Francisella tularensis: Possible Agent in Bioterrorism

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Francisella tularensis, the causative agent of *tularemia*, is a highly infectious gram-negative coccobacillus. Due to its high infectivity it is of major concern to public health officials as a possible biological weapon. Although accidental exposure can occur through arthropod bites, handling infected animals, or breathing in aerosols, cases are usually isolated and contained. In the event of an intentional exposure such as in a bioterrorist attack, inhalation of aerosols can result in devastating consequences with much causality. Although a vaccine is available, sufficient quantities may not be readily accessible in an actual attack. Therefore, it is very important for both medical professionals and public health officials to be prepared to contain and control the situation should it actually occur.

ABBREVIATIONS: BSL = biosafety levels; CDC = Centers for Disease Control and Prevention; FDA = Food and Drug Administration; IM = intramuscular; IV = intravenous; NHI = Neisseria/Haemophilus identification; PCR = polymerase chain reaction.

INDEX TERMS: bioterrorism; *Francisella tularensis*; tularemia.

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Focus Continuing Education Credit: see pages 40 to 42 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES
See learning objectives #15 through #20 on page 40.

There is an increasing threat that chemical and biological weapons will be used on civilian populations in an act of domestic or international terrorism. One of the identified diseases caused by an act of bioterrorism is tularemia, a systemic disease caused by *Francisella tularensis.* Human infections develop through a variety of mechanisms such as tick bites, direct contact with infected tissues, ingestion of contaminated water, food, or soil, and inhalation of aerosolized organism.[1-3]

Francisella tularensis, is a small (0.2 µm in width by 0.2 to 0.7 µm in length), faintly staining, gram-negative pleomorphic coccobacillus. It is aerobic, non-motile, and requires cystine for growth.[4-7] Although it does not produce spores, *F. tularensis* is a very hardy organism.[3] *F. tularensis* has a thin lipopolysaccharide-containing envelope that helps it survive for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses.[1]

There are several subspecies of *F. tularensis: F. tularensis* subspecies tularensis (type A), which is a common isolate of North America; *F. tularensis* subspecies holarctica, formerly palaearctica (type B), common in Europe and Asia; and *F. tularensis* subspecies mediaasiatica, found in central Asian countries of the former Soviet Union. Type A is highly infectious and virulent in humans and animals. Type B, which is considered less virulent, has three biovars: biovar I is erythromycin sensitive and primarily found in North America, Europe, Siberia, the Far East, and Kazakhstan; biovar II is erythromycin resistant and found in Eurasia; and lastly, biovar japonica is common in Japan.[8]

EPIDEMIOLOGY AND DISEASE PREVALENCE

Tularemia, also known as 'rabbit fever', 'deer fly fever', and 'lemming fever', has been reported throughout North America and continental Europe, Russia, China, and Japan. In the U.S., most

cases are said to be in rural and semi rural areas, specifically in Arkansas, Missouri, Oklahoma, Kansas, South Dakota, and Montana.[9] The disease is contracted in all months of the year. However, a higher incidence is seen in the summer when ticks and deer flies are abundant and arthropod-borne transmission is most common, and also during rabbit hunting season in early winter, when hunters and trappers who handle infected animal carcasses are mostly affected.[1,6] Unfortunately, worldwide incidence is not known, and the disease is probably greatly under recognized and under reported. Males are infected more often than females but the highest rate reported is among children from five- to nine-years old and among elderly people from 75- to 84-years old. Although the number of cases reported annually in the U.S. has dropped from 2,000 cases in 1930 to 124 to 200 cases in 1990, tularemia outbreaks continue to occur. In 2000, an outbreak took place in Martha's Vineyard. Fifteen cases of pneumonia-like disease with one death were reported.[9]

The natural reservoirs for *F. tularensis* are small and medium-sized mammals. In North America, Europe, and Japan, rabbits and hares carry the organism. Other animals that may carry and transmit the disease include beavers, muskrats, water and field voles, water and wood rats, squirrels, and lemmings. Arthropods such as ticks, mosquitoes, and biting flies are the primary vectors that transmit *F. tularensis.* However, ticks of the species *Amblyomma americanum, Dermacentor andersoni, Dermacentor variabilis, Ixodes scapularis, Ixodes pacificus,* and *Ixodes dentatus* are the predominant vectors found in the U.S., the former Soviet Union, and Japan. Biting flies common in the U.S. include the species *Chrysops discalis* (deer fly), *Chrysops aestuans, Chrysops relictus,* and *Chrysozona pluvialis.*[1]

PATHOGENESIS OF INFECTION

Tularemia can be acquired through insect bites, handling infected animals, contaminated food/water, or inhaling infected aerosols. *F. tularensis* is able to penetrate unbroken skin and infect mucous membranes, the gastrointestinal tract, and lungs. In addition, this organism has the ability to survive phagocytosis and is able to multiply within macrophages. It spreads to the different organs, especially the lymph nodes, lungs and pleura, spleen, liver, and kidney hematogenously. Suppurative lesions subsequently appear which may progress to a granulomatous form.[10,11] There are, therefore, several disease presentations and clinical forms that develop following exposure to *F. tularensis.*

Infections with *F. tularensis* may appear from one to 14 days with the typical period of three to five days after exposure or contact.[5,6] Signs and symptoms of tularemia vary depending on the mode of acquisition. In general, persons infected with *F. tularensis* often show flu-like symptoms. If the bacterium is inhaled, symptoms such as sudden onset of fever, muscle aches, weakness, headache, joint pain, and pneumonia may occur. Infected persons who develop pneumonia can experience difficulty breathing and chest pains, which can progress to respiratory failure. If not treated immediately, 40% of infected persons who develop pneumonia die. Depending on the route of exposure, other symptoms such as appearance of skin ulcers, sore throat, swollen glands, and inflammation of the eyes may occur.[12]

CLINICAL FORMS

Following inhalation of infectious aerosols, the common form used in an intentional release of *F. tularensis,* hemorrhagic inflammation of the airways that will possibly develop to bronchopneumonia may occur. Pneumonic tularemia, the most serious form of the disease requires only 10 to 50 organisms to initiate infection. This disease presentation may also result from a hematogenous spread or as a complication of both the ulceroglandular and the typhoidal form.

The organism produces multiple necrotizing granulomata that will eventually destroy alveolar septa and produce bronchopneumonia, bronchitis, or tracheitis. Some patients may develop nonproductive cough while others may not have any signs at all. Additional clinical features of the pneumonic form include pharyngitis, bronchiolitis, pleuropneumonitis, and hilar lymphadenitis, associated with other manifestations of systemic spread. Lung abscesses develop rarely. However, the most severe complications of pulmonary infection are severe pneumonia, respiratory failure, and sometimes death.[1,2,8,10,11]

Glandular and ulceroglandular tularemia

The most common form of tularemia, glandular and ulceroglandular tularemia, is generally acquired by handling infectious material such as a carcass or from an arthropod bite. It requires 10 to 50 organisms to cause an infection and the organism usually enters through the skin. Formation of a local cutaneous papule ensues at the site of inoculation within three to five days after exposure; then, the papule ulcerates to form an eschar or a dark scab over the site. The organism spreads to the lymph nodes where granulomas may develop. In the glandular form, although the regional lymph nodes are involved, ulceration at the site of inoculation is usually absent.[2,10]

Oculoglandular tularemia

Oculoglandular tularemia is the rarest form and develops after conjunctival exposure to the organism. The organism

spreads to the lymph nodes and produces focal necrosis and lesions.[2,10]

Oropharyngeal tularemia

Ingestion of contaminated food, drinking contaminated water, and sometimes inhaling infectious aerosols lead to oropharyngeal tularemia. An acute exudative or membranous pharyngitis or tonsillitis develops, accompanied by cervical lymphadenopathy.[1,10]

Typhoidal tularemia

Typhoidal tularemia is characterized by a systemic disease where there is no specific site of inoculation or specific infected site. There is also an absence of anatomic localization. The organism gets to the bloodstream through the skin or the mucous membrane and is disseminated to the target organs: lungs, lymph nodes, liver, spleen, and bone marrow. The infection may progress to sepsis leading to other types of serious diseases such as shock, organ system failure, adult respiratory distress, and disseminated intravascular coagulation (DIC).[1,2]

LABORATORY DIAGNOSIS

Specimen collection

The laboratory should be alerted to the need for special diagnostic and safety procedures if tularemia is suspected. *F. tularensis* is difficult to culture but can still be recovered from blood, ulcers, sputum, conjunctival exudates, pharyngeal exudates, and gastric washings. Acceptable specimens to recover *F. tularensis* include blood, respiratory secretions, cerebrospinal fluid, urine, and biopsies of tissue or scrapings of an ulcer. If biopsy or scrapings are not possible, a swab or aspirate of an ulcer, or tissue lesion exudates is acceptable alternatives. Organisms may also be recovered from tissues from infected animals and fluids from infected arthropods.[6,7]

Laboratory Findings

Clinical specimens should be inoculated onto supplemented agar, such as blood-cystine agar or chocolate agar. Grayish white colonies ranging from one to two millimeters wide will be evident on chocolate agar after 48 hours of incubation.[7] Because *F. tularensis* can show little to no growth on sheep blood agar after 48 hours, it is best for the physician to note when tularemia is suspected, so that the culture can be grown on a cystine containing media. The organism grows best on buffered charcoal yeast extract agar (BCYE agar), the media used for isolation of *Legionella* species.

Once *F. tularensis* has demonstrated growth on laboratory media, biochemical screening tests may be performed in Level A laboratories. Characteristics of *F. tularensis* include: oxidase negative, weakly positive for catalase, positive for beta-lactamase, negative for urease, and negative for satellitism.[7]

If the above features are present, the culture should be referred to a reference laboratory, or BSL-3 (Biological Safety Level-3) capable laboratory, to confirm identification. The state public health laboratory director should be notified that *F. tularensis* is suspected. The infection control department and the attending physician where the patient presented should also be informed. If bioterrorism is suspected, the organism should be preserved.[1] Identification should not be attempted in automated identification systems because they introduce the possibility for aerosol production and an even greater possibility for misidentification. It has been reported that the Vitek NHI card will identify *F. tularensis* as *Actinobacillus actinomycetemcomitans* with 99% assurance.[1]

Additional detection, confirmation, and characterization tests include Forshay's test which is a skin test antigen that gives a positive reaction in 90% of patients within the first seven days of the disease. Reference laboratories can perform additional tests to identify and confirm suspected isolates. Agglutination or microagglutination tests to identify *F. tularensis* by detecting immunoglobulin M and G (IgM, IgG) are frequently used. Serological tests are best used in forensic and epidemiological cases for confirmation. Molecular-based tests such as polymerase chain reaction (PCR) are considered very sensitive and specific in detecting the organism and are also used as confirmation test assays.[1] Antimicrobial susceptibility studies show that all strains of *F. tularensis* are susceptible to streptomycin, tetracycline, chloramphenicol, erythromycin, aminoglycosides, and fluoroquinolones. All strains produce beta-lactamase and are resistant to beta-lactam antibiotics and azithromycin.[1,10]

A POTENTIAL BIOLOGICAL WEAPON

It was first discovered during World War II by a Japanese researcher that *F. tularensis* could be used as a biological weapon. In the 1950s and 1960s, the U.S. developed aerosolized *F. tularensis* organisms to be used as weapons until 1973 when they were destroyed. Subsequently, the former Soviet Union developed antibiotic- and vaccine-resistant strains of *F. tularensis*. At that time (in 1969), the World Health Organization calculated the approximate number of people who would be infected with the dispersal of virulent *F. tularensis* aerosol of 50 kg over a metropolitan area with a population of 5 billion. The exposure would have produced 250,000 illnesses and 19,000 deaths. As a biological weapon, *F. tularensis* will most likely be in an infectious aerosolized form.[12,13]

CATEGORY

Microorganisms are classified by the CDC, into three different Categories, 'A', 'B', or 'C', dependent on their ability to multiply in the host as well as virulence, lethality, stability, infectivity, pathogenicity, mode of transmission, aggressive potential, availability, ease of production, and incubation period. *F. tularensis* is a Category 'A' organism. Category 'A' organisms can be easily disseminated or transmitted person to person, cause high rates

Table 1. Working group consensus recommendations for treatment of patients with tularemia in contained and mass casualty settings and for post exposure prophylaxis[16]

Patient category	Recommended therapy*	
Contained casualty		
Adults	Preferred choices	Alternative choices
	Streptomycin, 1g IM twice daily	Doxycycline, 100 mg IV twice daily
	Gentamicin, 5 mg/kg IM or IV once daily†	Chloramphenicol, 15 mg/kg IV four times daily
		Ciprofloxacin, 400 mg IV twice daily†
Children	Preferred choices	Alternative choices
	Streptomycin, 15 mg/kg IM twice daily (should not exceed 2 gm/dL)	Doxycycline If weight > = 45 kg, 100 mg IV If weight < 45 kg, give 2.2 mg/kg IV twice daily
	Gentamicin, 2.5 mg/kg IM or IV three times daily†	Chloramphenicol, 15 mg/kg IV four times daily†
		Ciprofloxacin, 15 mg/kg IV twice daily‡
Pregnant women	Preferred choices	Alternative choices
	Gentamicin, 5 mg/kg IM or IV once daily†	Doxycycline, 100 mg orally twice daily
	Streptomycin, 1 g IM twice daily	Ciprofloxacin, 400 mg IV twice daily†
Mass casualty setting and post exposure prophylaxis		
Adults	Preferred choices	
	Doxycycline, 100 mg orally twice daily	
	Ciprofloxacin, 500 mg orally twice daily†	
Children	Preferred choices	
	Doxycycline If > = 45 kg give 100 mg orally twice daily If < 45 kg then give 2.2 mg/kg orally twice daily	
	Ciprofloxacin, 15 mg/kg orally twice daily‡	
Pregnant women	Preferred choices	
	Ciprofloxacin, 500 mg orally twice daily†	
	Doxycycline, 100 mg orally twice daily	

* One antibiotic, appropriate for treatment for patient age, should be chosen from among the alternatives. Treatment with streptomycin, gentamicin, or ciprofloxacin should be continued for ten days; treatment with doxycycline or chloramphenicol should be continued for 14 to 21 days. Persons beginning treatment with intramuscular (IM) or intravenous (IV) doxycycline, ciprofloxacin, or chloramphenicol can switch to oral antibiotic administration when clinically indicated.

† Not a U.S. Food and Drug Administration-approved use.

‡ Ciprofloxacin dosage should not exceed 1 g/dL in children.

Source: http://www.bt.cdc.gov/Agent/Tularemia/tularemia-biological-weapon-abstract.asp.

of mortality, could cause public panic, and require special attention and public health awareness.[14] Fortunately, *F. tularensis* has not been identified as an organism that can be spread from person to person but is considered extremely hazardous.[2]

BIOLOGICAL SAFETY LEVELS

When handling clinical materials suspected to contain *F. tularensis*, BSL-2 practices and containment are required, while BSL-3 practices, containment, and facilities are used for all manipulations of cultures and for experimental animal studies. The protective clothing required when working with *F. tularensis* is a laboratory coat, impervious gloves, gown (with tight wrists and tie in the back), and facemasks for work in biosafety cabinets.[6,15]

WHERE TO SEND ISOLATES FOR IDENTIFICATION

When submitting the organism to a reference laboratory for positive identification, a gram stain smear should be prepared and an isolate should be sent. The original specimen should be saved, if possible, pending investigation into the occurrence. If necessary, the state laboratory may also request the original specimen.[7] According to CDC reports, a diagnosis of active *F. tularensis* infection can be made from identification in clinical specimens or through a four-fold change in titer of serum antibodies against *F. tularensis.* A presumptive diagnosis can be also be made when *F. tularensis* antigens are detected with fluorescent assays or by a single elevated antibody level.[4]

VACCINES AND TREATMENT

The preferred and most effective drug of choice to treat tularemia is streptomycin or gentamicin. Alternative choices are doxycycline, chloramphenicol, or ciprofloxacin. Tetracycline and chloramphenicol have the ability to control acute phases of tularemia; however, relapses occur. Post-exposure antibiotic prophylaxis is complicated because the preferred drug, streptomycin, must be administered parenterally.[8,11,16] Table 1 shows various treatment methods available.[16]

To prevent tularemia infections, a live attenuated strain of *F. tularensis* vaccine has been developed in the U.S. and is under review by the U.S. Food and Drug Administration.[10] Other prevention techniques involve taking safety precautions when dealing with suspected materials or animals and avoiding drinking untreated water.[1,11]

CONCLUSION

The events that took place in the U.S. on September 11, 2001 have had a profound effect on the entire country and the world. The terrorist attack on the World Trade Center and the subsequent anthrax bioterrorism attack brought to the forefront the vulnerability of people in the world to acts of terror. The threat of biological agents for use in biological warfare is of tremendous concern to public health officials and the public at large. Anthrax and smallpox are two agents globally known to the general public, however other agents including *F. tularensis* are not common public knowledge. The public should become aware of other potential agents in times of war and terrorism so as to know how to react in the event of an intentional exposure.

REFERENCES

1. Dennis, David T, Inglesby, and others. Tularemia as a biological weapon. JAMA 2001;285(21):2793-4.
2. Edlow J. (December 11, 2001). Tick-borne Disease, Tularemia. E-medicine. http://www.emedicine.com/EMERG/topic591.htm. Accessed April 5, 2003.
3. Disease Fact Sheet (July 2, 2001). Department of Health and Family Services. http://www.dhfs.state.wi.us/healthtips/BCD/Tularemia.htm. Accessed April 2, 2003.
4. Morbidity and Mortality Weekly Report: Tularemia – United States, 1990 – 2000. 2002;51(09);182-4. U.S. Centers for Disease Control. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5109al.htm. Accessed April 2, 2003.
5. Frequently Asked Questions (FAQ) About Tularemia. (October 3, 2002). U.S. Centers for Disease Control. http://www.bt.cdc.gov/agent/tularemia/faq.asp. Accessed April 2, 2003.
6. Material Safety Data Sheet: *Francisella tularensis.* (May 25, 2001). Office of Laboratory Security, Health Canada. http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/msds68e.html. Accessed April 2, 2003
7. Level A laboratory procedures for identification of *Francisella tularensis.* December 13, 2001. U.S. Centers for Disease Control-Laboratory Response Network. http://www.bt.gov/agent/tularemia/index.asp. Accessed April 2, 2003.
8. Johansson A, and others. Ciprofloxacin for treatment of tularemia in children. Pediatr Infect Dis J 2000;19(5):449-53.
9. Hornick R. Tularemia revisited. [Editorial]. N Engl J Med 2001;345(22):1637-9.
10. Ellis J, Oyston PCF, Green M, and others. Tularemia. Clin Microbiol Rev 2002;15(4):631-46.
11. Cooper D. (August 14, 2002). Tularemia. Medline plus. http://www.nlm.nih.gov/medlineplus/ency/article/000856.htm. Accessed April 4, 2003.
12. Tularemia (February 2003). CIDRAP and Infectious Disease Society of America. http://www.cidrap.umn.edu/content/bt/tularemia/biofacts/tularemiafactsheet.html. Accessed April 2, 2003.
13. What is Tularemia (April, 2003). South Dakota Department of Health. Accessed April 16, 2003.
14. Bioterrorism. 2002. Altruis Biomedical Network. http://www.e-bioterrorism.com. Accessed April 16, 2003
15. The 1, 2, 3s of Biosafety Levels. February 6, 1998. U.S. Centers for Disease Control. http://cdc.gov/od/ohs/symp5/jyrtext.htm. Accessed April 16, 2003.
16. Abstract: tularemia as a biological weapon: medical and public health. U.S. Centers for Disease Control. http://www.bt.cdc.gov/Agent/Tularemia/tularemia-biological-weapon-abstract.asp. Accessed April 16, 2003.