

Francisella tularensis as a Bioterrorist Agent

Agent: *Francisella tularensis* is a small Gram-negative coccobacillus. There are two main serotypes: Jellison Types A and B. Type A is considered the more virulent form. *F. tularensis* may be aerosolized in dry or wet form.

Disease: Tularemia

Incubation Period: 2-10 days

Signs/Symptoms: Tularemia is generally described in six forms that are not necessarily distinct from one another. They may overlap, particularly in the event of bioterrorism. The forms of tularemia are:

<i>Typhoidal</i>	This form is essentially <i>F. tularensis</i> bacteremia with fever, chills, headache, myalgias, malaise, sore throat, and anorexia. Abdominal pain, nausea, vomiting, and diarrhea may be present.
<i>Pneumonic</i>	This form is a pneumonia with dry, non-productive cough, dyspnea, pleuritic chest pain, and fever. Physical examination may reveal rales, consolidation, and a friction rub or signs of effusion. The pneumonic form can be caused by inhalation of organisms or by hematogenous spread subsequent to (ulcero)glandular or typhoidal forms.
<i>Oculoglandular</i>	This form is characterized by painful, often purulent, conjunctivitis with lymphadenopathy especially in the periauricular, submandibular, and cervical areas. Conjunctivitis is bilateral in less than 12% of patients. In the case of an aerosol exposure from bioterrorism bilateral conjunctivitis may be more common. Photophobia and excessive tearing may be early complaints in this form.
<i>Oropharyngeal</i>	This form is accompanied by a very painful sore throat; there may also be abdominal pain, nausea and vomiting.
<i>Ulceroglandular</i>	The characteristic symptoms of this form are regional lymphadenopathy with a papule that develops into an ulcer at the site of entry. Fever, chills, headache, malaise, anorexia, and fatigue usually are the first symptoms.
<i>Glandular</i>	This form is similar to the ulceroglandular form, but without skin or mucous membrane lesions.

Diagnosis:

Differential Diagnosis: The illness, while often severe, is generally nonspecific.

Typhoidal syndromes such as salmonellosis or rickettsial infections should be included in the differential diagnosis. Other causes of pneumonia such as infection with *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Coxiella burnetii*, or *Chlamydia psittaci*, as well as exposure to staphylococcal enterotoxin B should be considered. Large numbers of patients presenting with similar systemic illnesses, in whom a portion will have a pneumonia with non-productive cough may be indicative of tularemia. In fulminant pneumonias, plague and inhalational anthrax should also be included in the differential diagnosis.

Diagnosis is generally based on clinical suspicion.

Laboratory: *F. tularensis* is difficult to culture on standard media (e.g., sheep blood agar) and usually requires cysteine supplementation for good growth. Chocolate agar and Thayer-Martin agar will usually support growth of *F. tularensis* and may be useful for isolating this organism when other organisms are present. At 24 hours at 35°C, *F. tularensis* grows as a gray-white and opaque colony but is usually too small to be seen as individual colonies. It grows slowly at 35-37°C but poorly at 25-28°C. After 48-72 hours, the colonies are 1-2 mm in diameter, blue-white to gray in color, flat in elevation with an entire, smooth, and shiny surface. If *F. tularensis* is suspected, all additional manipulations should be within a biological safety cabinet. Isolates or clinical material should be sent to the Oregon State Public Health Laboratory (OSPHL) immediately. Confirmation of isolates may take up to 2-3 days.

F. tularensis stains poorly on Gram stain but does appear to be a gram-negative pleomorphic coccobacillus, seen mostly as single cells. It is difficult to see *F. tularensis* in Gram-stained smears or tissue biopsies.

Definitive diagnosis is usually made retrospectively by serology. Fourfold increase in tularemia tube agglutination or microagglutination titer is diagnostic of infection. Titers are usually negative during the first week of infection, positive in 50-70% of cases in the second week, and reach a maximum in 4-8 weeks. Cross-agglutination can occur with *Brucella* and *Proteus* species.

Early postexposure (0-24 hours) nasal swabs, sputum and induced respiratory secretions may be collected for culture, and for fluorescent antibody (FA) assay. During the clinical phase (24 - 72 hours) blood for serum may be collected in a tiger-top (SST) or red top tube. Blood for convalescent sera may be collected in tiger-top (SST) or red top tubes for serology.

Agglutination titers may be performed on single or paired sera collected at least seven days apart.

Specimens suspected of containing *F. tularensis* or isolates thought to be *F. tularensis* are considered to be “infectious agents” by the Department of Transportation (29 CFR Parts 171-189, <http://hazmat.dot.gov/rules.htm>) and must be shipped accordingly. Contact the OSHPL for further information on the shipping of infectious organisms. Prior to shipping call the OSHPL at (503) 229-5882 and Acute and Communicable Disease Prevention at (503) 731-4024. The address is 1717 SW Tenth Avenue, Portland, OR 97201. Prior notification is requested if you suspect *F. tularensis*.

Supportive Tests: Routine examination of the sputum is not helpful; however a false-positive direct fluorescent antibody stain for *Legionella* on bronchoscopy specimens has been reported. Infected pleural fluid is exudative, negative on Gram stain, and usually contains more than 1000 leukocytes/mm³; cells are predominantly lymphocytes, but neutrophilic effusions may occur.

Secondary pleuropulmonary involvement is common in typhoidal cases, with pleural effusions or pulmonary infiltrates being present in up to 45% of patients. In patients with the pneumonic form of disease, 25-30% will have radiographic infiltrates without any clinical findings of pneumonia. White blood cell count and sedimentation rate may be normal or elevated. Thrombocytopenia, hyponatremia, elevated serum transaminase, increased creatine phosphokinase, myoglobinuria and sterile pyuria are sometimes present. Radiologic evidence of pneumonia or mediastinal lymphadenopathy is usually evident. Acute radiographic changes may include subsegmental or lobar infiltrates, hilar adenopathy, pleural effusion, and apical or miliary infiltrates. Less common changes include ovoid densities, cavitation, and bronchopleural fistula.

Treatment: Streptomycin 30 mg/kg qd IM for 10-14 days, or gentamicin 3-5 mg/kg qd IV for 10-14 days.

Prophylaxis: A live, attenuated vaccine available as an investigational new drug is administered once by scarification. Doxycycline 100 mg q12h po for 14 days, or tetracycline 500 mg qid po for 14 days.

Infection Control: No evidence exists of person-to-person transmission. Standard precautions should be practiced; strict isolation is not required. Organisms are rendered harmless by mild heat (55°C for 10 minutes) and/or standard disinfectants

such as 0.5% sodium hypochlorite solution (1 part household bleach added to 9 parts water).

Report: Immediately report any suspect cases to your local health department or the Oregon Health Division at (503) 731-4024 during working hours (8:00 am to 5:00 pm Monday through Friday) or (503) 731-4030 nights, weekends and holidays.