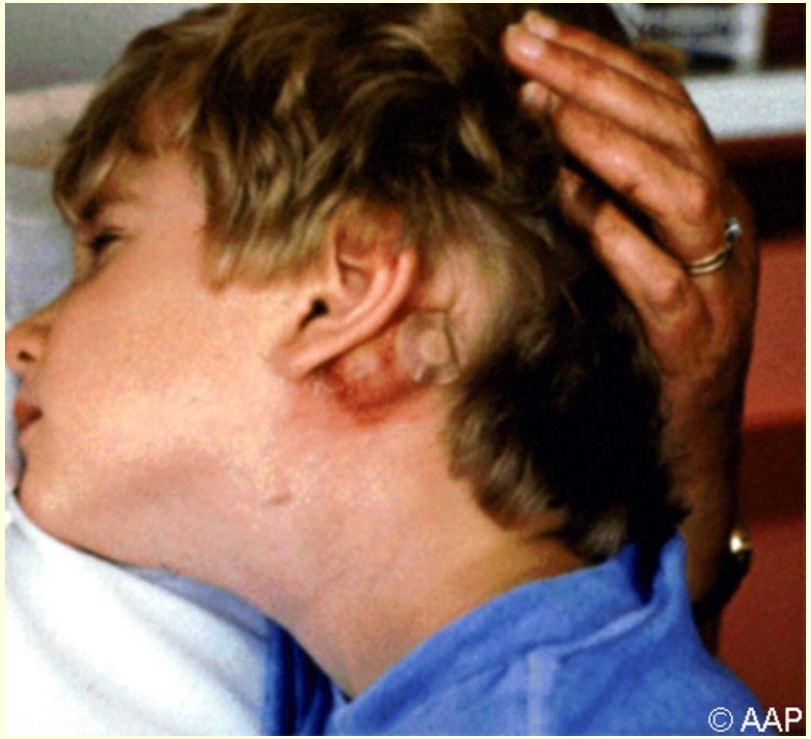
Epidemiology

Sources of the organism include approximately 100 species of wild mammals (eg, rabbits, hares, prairie dogs, and muskrats, rats, voles, and other rodents); at least 9 species of domestic animals (eg, sheep, cattle, and cats); blood-sucking arthropods that bite these animals (eg, ticks and deerflies); and water and soil contaminated by infected animals.

Ticks are the most important arthropod vectors, and most cases occur during late spring and summer months. Infection also may be acquired by direct contact with infected animals, ingestion of contaminated water or inadequately cooked meat, or inhalation of aerosolized organisms or contaminated particles related to lawn mowing, brush cutting, piling contaminated hay, or bioterrorism.

Person-to-person transmission does not occur. Organisms can be present in blood during the first 2 weeks of disease and in cutaneous lesions for as long as 1 month if untreated.

Pathogenesis



Tularemia is a relatively rare infection that can manifest with painful cervical adenitis.

A local abscess at the site of infection is followed by septicemia with rapid spread to the liver and spleen; 30 percent of untreated patients die.

Clinical Disease

The **incubation period** usually is 3 to 5 days, with a range of 1 to 21 days.

Most patients with tularemia experience an abrupt onset of fever, chills, myalgia, and headache. Illness usually conforms to one of the several tularemic syndromes. Most common is the ulceroglandular syndrome, characterized by a painful, maculopapular lesion at the entry site, with subsequent ulceration and slow healing associated with painful, acutely inflamed regional lymph nodes, which can drain spontaneously. The glandular syndrome (regional lymphadenopathy with no ulcer) also is common.

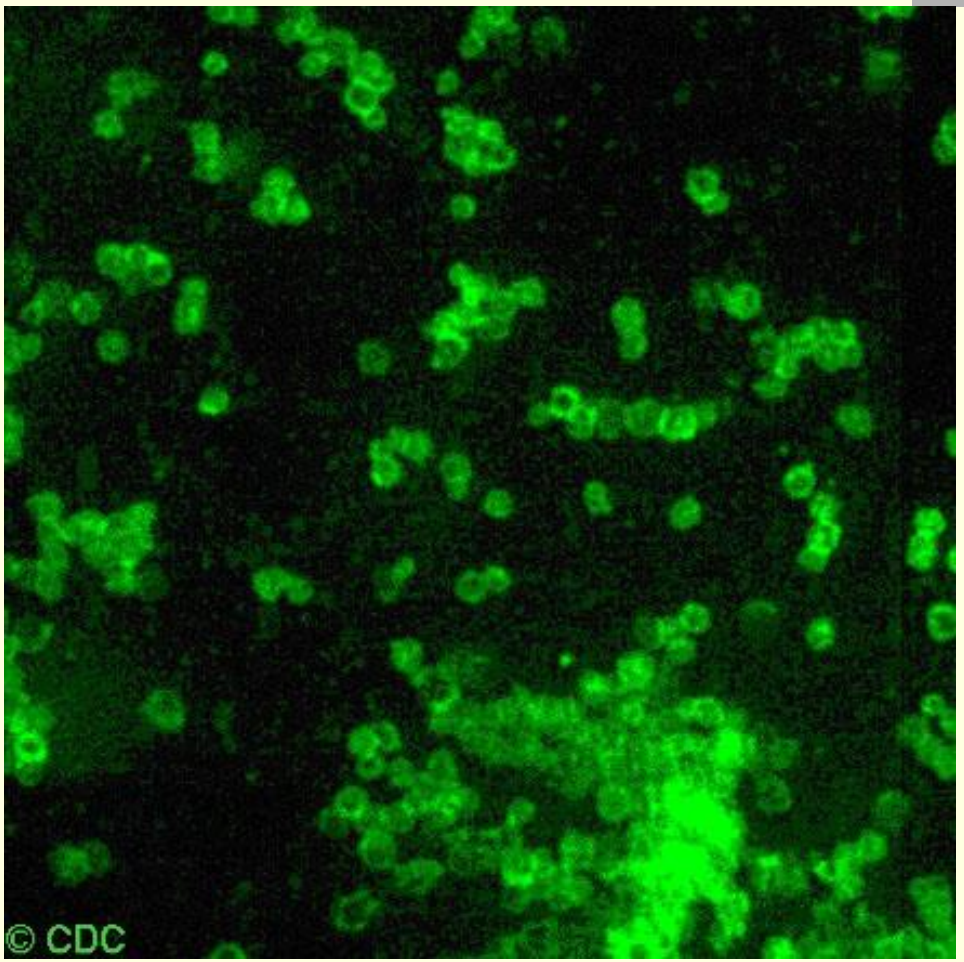
Less common disease syndromes are: oculoglandular (severe conjunctivitis and preauricular lymphadenopathy), oropharyngeal (severe exudative stomatitis, pharyngitis, or tonsillitis and cervical lymphadenopathy), typhoidal (high fever, hepatomegaly, and splenomegaly), intestinal (intestinal pain, vomiting, and diarrhea), and pneumonic. Pneumonic tularemia, characterized by fever, dry cough, chest pain, and hilar adenopathy, would be the typical syndrome after intentional aerosol release of organisms.

Diagnosis

Bacterioscopic method

F. tularensis may be identified through direct examination of secretions, exudates, or biopsy specimens using Gram stain, direct fluorescent antibody, or immunohistochemical stains. Microscopic demonstration of *F. tularensis* using fluorescent-labeled antibodies is a rapid diagnostic procedure.

Tularemia. *Francisella tularensis* (direct fluorescent antibody stain). Confirmation may be performed directly in tissue, sputum, or culture.



Biological method

Isolation of F. tularensis from infected animal can be difficult and slow. Best growth occurs on cysteine-glucose-blood agar, but plates should be incubated at 37°C for at least 3 weeks before being discarded as negative.

Serological method

Hemagglutinins appear in serum samples some 10 to 12 days after infection and slowly increase in titer for up to 8 weeks. A rising titer is always diagnostic of active disease. A single serum antibody titer of 1:128 determined by microagglutination (MA) or of 1:160 determined by tube agglutination (TA) is consistent with recent or past infection and constitutes a presumptive diagnosis.

Slide agglutination tests are less reliable than TA tests.

Polymerase chain reaction assays.

Treatment of tularemia

Streptomycin, gentamicin, or amikacin are recommended for treatment of tularemia. Duration of therapy usually is 7 to 10 days. A longer course is required for more severe illness. Alternative drugs for less severe disease include ciprofloxacin (which is approved only for specific indications in patients younger than 18 years of age), imipenem-cilastatin, doxycycline (which should not be given to children younger than 8 years of age unless the benefits of therapy are greater than the risks of dental staining, and chloramphenicol).

For specific prophylaxis is living, avirulent vaccine against tularemia. A living, avirulent vaccine against tularemia