Chapter 8

TULAREMIA

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INTRODUCTION

Francisella tularensis poses a substantial threat as a biological weapon, and it is viewed by most experts as a dangerous pathogen if weaponized. Both the United States and the former Soviet Union developed weaponized *F tularensis* during the Cold War.[1,2] It is unclear whether tularemia has ever been used deliberately as a biological weapon. The Japanese experimented with *F tularensis* as a biological weapon, but there is no documentation of its use in military operations.[3] There is also speculation that the former Soviet Union used *F tularensis* as a weapon against German troops in the Battle of Stalingrad during World War II.[2] Despite the tularemia outbreak among soldiers of both armies during this battle, some authors suggest that natural causes, as opposed to an intentional release, were responsible for the epidemic.[4] There was also speculation that *F tularensis* was used as a biological weapon by Serbia in the Kosovo conflict, although the subsequent investigation suggested the observed cases were not caused by an intentional release.[5,6]

F tularensis has been included in the list of Centers for Disease Control and Prevention Category A threat organisms because of the infectivity with exposure to low numbers of organisms, the ease of administration, and the serious consequences of infection.[1] Tularemia's effectiveness as a biological weapon includes a nonspecific disease presentation, high morbidity, significant mortality if untreated, and the limited ability to obtain a rapid diagnosis. Although tularemia responds to antibiotics, the use of an antibiotic-resistant strain can make these countermeasures ineffective.

INFECTIOUS AGENT

Tularemia was named after Tulare County, California, where an epidemic disease outbreak resembling plague occurred in ground squirrels in 1911. McCoy and Chapin successfully cultured the causative agent and named it *Bacterium tularense*.[7] Wherry and Lamb subsequently identified the pathogen as the cause of conjunctival ulcers in a 22-year-old man.[8] Edward Francis made significant scientific contributions to the understanding of the disease in the early 20th century, including naming it "tularemia."[9]

F tularensis is an aerobic, gram-negative coccobacilli. *F tularensis* is not motile, and appears as small (approximately 0.2–0.5 μm by 0.7–1.0 μm),[10] faintly staining gram-negative bacteria on Gram's stain (Figure 8-1). *F tularensis* was formerly included in the *Pasteurella* and the *Brucella* genera. Eventually a new genus was created, and the name *Francisella* was proposed in tribute to Edward Francis.[11] A closely related species, *Francisella philomiragia*, has also been described as a human pathogen.[12,13] *F tularensis* is considered to have four subspecies: (1) *tularensis*, (2) *holarctica*, (3) *mediasiatica*, and (4) *novicida*.[14] *F tularensis* subspecies *tularensis*, also known as Type A (or biovar A), occurs predominantly in North America and is the most virulent subspecies in both animals and humans. This subspecies was recently divided into A.I. and A.II. subpopulations. Subpopulation A.I. causes disease in the central United States, and subpopulation A.II. is found mostly in the western United States.[15] *F tularensis* subspecies *holarctica* (formerly described as *palearctica*), also known as Type B (or biovar B), is found in Europe and Asia, but also occurs in North America. *F tularensis* subspecies *holarctica* causes a less virulent form of disease than subspecies *tularensis*, but has been documented to cause bacteremia in immunocompetent individuals.[16,17] Before antibiotics, *F tularensis* subspecies *tularensis* resulted in 5% to 57% mortality, yet *F tularensis* subspecies *holarctica* was rarely fatal.[18] Unlike these other subspecies, *F novicida* rarely causes human disease.[12] *F tularensis* subspecies *mediasiatica* has been isolated in the central Asian republics of the former Soviet Union, and it appears to be substantially less virulent in a rabbit model compared to *F tularensis* subspecies *tularensis*.[19,20] The four subspecies can be distinguished with biochemical tests and genetic analysis.

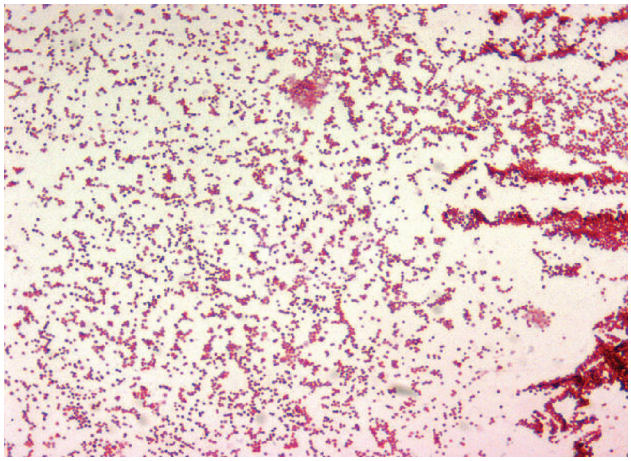

Fig. 8-1. Gram's stain of *Francisella tularensis*.
Photograph: Courtesy of Dr Larry Stauffer, Oregon State Public Health Laboratories, Centers for Disease Control and Prevention, Atlanta, Georgia, Public Health Image Library, #1904.

THE CLINICAL DISEASE

Tularemia is an infection with protean clinical manifestations. Healthcare providers need to understand the range of possible presentations of tularemia to use diagnostic testing and antibiotic therapy appropriately for these infections. Most cases of naturally occurring tularemia are ulceroglandular disease, involving an ulcer at the inoculation site and regional lymphadenopathy. Variations of ulceroglandular disease associated with different inoculation sites include ocular (oculoglandular) and oropharyngeal disease. Occasionally patients with tularemia present with a nonspecific febrile systemic illness (typhoidal tularemia) without evidence of a primary inoculation site. Pulmonary disease from *F tularensis* can occur naturally (pneumonic tularemia), but is uncommon and should raise suspicion of a biological attack, particularly if significant numbers of cases are diagnosed. Because of the threat of this microorganism as a biological weapon, clusters of cases in a population or geographic area not accustomed to tularemia outbreaks should trigger consideration for further investigation.[21] Rotz et al provide criteria for determining the likelihood that a tularemia outbreak is caused by intentional use of tularemia as a biological weapon.[21] A tularemia outbreak in US military personnel deployed to a nonendemic environment would be one example of an incident that should be investigated. The investigation should yield the likely cause of the outbreak, which could be varied (exposure to infected animals, arthropod-borne, etc). By determining the cause of tularemia, it may be possible to implement control measures, such as water treatment or use of an alternative water supply if the outbreak is traced to a waterborne source.

Epidemiology

F tularensis subspecies *tularensis* (Type A) is the most common *F tularensis* subspecies causing clinical tularemia in North America.[10] Type A was once thought not to occur in Europe, but a type A strain has recently been isolated from flea and mite parasites of small rodents trapped in Slovakia.[22] *F tularensis* subspecies *holarctica* (type B), found throughout the Northern Hemisphere, is less pathogenic.[1] In the United States an average of 124 tularemia cases per year were reported from 1990 through 2000.[23] Over half of all cases reported came from Arkansas, Missouri, South Dakota, and Oklahoma, where the foci of infection are well-established. Tularemia can be transmitted by direct contact with infected animals or their tissues, ingestion of undercooked infected meat or contaminated water, animal bites or scratches, arthropod bites, and inhalation of an aerosol or contaminated dust.

Various epidemiological categories of tularemia have been suggested, often dependent on the infective vector, mode of infection, or occupation of the infected individuals.[18]

Direct Contact

In 1914 a meat cutter with oculoglandular disease, manifested by conjunctival ulcers and preauricular lymphadenopathy, had the first microbiologically proven human tularemia case reported.[8] An early review of tularemia established that a majority of human cases (368 of 488, or 75%) in North America resulted from dressing and eating wild rabbits.[9] Other wild mammals may potentially serve as sources for tularemia transmission from direct contact, such as wild prairie dogs that are captured and sold as pets.[24]

Food and Water Ingestion

Tularemia can also be contracted by eating meat from infected animals[9] or food contaminated by infected animals.[25] Water can also become contaminated from animals infected with tularemia and cause human infection. During March through April 1982, 49 cases of oropharyngeal tularemia were identified in Sansepolcro, Italy.[26] The case distribution in this city suggested that a water system was the source. The infected individuals had consumed unchlorinated water, and a dead rabbit from which *F tularensis* was isolated was found nearby.[26] Waterborne transmission of ulceroglandular tularemia also occurred during a Spanish outbreak among 19 persons who had contact with river-caught crayfish.[27] Contaminated water may have contributed to recent outbreaks of oropharyngeal tularemia in Turkey[28] and Bulgaria.[25] It is unclear how *F tularensis* survives in water, but it may be linked to its ability to survive in certain protozoa species such as *Acanthamoeba castellanii*.[29]

Mammalian Bites and Arthropod Vectors

Mammalian bites are another source of tularemia transmission to humans. Instances of human transmission from the bites or scratch of a cat, coyote, ground squirrel, and a hog were documented over 80 years ago.[9] In April 2004 a 3-year-old boy from Denver, Colorado, contracted tularemia from a hamster bite, providing evidence of disease transmission from these pets.[30]

Transmission of tularemia by the bites of ticks and flies is also well-documented.[10] *Dermacentor* species

ticks (dog ticks) are important vectors in areas where enzootic transmission occurs in North America[31] and Europe.[32] *Ixodes* species ticks may also contribute to *F tularensis* transmission.[33] In Utah during the summer of 1971, 28 of 39 tularemia cases were contracted from deerfly (*Chrysops discalis*) bites.[34] An epidemic of 121 tularemia cases (115 ulceroglandular) in Siberia from July through August 1941 may have resulted from transmission of *F tularensis* by mosquitoes, midges (*Chironomidae*), and small flies (*Similia*).[35]

Aerosol Transmission

The largest recorded pneumonic tularemia outbreak occurred in Sweden during the winter of 1966 through 1967, when 676 cases were reported.[36] Most of the cases occurred among the farming population, 71% among adults older than 45 years and 63% among men. The hundreds of pneumonic cases likely resulted from contact with hay and dust contaminated by voles infected with tularemia. *F tularensis* was later isolated from the dead rodents found in barns, as well as from vole feces and hay.

In the summer of 2000, an outbreak of primary pneumonic tularemia occurred in Martha's Vineyard, Massachusetts.[37] Fifteen confirmed tularemia cases were identified, 11 of which were the pneumonic form of tularemia. One 43-year-old man died of primary pneumonic tularemia. Epidemiological analysis revealed that using a lawn mower or brush cutter was significantly associated with illness in the 2 weeks before presentation of this case.[38] Feldman et al proposed that in Martha's Vineyard, *F tularensis* was shed in animal excreta, persisted in the environment, and was transmitted to humans after mechanical aerosolization by mower or brush cutter and subsequent inhalation.[38] The strong epidemiological link with grass cutting adds plausibility to this explanation.[39] A seroprevalence survey conducted in 2001 in Martha's Vineyard demonstrated that landscapers were more likely to have antibodies to *F tularensis* than nonlandscapers, suggesting an increased occupational risk for tularemia.[38]

The only other previously reported outbreak of pneumonic tularemia in the United States occurred at Martha's Vineyard during the summer of 1978.[40] In a single week, seven persons who stayed together in a vacation cottage eventually developed typhoidal tularemia. A search for additional cases on the island uncovered six other tularemia cases (five typhoidal and one ulceroglandular). No confirmed source for the disease exposure was discovered. Tularemia had been reported sporadically since the introduction of rabbits to Martha's Vineyard in the 1930s,[40] and pneumonic tularemia was initially reported in Massachusetts in 1947.[41]

Tularemia in an Unusual Setting

Some tularemia cases have occurred in geographic areas where the disease has never been reported. An orienteering contest on an isolated Swedish island in 2000 resulted in two cases of ulceroglandular tularemia.[42] These cases were theorized to have occurred from contact with migratory birds carrying the microorganism. The social disruption caused by war also has been linked to tularemia outbreaks. During World War II, an outbreak of over 100,000 tularemia cases occurred in the former Soviet Union,[4] and outbreaks with hundreds of cases after the war occurred in Austria and France.[43] Outbreaks of zoonoses during war since that time have led to speculation that these epidemics were purposefully caused. For example, no tularemia cases had been reported from Kosovo between 1974 and 1999, and tularemia was not previously recognized endemically or enzootically in the Balkan countries.[5] However, after a decade of warfare, an outbreak of over 900 suspected tularemia cases occurred in Kosovo during 1999 and 2000, leading researchers to investigate claims of use of this agent as a biological weapon by the Serbs against the Albanian inhabitants of the country.[5,6] The Kosovo outbreak and subsequent investigation are described in detail in chapter 3, Epidemiology of Biowarfare and Bioterrorism.

Laboratory-acquired Tularemia

Soon after the discovery of *F tularensis* as a pathogen, cases of laboratory-acquired infection were recognized. Edward Francis observed that many laboratory personnel working with the pathogen, including himself, became infected.[9] Six tularemia cases occurred during US Public Health Service laboratory investigations of tularemia outbreaks from 1919 through 1921.[44] Tularemia is the third most commonly acquired laboratory infection,[45] and recent laboratory-acquired infections of tularemia emphasize the laboratory hazard that this organism presents.[46] Because of the extreme infectivity of this microorganism, investigators of a 2000 outbreak in Kosovo chose not to culture the organisms from patients, but instead relied on empirical clinical evidence of tularemia cases.

Pathogenesis

For infection to occur, bacterial pathogens must traverse the normal skin and mucosal barriers that typically prevent microorganisms from entering the body. Breaks in the skin from lacerations or abrasions provide opportunity for *F tularensis* transmission and infection. Arthropod vectors can bypass the skin defenses with

a bite, thus inoculating the organism directly into the host. However, the portal of entry can also be mucous membranes in the respiratory tract, ocular membranes, or the gastrointestinal tract.

One of the remarkable attributes of *F tularensis* is the low infectious dose. As few as 10 organisms can produce an infection when injected subcutaneously into human volunteers, and only 10 to 50 organisms are required when administered to human volunteers by aerosol.[47,48] Recent investigations have attempted to elucidate the unique characteristics that allow *F tularensis* to cause infection at such a low number of organisms. As an intracellular pathogen, *F tularensis* has developed the means to survive in the typically hostile environment inside macrophages by interfering with multiple aspects of macrophage function. On initial entry into the macrophage, *F tularensis* uses a bacterial acid phosphatase, AcpA, to inhibit the bactericidal respiratory burst response of the macrophage.[49,50] Additionally, both *F tularensis* Type A and B can inhibit acidification of the phagosome after entry into the macrophage, escape from the phagosome, and reside in the macrophage cytoplasm.[51,52] Another survival mechanism of *F tularensis* is the interference with the normal macrophage response by inhibiting Toll-like receptor signaling and cytokine secretion, as demonstrated in experiments with murine macrophages and the live vaccine strain (referred to as LVS, which is subspecies *holarctica* or a Type B strain) of *F tularensis*.[53] An absence of Toll-like receptor signaling inhibits the typical robust innate immune response that could eliminate the bacteria. Replication of the organism in the macrophage begins slowly, but eventually large numbers of organisms can be found in a single macrophage.[52,54,55] Although *F tularensis* may initially delay apoptosis (programmed cell death) of the macrophage, the organism eventually induces apoptosis through mechanisms similar to intrinsic cellular signals.[56] Researchers have identified only some of the factors required by *F tularensis* for survival in macrophages, including *IglC*, a 23-kDa protein that most likely affects Toll-like receptor-4 signal transduction,[53,57] and the *MglAB* operon that regulates transcription of virulence factors.[58] The MinD protein functions as a pump for substances containing free radicals such as hydrogen peroxide, allowing the organism to resist oxidative killing.[59]

The early innate immune response to *F tularensis* involves intracellular killing of the pathogen by the macrophages and proinflammatory cytokine secretion. Murine experiments have demonstrated the importance of an effective early cytokine response. Interferon-γ-deficient mice die from sublethal doses of LVS[60] and tumor necrosis factor-α is at least as important as interferon-γ for control of *F tularensis* infection.[61,62] The host defense within macrophages appears to be crucial at controlling infection by *F tularensis*. In human monocytes/macrophages, LVS strain and *F novicida* induced the processing and release of interleukin (IL)-1β, an essential component of the inflammatory immune response.[63] However, killed bacteria did not induce this response, but did induce the early phases required for IL-1β, such as mRNA transcription. The results suggest that only live *Francisella* can escape from the phagosome, and thus trigger the function of caspase-1, which converts the precursor of IL-1β to its active form. In mice deficient in caspase-1 as well as ASC, an adaptor protein involved in host cell death, substantially higher bacterial loads were observed, as well as early mortality, compared to normal mice.[64] Neutrophils perform an important function in limiting the spread of *F tularensis* after inoculation. Experiments have demonstrated that neutrophils can kill *F tularensis*,[65] and mice depleted of neutrophils appear susceptible to infection with *F tularensis* LVS.[66]

The late adaptive immune response to *F tularensis* requires an intact cell-mediated immune system, particularly in resolving the initial infection and in producing long-term immunity.[67] There is no clear immunodominant epitope on any one *F tularensis* virulence protein that stimulates the required cell-mediated response; however, studies have demonstrated that multiple protein/peptides are required.[68] Vaccination with *F tularensis* LVS appears to produce a long-term memory T-cell response (as measured by lymphocyte stimulation),[69] but it is unclear what degree of long-term protection is conferred by this response. Both $CD4^+$ and $CD8^+$ lymphocytes are required for an effective cell-mediated response to *F tularensis*.[60] The protective memory response is dependent on a robust proinflammatory cellular response, because administration of anti-interferon-γ and anti-tumor necrosis factor-α antibodies to previously vaccinated mice dramatically lowers the lethal infective intradermal dose of *F tularensis*.[62] This response initially appears 2 to 4 weeks after initial infection,[70-72] and it can remain detectable for many years.[69,73]

The importance of humoral immunity in the defense against tularemia is not completely understood, but it appears that the humoral response by itself provides little or no value in protecting the host.[74] When laboratory workers received a formalin-killed whole-cell vaccine developed by Foshay et al,[75] a strong humoral response was elicited but was not protective against cutaneous[48] or respiratory[47] challenge. The failure of this vaccine suggested that the formalin inactivation procedures destroyed some of the essential protective antigens or that these protective antigens were

not expressed in vitro. A persistent humoral response does develop during human infection and after vaccination. Waag et al reported that sera from five of nine vaccinees resulted in Western blot banding profiles that were identical to *F tularensis* lipopolysaccharide.[70] Investigations focused on identifying protective antigens are ongoing, particularly in animal models.[14] Unfortunately, the antigens that induce humoral immunity appear to be different than antigens inducing cell-mediated immunity, making determinations of the most immunogenic antigen challenging.[74] The ultimate goal of these investigations is to optimize the cell-mediated immune response to *F tularensis*, thereby suggesting improvements to prophylactic and therapeutic strategies.

In addition to understanding the interaction of *F tularensis* with the immune system, substantial research has focused on the poorly understood virulence factors of *F tularensis*.[10] The lipopolysaccharide capsule of many gram-negative pathogens elicits a profound proinflammatory immune response, which can lead to the clinical manifestations of septic shock.[76] However, although *F tularensis* lipopolysaccharide can elicit a strong humoral response, it does not induce significant tumor necrosis factor-α and nitric oxide production in macrophages or IL-1 from polymorphonuclear cells,[77] in contrast to lipopolysaccharide from other gram-negative pathogens. *F tularensis* does have virulence factors allowing for survival within macrophages and possibly other cells, and *iglC*, *mglAB*, and *minD* genes were previously mentioned. Advances in genetic manipulation will enhance understanding of the role of specific genes in the pathogenesis of this organism.[14] One promising technique is allelic replacements, with successful studies on *F tularensis* recently conducted.[78]

Clinical Manifestations

Tularemia has a diversity of clinical presentations, and it is likely that many cases are unrecognized, especially because of the diagnostic challenges associated with this infection.[79] The disease manifestations of tularemia have been classified into two groups (ulceroglandular and typhoidal),[80] or more specific categories (ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, pneumonic, and septic). The more specific classification is preferred[1,11] because direct pulmonary inoculation is probably a presentation clinically distinct from the nonspecific syndrome of typhoidal tularemia. Symptoms overlap among these seven categories.[81] Patients with glandular tularemia forms (ulceroglandular and oculoglandular) usually present with ulcerative skin lesions. However, a distinct clinical presentation of lymphadenopathy greater than 1 cm and no skin lesions is well-described and known as glandular tularemia. Patients with typhoidal tularemia lack mucosal or cutaneous lesions and are less likely to present with lymphadenopathy, but have various systemic symptoms including fever, weight loss, and possible signs of an atypical pneumonia.[10]

Clinical symptoms in cases of ulceroglandular tularemia typically appear after an incubation period of 3 to 6 days.[80] These manifestations of disease include fever (85% of cases), chills (52% of cases), headache (45% of cases), cough (38% of cases), and myalgias (31% of cases). The fever may be associated with pulse-temperature disassociation (42% of cases in one series)[80] (the pulse increases fewer than 10 beats per minute per 1°F increase in temperature above normal), although this finding is not specific for tularemia. Other nonspecific complaints include chest pain, vomiting, arthralgia, sore throat, abdominal pain, diarrhea, dysuria, back pain, and nuchal rigidity.[80,81]

A persistent ulcer is the hallmark of ulceroglandular tularemia. Ulcers generally range in size from 0.4 cm to 3.0 cm and occasionally have raised borders. The location of the lesion may provide an indirect clue as to the route of exposure: inoculation from an arthropod vector, such as a tick, is more likely on the lower extremities, and exposure to a mammal with tularemia tends to cause lesions on the upper extremities.[80] Lesions are typically associated with regional lymphadenopathy, and a lack of lymphadenopathy may suggest another etiologic agent.[80] Enlarged lymph nodes can occur singly, in groups, or enlarged in a sequential fashion along the lymphatic tracts (sporotrichoid pattern). The lymph node is typically painful and may precede, occur simultaneously, or follow the appearance of the cutaneous ulcer in ulceroglandular disease.[81]

Oculoglandular tularemia is similar to the ulceroglandular form, with ocular erythema and exudative conjunctivitis as key distinguishing features. The mechanism of exposure is usually from contact with infected mammals. One case report describes infection after tick removal; the tick contents were inadvertently inoculated into the eye.[82] Food and water contamination can also lead to oculoglandular infection.[25]

In one series pharyngitis was observed in 24% of patients with tularemia.[80] Possible findings on examination include erythema, exudates, petechiae, hemorrhage, or ulceration. Other findings may include retropharyngeal abscess or suppuration of the regional lymph nodes. The nonspecific mild symptoms of pharyngitis associated with the other forms of tularemia should be distinguished from the severe, usually exudative, pharyngitis of the oropharyngeal form of tularemia.[81] Severe exudative pharyngitis suggests

ingestion of contaminated food or water as the likely source of infection. The appearance of pharyngitis may be linked to lower respiratory tract disease, or possibly to ingestion as the route of exposure. Oropharyngeal signs and symptoms and cervical adenitis have been the primary manifestation of recent outbreaks in Turkey (83% of cases)[28] and Bulgaria (89% of cases),[25] and these outbreaks appear to be associated with a contaminated water source.

The overall incidence of symptoms of lower respiratory tract disease in patients with tularemia is high, ranging from 47% to 94%.[80,83] These percentages are influenced by the route of exposure and the diagnostic approach to a patient with tularemia. The routine use of chest radiographs increases the likelihood of detecting mild or asymptomatic respiratory infections. Additionally, case series may only involve patients who are hospitalized, or receive a thorough evaluation, and may not include milder case presentations. Pneumonic tularemia can result from cases of ulceroglandular or glandular tularemia, with an onset ranging from a few days to months after the appearance of initial nonpulmonary symptoms.[83] Approximately 30% of patients with ulceroglandular disease and 80% of patients with typhoidal tularemia also have pulmonary signs and/or symptoms consistent with pneumonia.[80] Pneumonic tularemia can also occur from direct inhalation of the organism, which has been demonstrated in human experimental models.[47,84] In experimental infections of humans, cases were characterized by abrupt onset of fever, headache, sore throat, malaise, myalgias, coryza, and cough, which was typically nonproductive.[84] Chest radiographic findings in pneumonic tularemia are highly variable and nonspecific[85] because they can mimic findings in bacterial pneumonias, tuberculosis, lymphoma, or lung carcinoma.[83] Patients can have infiltrates consistent with pneumonia and hilar adenopathy. In patients with pneumonia, 15% have an associated pleural effusion. Other less common findings include interstitial infiltrates, cavitary lesions, and bronchopleural fistulas.

A recent pneumonic tularemia outbreak in Martha's Vineyard, Massachusetts, provides an instructive example of tularemia's diagnostic challenges. The index case was a Connecticut resident with a second home at Martha's Vineyard. His family physician in Connecticut empirically treated this case of "summer pneumonia." Hospital clinicians in Martha's Vineyard noticed the outbreak over a month later while searching for the cause of another pneumonic summer illness.[37,86] After seeing news accounts of the Martha's Vineyard tularemia outbreak, the Connecticut man reported to Connecticut health authorities with a history of symptoms, exposure risk, and laboratory tests compatible with tularemia.

Other examples of pneumonic tularemia have presented as diagnostic challenges. In 1994 a California case of community-acquired pneumonia was recognized as typhoidal tularemia in a 78-year-old with an absence of any epidemiological association for the illness.[87] A decade earlier, of the 96 patients with tularemia presenting to a Veteran's Hospital in Arkansas, five had pneumonic tularemia.[88]

The clinical manifestations of typhoidal and septic forms of tularemia overlap. Septic tularemia can be considered the result of clinical progression of any of the other forms of tularemia to a state of septic shock. Typhoidal tularemia presents as a nonspecific febrile syndrome, with or without lymphadenopathy, that can lead to death if untreated.[81] This presentation mimics an extensive number of other disease entities, making the diagnosis challenging. A wide range of additional clinical manifestations has been described with all forms of tularemia, including pericarditis, enteritis, appendicitis, peritonitis, erythema nodosum, and meningitis.[79,80,89]

The laboratory findings with tularemia are nonspecific. Hemoglobin and platelet counts are typically normal, and the white blood cell count is usually only mildly elevated, with no alteration in the normal cell differential.[80] Microscopic pyuria may be observed.[80] One case series describes tularemia associated with skeletal muscle abscesses, elevated creatine kinase, and rhabdomyolysis.[90] Nonspecific elevations of liver transaminases and alkaline phosphatase may be observed with tularemia. The cerebrospinal fluid is usually normal, but may have mildly abnormal glucose, protein, and cell counts.[80]

Untreated tularemia patients usually have a prolonged illness lasting for months. The disease can be fatal, although rarely in ulceroglandular tularemia with antibiotic intervention. Before the use of streptomycin for therapy, tularemia—particularly the typhoidal form—had a mortality rate of 33%.[81] No specific infection control practices are recommended for tularemia, other than universal precautions, because no documented cases of human-to-human transmission exist.[1] However, special precautions are needed for the clinical microbiology laboratory because of the high incidence of laboratory-acquired infection[91] (see "Issues for Laboratory Workers").

Diagnosis

The diagnosis of tularemia is difficult because the clinical presentations for the various forms are not specific and diagnostic modalities have limitations. In a scenario in which *F tularensis* is used as a biological weapon, a rapid increase in pneumonic cases may be

the initial clue implicating a biological weapon attack. In this scenario, either astute clinical judgment[92] or epidemiological syndromic surveillance[93] would be useful in detecting the attack.

Bacterial Culture Techniques

The diagnosis of tularemia by culture can be challenging because the organism grows poorly on routine culture medium. Although positive cultures have been obtained from the blood,[94-96] cultures from ulcer sites, sputum, gastric washings, and pharyngeal and conjunctival exudates are usually negative.[10] *F tularensis* is difficult to grow using standard media, but medium supplemented with cysteine or other sources of sulfhydryl groups can enhance recovery.[10,97] Cysteine glucose blood agar has been the traditional medium of choice.[98] Charcoal yeast extract agar and Thayer-Martin agar are two additional preparations that may support the growth of *F tularensis*. The colonies appear gray-white on chocolate or Thayer-Martin agar (Figure 8-2). The organism is optimally grown in a CO_2 incubator and tends to grow more slowly than bacteria routinely encountered in clinical practice. The fastidious growth characteristics of *F tularensis* can often make the diagnosis of tularemia difficult, particularly when only routine culture techniques are used. However, some strains of *F tularensis* do not have these fastidious growth requirements.[99] The organism may be identified with biochemical testing, but automated identification systems in microbiology laboratories may misidentify the pathogen.[100] The samples should be referred to a specialized laboratory. Blood cultures are rarely positive, even in cases of severe disease.[16] Occasionally, positive blood cultures have been observed in immunocompromised persons (infected with the less virulent subspecies *holarctica*), and have been discovered when blind subculture of blood cultures has been conducted.[96]

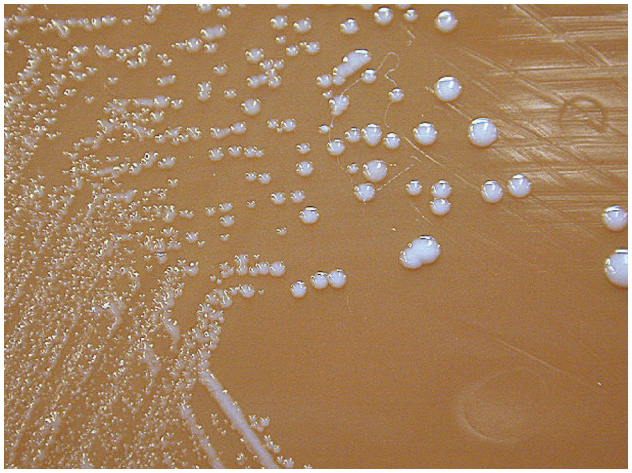

Fig. 8-2. Chocolate agar plate of *Francisella tularensis*. Photograph: Courtesy of Dr Larry Stauffer, Oregon State Public Health Laboratories, Centers for Disease Control and Prevention, Atlanta, Georgia, Public Health Image Library, #1912.

Serology

Traditionally, tularemia diagnosis has been based on serology, with a 4-fold rise in antibody titer as an acceptable diagnostic criterion. When using a microagglutination test, levels of antibody may be measurable within 1 week after infection, although significant levels usually appear in 2 weeks. An agglutination titer of greater than 1:160 tends to be specific for *F tularensis* infection. These criteria are used in a major case series on tularemia.[80]

The limitations of serologic diagnosis are as pertinent to tularemia as they are to other infections. This technique depends on obtaining acute and convalescent sera, which may not be practical, especially if the suspicion of tularemia is delayed because of a nonspecific presentation.[101] Antibodies to *F tularensis* may cross-react with other bacteria, such as *Brucella*, *Proteus*, and *Yersinia* species, which decreases the specificity of serology-based assays. Antibiotic therapy can blunt the serologic response, which could mask the convalescent rise in titer needed to confirm the diagnosis. Finally, antibody levels against *F tularensis* can persist for years, so distinguishing between acute and remote infection may be difficult. For all of these reasons, the development of better diagnostic capabilities for tularemia has become imperative.[1]

Rapid Diagnostic Methods

The most promising recent development in tularemia diagnosis has been the application of polymerase chain reaction (PCR) technology. *F tularensis* can be detected by standard PCR of the 16S rRNA gene[102,103] and the genus-specific *tul4* gene encoding a 17-kd membrane lipoprotein.[102,104-106] Other PCR assays have been designed to target *fopA*, a locus encoding an outer membrane protein.[104,107,108] PCR testing of tissue specimens has been performed with mouse models,[109] rabbit tissue,[110] and humans with ulceroglandular tularemia.[102,111] However, PCR as a diagnostic test has some limitations. The limit of detection of *F tularensis* in blood samples may be suboptimal because of the

presence of PCR inhibitors[10] or other unknown confounding factors. Antigen-detection techniques have also been suggested for *F tularensis*,[110,112] although extensive data on the specificity and sensitivity of these techniques have not been published. These techniques offer the potential of rapid detection, but have not been extensively used in human clinical case scenarios.

Treatment

Antibiotics usually provide curative therapy for tularemia, with resulting mortality rates of only 1% to 2.5%.[1,80] Mortality varies, depending on type of infection (ulceroglandular vs typhoidal), overall health of the infected individual, and rapidity after infection that antimicrobial therapy was initiated. Streptomycin has traditionally been used to treat tularemia, with individuals often demonstrating a clinical response within 48 hours of administration.[1,10,113] Relapses with streptomycin rarely occur. Gentamicin or other aminoglycosides are thought to be as effective as streptomycin and are often listed as reasonable alternatives in clinical practice reviews,[1,114,115] but no controlled trials have been reported. Beta-lactam antibiotics such as ceftriaxone[114] are typically ineffective.

Antibiotics other than the aminoglycosides have been proposed for treating tularemia. Tetracycline and doxycycline are effective, but are associated with a higher relapse rate than the aminoglycosides.[1,101,114] Chloramphenicol is another alternative,[1] but it is rarely used in the United States. The fluoroquinolones offer an additional treatment option,[116-118] especially with the high bioavailability of oral preparations. Although extensive clinical data are lacking for the fluoroquinolones, one report of a tularemia outbreak in Spain noted a 5% failure rate for ciprofloxacin, compared to a 23% failure rate for streptomycin and 43% failure rate for doxycycline.[101] However, the number of patients treated with streptomycin in this study was 94, compared to only 22 being treated with ciprofloxacin. The use of combination antibiotic therapy has not been studied for severe tularemia cases, nor has the antimicrobial susceptibility of antibiotic-resistant strains been extensively studied. The treatment options are summarized in Table 8-1. The general recommendations for length of therapy depend on the antibiotic used. Aminoglycosides and ciprofloxacin are thought to have a low incidence of relapse and, therefore, a course of 10 days is recommended.[1] For doxycycline and chloramphenicol, a longer course of 14 to 21 days is indicated.[1]

TABLE 8-1

ANTIBIOTICS FOR THE TREATMENT OF TULAREMIA*

Patient Group	Preferred Antibiotic	Dose	Alternate	Dose
Adults	Streptomycin	1 g IM twice daily	Doxycycline	100 mg IV twice daily
	Gentamicin*	5 mg/kg IM or IV once daily	Ciprofloxacin*	400 mg IV twice daily
			Chloramphenicol*	15 mg/kg IV four times a day
Children	Streptomycin	15 mg/kg IM twice daily	Doxycycline	If weight is > 45 kg, 100 mg IV twice daily; if weight is < 45 kg, 2.2 mg/kg IV twice daily
	Gentamicin*	2.5 mg/kg IM or IV three times daily	Ciprofloxacin*	15 mg/kg IV twice daily
			Chloramphenicol*	15 mg/kg IV four times daily
Pregnant Women	Gentamicin*	5 mg/kg IM or IV once daily	Doxycycline	100 mg IV twice daily
	Streptomycin	1 g IM twice daily	Ciprofloxacin†	400 mg IV twice daily

*Recommendations are from the Working Group on Civilian Biodefense, and assume a contained casualty setting. Recommendations would differ in a mass casualty scenario.
†Usage is not approved by the Food and Drug Administration.
IM: intramuscular.
IV: intravenous.
Source: Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA.* 2001;285:2763–2773.

PROPHYLAXIS

Postexposure Prophylaxis

Recent consensus recommendations have addressed the issue of postexposure prophylaxis after the use of *F tularensis* in a biological attack.[1] These recommendations have suggested that antibiotics are indicated, especially if the exposure is thought to be recent. Data from human challenge models have suggested that tetracycline can be used to prevent infection after exposure.[119] In an experiment in which volunteers received tetracycline within 24 hours after airborne exposure to *F tularensis*, no tularemia symptoms were detected in 8 volunteers receiving 2 g per day for 14 days, or in 8 volunteers receiving 1 g per day for 28 days. In a group in the same experiment receiving 1 g per day for 15 days, 2 of 10 volunteers developed symptoms after therapy was discontinued. Therefore, if patients can be treated in the early incubation period, oral therapy with either ciprofloxacin or doxycycline (a compound closely related to tetracycline) for 14 days is suggested. However, if the exposure is not detected immediately and it is suspected that individuals were exposed more than a few days ago, a "fever watch" is recommended, involving self-monitoring for constitutional symptoms such as a fever or flu-like illness.[1] Individuals who develop these symptoms should be presumptively treated as if they had tularemia. Consensus statements for postexposure prophylaxis are described in Table 8-2.

Vaccination with Live Vaccine Strain

A live vaccine for *F tularensis* was first developed in the former Soviet Union in the 1930s and reportedly used to safely vaccinate millions of individuals.[120] This vaccine, developed from a Type B strain, was transferred in 1956 to the United States,[121] where researchers Eigelsbach and Downs further characterized the strain, designating it as the LVS of *F tularensis*.[122] It is the only tularemia vaccine available in the United States and is currently in Food and Drug Administration Investigational New Drug status. This vaccine has been administered to hundreds of recipients since the 1950s at the US Army Medical Research Institute of Infectious Diseases (USAMRIID). The vaccine is administered by a scarification process (similar to smallpox vaccination) to the volar surface of the forearm. A small papule forms initially, developing occasionally into a pustule and ulcer. Most vaccine recipients develop a minor scab, and few have systemic side effects. In human challenge studies, the vaccine protected against low-dose respiratory challenge and partially protected against high-dose respiratory challenge with virulent Type A strains.[47,84] Alternative vaccine strategies have been the focus of considerable research, but none of these candidate vaccines are ready for human use.

F tularensis LVS has been studied extensively in mice, but significant differences exist in the immune response of mice to this Type B strain and the immune response of humans to Type A strains. LVS can be fatal in mice when administered as an intraperitoneal injection, yet it can confer protective immunity if given as an intradermal injection.[60] Intradermal administration of LVS can also protect mice from a lethal challenge dose of virulent strains of *F tularensis*. Mice can be protected from the virulent form of *F tularensis* as early as 2 to 3 days after intradermal injection of LVS.[123] Injections of bacterial DNA (as unmethylated CpG motifs) can also confer a similar early protective response.[124] The prompt development of immunity after vaccination in mice suggests that the protective mechanisms are attributable to innate immunity[60] because an adaptive response would require more time to develop. It is unknown whether the vaccine in humans induces an early immune response that is protective. This type of early protection after vaccination would be useful in the military environment because unexposed soldiers may be rapidly protected from further intentional use of *F tularensis* as a weapon.

TABLE 8-2

ANTIBIOTICS FOR POSTEXPOSURE PROPHYLAXIS*

Type of Patient	Preferred Antibiotic	Therapy
Adult	Doxycycline	100 mg orally twice daily
	Ciprofloxacin†	500 mg orally twice daily
Children	Doxycycline	If weight is > 45 kg, 100 mg orally twice daily; if weight is < 45 kg, 2.2 mg/kg orally twice daily
	Ciprofloxacin†	15 mg/kg orally twice daily
Pregnant Women	Ciprofloxacin†	500 mg orally twice daily
	Doxycycline	100 mg orally twice daily

*Recommendations are from the Working Group on Civilian Biodefense.
†Usage is not approved by the Food and Drug Administration.
Source: Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA*. 2001;285:2763–2773.

The correlates of immune response to vaccination have been suggested by prior investigations, but are not definitively established. Before the use of LVS, a killed *F tularensis* vaccine was used.[75] This vaccine was documented to elicit a serologic response, but was not protective. Markers of cell-mediated immunity, such as delayed-type hypersensitivity testing, have also been correlated with protection after vaccination.[74]

The LVS tularemia vaccine is offered at the Special Immunizations Clinic at USAMRIID for laboratory workers at risk for exposure to *F tularensis*. This vaccine has some efficacy, as documented in a human challenge model; however, this protection is not 100%, particularly at high-dose aerosol challenges.[47,84] In addition, an epidemiological study showed that the incidence of typhoidal tularemia in laboratory workers decreased after the introduction of vaccination with LVS.[125] The primary disadvantages are the potential hazards associated with a live vaccine (such as potential dissemination and severe infection in immunocompromised individuals), and the lack of effectiveness against high-dose respiratory challenge. For these reasons, there is much interest in the development of a subunit *F tularensis* vaccine.[10,14] Promising vaccine candidates are being explored.[14]

ISSUES FOR LABORATORY WORKERS

Tularemia is considered a significant hazard for laboratory workers.[91] All experiments that involve using the live virulent form of *F tularensis* should be conducted in biosafety level 3 containment. Additionally, vaccination may augment personal protective measures in diminishing the risk of laboratory-acquired infections. A retrospective review of tularemia cases at USAMRIID was conducted, documenting that typhoidal tularemia incidence dropped substantially after the live vaccine was instituted, decreasing from 5.70 to 0.27 cases per 1,000 at-risk employee-years.[125] The occurrence of ulceroglandular tularemia did not decline significantly (from 0.76 to 0.54 cases per 1,000 at-risk employee-years), but milder symptoms were observed in the recipients of the LVS vaccine.[125] Another review of occupational exposures at USAMRIID suggested that the incidence of tularemia (15 cases/year) did not decrease with the introduction of biosafety cabinets, but did decline after LVS vaccination was introduced.[126]

USE OF TULAREMIA AS A BIOLOGICAL WEAPON

Tularemia could be used as a biological weapon in a number of scenarios, causing varying degrees of casualties. The most dangerous scenario involves an aerosol release with large numbers of persons exposed. Additional complications would result if an antibiotic-resistant strain, as is claimed to have been developed in the former Soviet Union, were used.[2]

Researchers have estimated that a large-scale aerosol release of 50 kg over a large metropolitan area could cause 250,000 incapacitating casualties.[127] Most of those affected could present with a nonspecific febrile illness 3 to 5 days after exposure (range: 1–14 days, depending on the inoculum of exposure), and would subsequently develop pulmonary symptoms consistent with pneumonic tularemia.[1] However, because of the aforementioned difficulties in tularemia diagnosis and the nonspecific clinical presentation, the determination of tularemia as the causative agent may be delayed. The initial presentation of cases may be difficult to distinguish from a natural influenza outbreak or other respiratory pathogens.[1] Tularemia may also be confused with another biological weapon. Epidemiological clues to distinguish tularemia from plague or anthrax is the clinical course (slower with tularemia), case fatality rate (higher with plague[128] or anthrax[129]), and possibly the pattern of pulmonary manifestations observed on chest radiograph, such as the large pleural effusions and mediastinal widening characteristic of inhalational anthrax.[130] Pulmonary tularemia may be difficult to distinguish from Q fever, another potential biological weapon agent.

SUMMARY

Tularemia constitutes a substantial threat as a biological weapon. The variety of clinical manifestations of tularemia infection and the benefits of early antibiotic intervention necessitate a high degree of suspicion from healthcare providers. Familiarization with the variety of epidemiological and clinical manifestations of this disease, along with available diagnostic tests and countermeasures allow healthcare professionals to minimize the impact of its use. Although the current LVS vaccine provides a preventive option against tularemia, much interest remains in the development of a more effective vaccine. Further research will likely continue to elucidate the pathogenesis of this organism and yield improved preventive, diagnostic, and therapeutic options.

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