

Tularemia – A Review with Concern for Bioterrorism

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Introduction

Tularemia has been classified by the CDC as a Category A biological weapon (Aquino & Wu, 2011, McGovern, Christopher, & Eitzen, 1999). As such, it has the potential to create a significant amount of morbidity and mortality under the right set of circumstances. However, despite occupying the same niche as botulism, anthrax, viral hemorrhagic fever, smallpox, and plague, tularemia lacks sufficient gravitas to be on the healthcare radar. Therefore, it is not as well-known as its Category A companions and is not typically taught in most bioterrorist classes. Nevertheless, it has attained the dubious distinction as a Category A agent due to the following properties and characteristics:

1. It's highly infectious to both humans and animals;
2. It is easily obtainable from an extended list of natural animal reservoirs;
3. It can be easily cultured in large quantities;
4. It has a tenacious ability to survive in the environment for extended periods of time;
5. It is without a satisfactory vaccine. (Maurin, 2017).

Therefore, the purpose of this paper is two-fold: 1) Summarize the relevant characteristics of the bacterium, *Francisella tularensis* and list the human effects of tularemia within the natural environment; and 2) Present tularemia's potential as an agent of

bioterrorism and discuss various strategies to prepare for and respond to an intentional tularemia assault.

History of *Francisella tularensis*

Evidence of tularemia among human populations first surfaced in the late 19th Century in the United States, Norway, Russia and Japan. In 1911, George McCoy and Charles Chapin of the United States Public Health Service isolated the tularemia bacteria in California ground squirrels. In 1928, Dr. Edward Francis of the United States Public Health Service linked the causal bacteria agent of deer-fly fever to *Bacterium tularensis*. McCoy and Chapin (Nigrovic & Wingerter, 2008) renamed it *Francisella tularensis* in Dr. Francis' honor in 1947 (Ellis, Oyston, Green, & Titball, 2002, GlobalSecurity.org, 2011).

In the 1930's and 1940's, large waterborne outbreaks occurred in Europe and the Soviet Union (Karpoff & Antonoff, 1936). The largest recorded airborne tularemia outbreak occurred in 1966-1967 in an extensive farming area of Sweden (Dahlstrand, Ringertz, & Zetterberg, 1971) involving more than 600 patients most of whom acquired infection secondary to aerosolization of the bacteria during farm work. (Dennis et al., 2001).

Microbiology

F. tularensis is a gram-negative, aerobic, non-motile enveloped coccobacillus. (Image 2) Of four different sub-species,

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Jellison type A is the most common in the USA and the most virulent in humans.

It is an extremely hardy organism surviving for weeks in soil, water and animal carcasses and for months to years in a frozen environment (Darling & Catlett, 2002, Evans & Friedlander, 1997).

It's difficult to see under light microscopy and does not grow in commercial blood culture media. Cysteine glucose blood agar is the growth medium of choice (Gimenez-Garcia, 2016). Visible growth is observed in 2-5 days at 35°C (Penn 2012). When suspecting *F. tularensis*, culturing should be conducted under biosafety level 3 conditions because of a risk of aerosolization (Centers for Disease Control & Prevention, 2011, Nigrovic & Wingerter, 2008).

Epidemiology

Historically, tularemia has been almost entirely a rural disease, primarily in the northern hemisphere from 30° to 70° north latitude. Very few outbreaks have been discovered in urban and suburban areas. In fact, multiple cases in urban areas is highly suggestive of intentional dissemination. In the United States, it has been reported in every state except Hawaii (Centers for Disease Control & Prevention, 2002), but the majority of cases occur in Arkansas, Oklahoma, Missouri and South Dakota (Pedati, C., et al., 2015).

Reservoirs of *F. tularensis* include mice, rabbits, hares, squirrels and water rats. It has also been recovered from water, soils and vegetation. (Centers for Disease Control & Prevention, 2015; Centers for Disease Control & Prevention, 2016) People at highest risk are hunters, meat handlers, farmers and lab workers (Southern Illinois University School of Medicine, 2012).

Pathogenesis

Although it is unlikely to spread from person to person, *F. tularensis* remains a very

virulent pathogen. It can infect humans through skin, mucous membranes, GI tract and lungs. Inoculation or inhalation of as few as 10 organisms can cause disease (Dennis et al 2001, Holland et al 2016). However, infection can occur through bites from infected ticks, deerfly or other insects, handling infected animal carcasses, eating or drinking contaminated food or water, or breathing in aerosolized bacteria. (Image 1)

Within target organs of lymph nodes, lungs, pleura, spleen, liver and kidney, the bacterium multiples within macrophages degrading their innate protective role. There is a lack of an acute inflammatory response by the host during the early phases of the disease (Holland, 2016). *F. tularensis* produces local suppurative necrosis but spreads rapidly to regional lymph nodes. Bacteremia occurs during the early phase of the infection; untreated, patients progress to disseminated disease and death.

Clinical Features

The incubation period is typically 3 to 6 days (Range: Hours up to 3 weeks) (Evans et al., 1985, Thomas & Schaffner, 2010).

Early symptoms of tularemia may be nonspecific: sudden fever (38°C-40°C), coryza, chills, rigors, headaches, diarrhea, muscle aches (especially the lower back), arthralgias, dry cough, and/or progressive weakness (Dennis et al., 2001). A pulse-temperature dissociation (Faget sign: fever and bradycardia) has been noted in as many 42% of patients (Evans et al., 1985). The more classical manifestations of the disease depend on how a person is exposed to the *F. tularensis* bacteria (Evans et al., 1985, Thomas & Schaffner, 2010; Center for Infectious Disease Research and Policy, Saslaw et al., 1961; Centers for Disease Control and Prevention 2015). They are divided into seven types:

A. Ulceroglandular

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- a. Routes of infection:
 - i. The handling of contaminated animal carcasses
 - ii. Bite from an infected arthropod
 - b. Manifestations (Images 3-6)
 - i. Development of an ulcer at the inoculum site
1. A local cutaneous papule appears at the inoculation site at the time of onset of generalized symptoms, becomes pustular, and ulcerates within a few days of its first appearance. The chancre-like ulcer (0.4 to 3.0 cm in diameter) is tender, generally has an indolent character, and may be covered by an eschar. (Dennis et al., 2001; Karwa, Currie, & Kvetan, 2005). Some ulcers have heaped-up borders at the site of bacterial inoculation in 60% of patients (Evans & Friedlander, 1997).
 - ii. Regional lymphadenopathy (painful)
 2. Typically, one or more regional afferent lymph nodes become enlarged and tender within several days of the appearance of the papule. Despite appropriate antibiotics, the affected nodes may become fluctuant and rupture (Dennis et al., 2001) or the ulcer and lymphadenopathy may persist for months (Dennis et al., 2001, Karwa et al., 2005).
 3. Glandular (Image 7)
 - a. Routes of infection:
 - i. The handling of contaminated animal carcasses
 - ii. Bite from an infected arthropod
 - b. Manifestations
 - i. Regional lymphadenopathy only (painful)
 1. About 85% of patients develop tender lymphadenopathy that sometimes presents as fluctuant buboes (Evans & Friedlander, 1997).
 4. Oculoglandular
 - a. Route of infection
 - i. Direct contamination of the eye with *F. tularensis*
 - b. Manifestations
 - i. Ocular pain
 - ii. Conjunctival injection and swelling
 - iii. Discharge
 - iv. Ulcers may develop on the inner aspect of the eyelid
5. Oropharyngeal
 - a. Routes of infection
 - i. Eating or drinking food and water contaminated by *F. tularensis*
 - ii. Inhalation of the aerosolized bacteria
 - b. Manifestations
 - i. Pharyngitis
 - ii. Tonsillitis
 - iii. Regional lymphadenopathy (cervical/periauricular)
 - iv. Nausea, vomiting, and diarrhea possible
 6. Pneumonic
 - a. Routes of infection
 - i. Inhalation of aerosolized *F. tularensis* (primary)
 1. Pharyngeal ulcers may accompany aerosol-induced disease (Evans & Friedlander, 1997).
 - ii. Spread from a primary site to the lungs (secondary)
 - b. Manifestations
 - i. Fever (sudden onset)
 - ii. Pharyngitis, headache, chills, myalgias, arthralgias
 - iii. Non-productive cough (typical)
 - iv. Debilitation
 - c. Pneumonic pathogenesis: Using animal models, it is hypothesized that Tularemia's pneumonic manifestations are due to the

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bacteria's ability to invade and replicate within macrophages, dendritic cells and epithelial cells. Exponential replication within the cells leads to the induction of autophagy and cell death. (Holland, et al., 2016)

7. Typhoidal

- a. Route of infection
 - i. Non-specific
- b. Manifestations
 - i. Systemic
 - ii. Severe: Fever, chills, headaches
 - iii. Sometimes: Prominent gastrointestinal manifestations, such as diarrhea and pain (Avery & Barnett, 1967).
 - iv. No obvious site of infection

1. No localized signs or symptoms; A morbilliform eruption has been reported in a minority of patients with systemic disease (Evans, et al., 1985).

8. Septic

- a. Route of infection
 - i. Non-specific
- b. Manifestations
 - i. Severe: Fever, chills, headaches
 - ii. Obtundation, coma
 - iii. Significant morbidity and mortality

2. Septic shock, ARDS, multisystem organ failure

Any form of tularemia may be complicated by hematogenous spread, resulting in secondary pleuropneumonia, sepsis, and rarely, meningitis (Stuart & Pullen, 1945a).

In untreated tularemia, symptoms (sweats, fever and chills, progressive weakness, malaise, anorexia, and weight loss) often persist for weeks and, sometimes, for months, usually with progressive debility.

Diagnosis

The diagnosis of tularemia requires a strong index of suspicion. This can be somewhat difficult since the incidence is low and the initial manifestations are non-specific. Once suspected, notification of public health is required. Tularemia is a reportable disease (Aquino & Wu, 2011). Cultures of all relevant specimens should be obtained. Growth of *F tularensis* in enriched culture medium may take up to 48 hours under ideal conditions (Image 9). However, it may require up to ten days for bacterial growth to occur. The lab, once tularemia is being considered, must institute special diagnostic and safety procedures. Other diagnostic modalities include Gram-stains of clinical specimens (Image 2), DFA, immunofluorescent staining, serology, specifically microagglutination studies and Polymerase Chain Reaction (PCR) (Adalji, 2015, Centers for Disease Control and Prevention. (2016). Mortality

Prior to the advent of antibiotics, the overall mortality from the more severe type A strains ranged from 5% to 15%. For untreated pneumonic and severe systemic forms, mortality rates as high as 30% to 60% were seen (Holland et al. 2016). Mortality drops to 1% to 2.5% with timely diagnosis and appropriate treatment (Evans, et al., 1985).

Treatment

The key to successful treatment of tularemia is early diagnosis and initiation of appropriate antibiotic therapy. Treatment for uncomplicated, naturally-acquired tularemia usually consists of a ten-day course of an

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aminoglycoside (e.g. streptomycin or gentamicin). Alternatives to the aminoglycosides include doxycycline and ciprofloxacin. Since doxycycline is bacteriostatic it should be dosed for 14 days in uncomplicated cases and, while the fluoroquinolones are effective against tularemia, they are not FDA-approved. (Adalji, 2015; Centers for Disease Control and Prevention, 2016).

However, because *F. tularensis* infections are rare, no randomized studies compare the various treatment regimens or duration of therapy. Nevertheless, it must be understood that streptomycin remains the drug of choice “based on experience, efficacy and FDA approval.” Gentamicin has a lower success rate. (Centers for Disease Control and Prevention, 2016).

Prevention/Vaccination

In endemic regions, wearing protective clothing, using chemical insect repellants and removing ticks promptly mitigate tularemia. Infected animals and lab specimens should be handled by trained individuals.

F. tularensis is easily killed by disinfectants (1% hypochlorite, 70% ethanol, and formaldehyde). It is inactivated by moist heat (121°C; 15-minute exposure) and dry heat (170°C; one-hour exposure). However, it remains viable at freezing temperatures for months to years (State of New Jersey Department of Agriculture, 2003).

Vaccine research has been an ongoing endeavor. (Nigrovic & Wingerter, 2008).

Vaccines using live, attenuated virus have been found to lessen symptoms minimally and do not confer long-term immunity. The goal is to develop a vaccine that creates long-term immunity and is powerful enough to protect against an inhalation exposure greater than 50 CFU. (Roberts, 2017).

Standard precautions are sufficient for infected patients since human to human transmission has not been reported.

The History of *F. tularensis* as a Biological Weapon

Recognizing that, historically, tularemia’s virulence had the potential for causing significant morbidity and mortality, it was a natural progression for state aggressors to consider tularemia as a prospective candidate for biological weaponization (Dennis et al., 2001, Evans & Friedlander, 1997). The fact that the microorganism was easy to obtain and to cultivate made it economically attractive for research and development (Adalja et al, 2015, Maurin, 2015).

Tularemia in the History of Biological Warfare (Dennis, et al., 2001, Jacoby, 2015)

- 1932-1945: *F. tularensis* was included in Japan’s germ warfare research program in Manchuria.
- 1940-1945: A former Soviet Union bioweapons expert implicated the Soviet hierarchy as being responsible for tularemia outbreaks affecting tens of thousands of Soviet and German soldiers along the Eastern European Front during World War II.
- 1950s-1960s: The U.S. military developed aerosolized weaponry that facilitated the airborne dissemination of *F. tularensis*. Additionally, its scientists, via voluntary human experimentation, uncovered the microorganism’s pathophysiology and developed novel vaccines and chemoprophylactic guidelines.
- 1960s (late): *F. tularensis* was stockpiled by the United States as a biological weapon.
- 1960s-1990s: According to a former Soviet Union bioweapons expert, the Soviets advanced tularemia research by weaponizing the bacteria and developing

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strains that were resistant to current antibiotics and vaccines.

- 1969: An expert committee commissioned by the World Health Organization declared that an aerosol attack with 50 kg. of *F tularensis* over an urban population of 5 million inhabitants would cause 250 000 casualties and 19 000 deaths.
- 1970: The U.S. bioweapons program was terminated.
- 1973: The U.S. biological weapons arsenal was dismantled and all biological stocks destroyed save for those needed for defensive medical research.
- 1997: A CDC published its findings estimating that such an attack published by WHO in 1969 would cost \$5.4 billion per 100 000 persons exposed.

Tularemia as a Bioterrorism Agent

An occult biological terrorist assault would be difficult to uncover in its initial stages (McDade, J.E. et al, 1998). Such was the case in The Dalles, Oregon at which an intentional Salmonella food-borne attack sickened over 700 inhabitants and hospitalized approximately seventy of them (Torok, T.J. et al., 1997). A similar lack of awareness about the possibility of a terrorist attack occurred during the initial stages of the anthrax attack in the eastern U.S. in 2001. (Bush et al, 2001).

The ignorance associated with the lack of an early diagnosis of a bioterrorist attack is related to:

- 1) Incubation Period. Depending on the degree of exposure and the underlying physiological and medical susceptibilities of the victim, manifestations will develop at varying time intervals from person-to-person.
- 2) Layered fabric of the American healthcare infrastructure. As the victims begin

developing symptoms, they have a multiplex of options to receive care (private clinicians' offices, clinics, emergency departments, etc.). Therefore, no one venue or individual will have a bird's-eye view of what occurred in the community within the past week.

- 3) Inconsistent bioterrorism education for all healthcare providers (physicians, nurses, physician assistants, nurse practitioners, etc.) (Bork, C. E, et al, 2012).

While some of the Category A agents have stigmata that may facilitate an early diagnosis, this review will limit itself to those agents that begin as an influenza-like illness (ILI) and for which an early diagnosis will be difficult, especially during influenza season. While local public health systems have invested in sophisticated syndromic surveillance systems, the average primary care practitioner should still acquire an "awareness level" education of bioterrorism. Clues of a possible bioterror attack include:

- 1) An increase in the number of patients presenting with acute respiratory or ILI complaints;
- 2) An increase in the severity of respiratory manifestations in previously healthy populations.
- 3) An increase in the death rate of patients with ILI.

However, given the notoriety of anthrax and plague within the traditional bioterrorist lexicon, tularemia may be overlooked as another inhalational Category A agent. Inhalational anthrax may be differentiated by:

1. Rapid clinical deterioration in a patient with an ILI syndrome;
2. Absence of pneumonic infiltrates;
3. Copious pleural effusions;
4. Mediastinal widening on imaging; (Jernigan, J.A. et al., 2001).

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Pneumonic plague, in addition to a rapid clinical deterioration, is specifically characterized by

1. Pneumonic infiltrates
2. Hemoptysis
3. Ischemic manifestations of fingers, toes, nose, and ears.

In contrast, inhalation tularemia, will have a progression of symptoms that is considerably slower than that of plague or anthrax and whose severity of symptoms will not be as marked initially. The classical inhalational tularemia patient, likely afflicted with the Type A or Type B strain, can be expected to manifest a prodrome consisting of a fever up to 40°C, headache, chills, coryza, and diffuse myalgias. In due course, the respiratory component will dominate starting with a pharyngitis and a complex of presentations such as bronchiolitis, pleuropneumonitis, hilar lymphadenitis, and classic pneumonia. While the pulmonary aspect may be the principal area of involvement, the possibility that the disease can progress beyond the pulmonary architecture is not unfathomable, especially in the very young, the geriatric population, and the immunosuppressed. Therefore, typhoidal tularemia and tularemia sepsis, as described earlier, may develop.

With regard to pulmonary imaging, a wide variety of radiographic signs may be seen: peribronchial infiltrates, pneumonic infiltrates in one or more lobes, pleural effusions, hilar lymph adenopathy and lung abscesses. (CDC, 1998)

Once tularemia is added to the differential diagnosis there exists multiple ways to confirm the diagnosis:

- Gram stain of respiratory secretions: Small, Gram-negative coccobacilli;
- Cultures of the sputum, tracheobronchial secretions, and blood;
- Direct fluorescent antibody (DFA) staining;
- Polymerase chain reaction (PCR);
- Microagglutination assay;
- Virulence testing;
- Molecular genetic characterization;

F. tularensis, as a facultative intracellular microorganism, is naturally resistant to many classes of antibiotics. While the aminoglycosides, the tetracyclines, and the fluoroquinolones are endowed with the appropriate intracellular pharmacokinetics and pharmacodynamic properties to treat tularemia, antibiotic-resistant variations of the bacterium have been created in the laboratory and should be anticipated (Maurin, 2015). Even without bacterial micromanipulation, the preferred antibiotics are not 100% effective, regardless if administered early in the course of the disease process. Reports indicate that up to 15% of tularemia patients will experience therapeutic failures and disease relapses. (Maurin, 2015).

Beyond The Patient and Into The Community

Once a community has come under assault, victims will begin presenting with manifest illness within one week of exposure. This is the acute phase of a tularemia attack. However, the features of tularemia and its etiologic agent are such that the bacterium can remain in the community environment for a prolonged period of time, based on its environmental hardiness and its number of zoonotic reservoirs. Over time, significant segments of the local animal and arthropod population can be infected and may transmit *F. tularensis* to a heretofore naïve human population. Therefore, secondary outbreaks among humans can occur over the following weeks and months secondary to arthropod

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bites and normal animal-human contact. It is, then, within the realm of possibility that in these second-, third-, and fourth-generation patients, the other clinical forms of tularemia (viz. ulceroglandular, conjunctival, oropharyngeal, etc.) may develop as the bacterium gains a foothold in the local community. What began as an intentional infectious disease epidemic, may metamorphose into an endemic disease process that will tax the socio-economic fabric of a community and wreak medical and psychological havoc upon its citizens. How “climate change” will impact Tularemia and other infectious diseases remains controversial. (Altizer et al., 2013).

Mitigation and response efforts are complicated. A vaccine has yet to be licensed for humans in this country, although novel vaccines are under development. In fact, one or more vaccines in development may become available to the general public under the FDA’s “Emergency Use of an Investigational Drug or Biologic” guidelines. (FDA, 2016). Currently, antibiotic prophylaxis has been proposed with administration of the fluoroquinolones (Maurin, 2015). However, the extent to which vaccines and antibiotics should be instituted prophylactically will be dictated by factors that may be based upon fear and confusion as well as sound medical science:

- The estimated size of the exposed population;
- CDC guidelines among those of other reputable institutions;
- State and Federal response assets;
- Antibiotic resources available locally and via the Strategic National Stockpile;

- Development and activation of PODs [Points of Distribution]) for the dissemination of resources to meet the medical and physical needs of the public (FEMA/USACE, 2010);
- Development and activation of Alternative Care Facilities to unburden the healthcare infrastructure (Lam, C. et al., 2006);
- Legitimate requirements of the 24/7 news cycle;
- Hyperbolic bombast of the pseudo-experts (e.g. “fake news”);
- Social media

What weight each of these factors carries has not been fully vetted in the 21st century. The success or failure of a community’s response to an intentional tularemia attack will depend upon how well it recognizes and meets each of these challenges before, during, and after the attack. A benchmark for ensuring success is having an educated, well-informed healthcare infrastructure.

Conclusion

Tularemia is a rare, zoonotic, rural disease that can be considered to be of little consequence in most industrialized nations. However, because of its special attributes, it has the potential of creating significant morbidity and mortality in an unwary public and its healthcare infrastructure. That infrastructure should receive the necessary education, not only to recognize and treat the disease in a patient, but also, to become a vital component of the public health response to meet the medical, physical, and psychological demands of the community.

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Image 1: A male “brown dog tick”: A vector of *F tularensis*. Photo credit: CDC/Gathany, 2005.

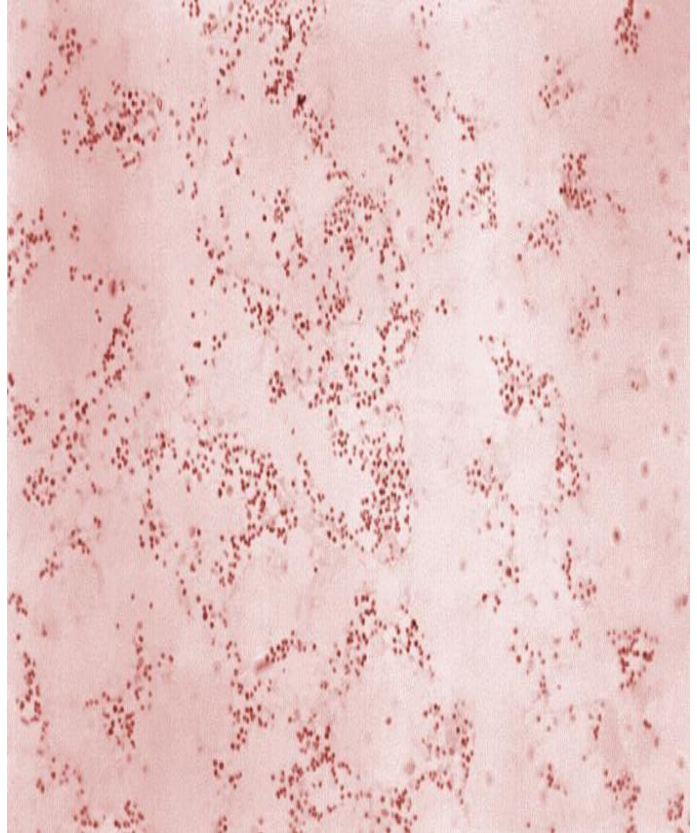


Image 2: This Gram-stained photomicrograph reveals numerous Gram-negative *Francisella tularensis* bacteria. Credit: CDC/ Dr. W.A. Clark, 1977;

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Image 3. A muskrat trapper contracted tularemia manifesting as a cutaneous lesion on his left lateral forehead. CDC/ Dr. Roger A. Feldman, 1972. Public domain.



Image 4. A Tularemia lesion on the dorsal skin of right hand. Photo credit: CDC

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Image 5. A Tularemia lesion on the dorsal skin of the right hand,

Photo credit: CDC



Image 6. Thumb with skin ulcer of tularemia..

Photo credit: CDC

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Image 7. Cervical lymphadenitis in a patient with pharyngeal tularemia. Patient has marked swelling and fluctuant suppuration of several anterior cervical nodes. Infection was acquired by ingestion of contaminated food or water. Source: World Health Organization.



Image 9. This photograph depicts the colonial morphology displayed by Gram-negative *Francisella tularensis* bacteria, which was grown on a medium of chocolate agar, for a 24 hour time period, at a temperature of 37°C. Photo credit: CDC/Seidel.