

# Chapter 11

## TULAREMIA

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## INTRODUCTION

Tularemia is a life-threatening, debilitating disease caused by infection with the bacterium *Francisella tularensis*. This bacterium is one of the most infectious microorganisms known and poses a substantial threat as a potential biological weapon.<sup>1</sup> Both the United States and the former Soviet Union developed weaponized *F tularensis* during the Cold War.<sup>12</sup> The Japanese experimented with *F tularensis* as a biological weapon,<sup>3</sup> but no documentation shows that it was deliberately used as a biological weapon. There is also speculation that the former Soviet Union used *F tularensis* as a biological weapon against German troops in the Battle of Stalingrad during World War II.<sup>2</sup> However, other authors suggest that natural causes, as opposed to an intentional release, were responsible for the tularemia epidemic that occurred in both armies during this battle.<sup>4</sup> There was similar speculation that *F tularensis* was used as a biological weapon by Serbia during the

Kosovo conflict, although subsequent epidemiological investigations suggest that the observed cases were not caused by an intentional release.<sup>5,6</sup>

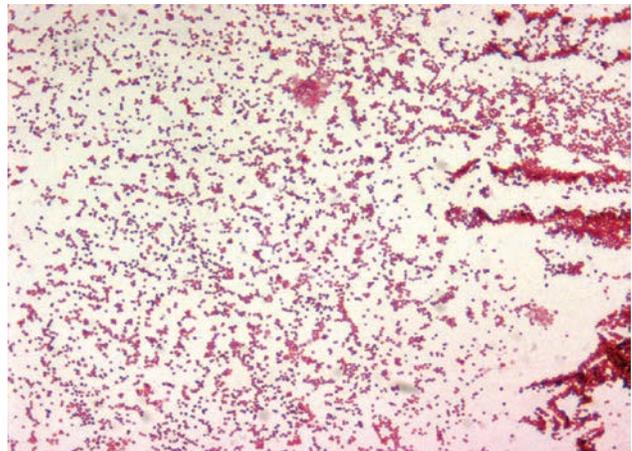
Given its highly pathogenic nature, low infectious dose, and ability to infect via aerosol, *F tularensis* is classified by the US Department of Health and Human Services as a tier 1 select agent. This classification is reserved for those pathogens deemed to pose the highest risk for intentional misuse. The nonspecific disease presentation of tularemia, the high morbidity, the significant mortality if untreated, and the limited ability to obtain a rapid diagnosis are other characteristics that contribute to the effectiveness of *F tularensis* as a potential biological weapon. Although tularemia responds to antibiotic therapy, the intentional use of a genetically modified antibiotic-resistant strain could make these countermeasures ineffective.

## INFECTIOUS AGENT

Infection associated with *F tularensis* was first identified in 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 tularensis*.<sup>7</sup> Subsequently, Wherry and Lamb identified this pathogen as the cause of conjunctival ulcers in a 22-year-old man.<sup>8</sup> Edward Francis' pioneering work significantly increased the understanding of human disease associated with this pathogen in the early 20th century. He described the clinical syndromes associated with *Francisella* infection and named it "tularemia."<sup>9</sup> *F tularensis* was formerly included in both the *Pasteurella* and the *Brucella* genera. However, as mounting scientific data supported the creation of a new genus for this remarkable pathogen, this bacterium was assigned to its own genus and the name *Francisella* was proposed in tribute to Edward Francis.<sup>10</sup>

*F tularensis* is an aerobic, nonmotile bacterium and member of the *Gammaproteobacteria*. By Gram stain, it appears as a small (approximately 0.2–0.5  $\mu\text{m}$  by 0.7–1.0  $\mu\text{m}$ ),<sup>11</sup> faintly staining coccobacillus (Figure 11-1). *F tularensis* is considered to have four subspecies: (1) *tularensis*, (2) *holarctica*, (3) *mediasiatica*, and (4) *novicida*.<sup>12</sup> *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 based on extensive genotyping of isolates. Subpopulation A.I. causes disease in the central United States, and subpopulation A.II. is found mostly in the western United

States.<sup>13</sup> *F tularensis* subspecies *holarctica* (formerly described as *palaearctica*), also known as type B (or biovar B), is found throughout the Northern Hemisphere. *F tularensis* subspecies *holarctica* typically causes a less clinically severe disease than subspecies *tularensis*, but has been documented to cause bacteremia in immunocompetent individuals.<sup>14,15</sup> Before antibiotics, *F tularensis* subspecies *tularensis* resulted in 5% to 57% mortality, yet *F tularensis* subspecies *holarctica* was rarely fatal.<sup>15</sup> *F tularensis* subspecies *mediasiatica* has



**Figure 11-1.** 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, Image 1904.

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*.<sup>16,17</sup>

The clinical significance of this subspecies is not known. *F tularensis* subspecies *novicida*, also referred to as *F novicida*, is believed to be avirulent in healthy humans. Reported cases associated with this subspecies

usually involve patients with other underlying health conditions.<sup>18</sup> The four subspecies can be distinguished using biochemical tests and genetic analysis. Another closely related species, *Francisella philomiragia*, has also been described as a human pathogen.<sup>19,20</sup> However, similar to *F tularensis* subspecies *novicida*, infections attributed to *F philomiragia* were typically found in patients with underlying health conditions.<sup>19,20</sup>

## 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 the cause is not readily discernable and 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.<sup>21</sup> Rotz et al provide criteria for determining the likelihood that a tularemia outbreak is caused by intentional use of *F tularensis* as a biological weapon.<sup>21</sup> A tularemia outbreak in US military personnel deployed to a nonendemic environment would be one example of an incident that should be investigated. An investigation should yield the likely cause of the outbreak, which could be varied (exposure to infected animals, arthropod borne, etc). By determining the source of the outbreak, 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 virulent subspecies and found predominantly in North America. This subspecies has recently been genetically subdivided into two subpopulations, A.I. and A.II. The subpopulations are distinct in mortality rates, geographic distribution, transmission vectors,

and hosts.<sup>22–27</sup> *F tularensis* subspecies *holarctica* (type B), which is found throughout the Northern Hemisphere, is associated with a milder form of disease. In the United States, 90 to 154 cases of tularemia have been reported yearly from 2001 to 2010, according to the Centers for Disease Control and Prevention.<sup>28</sup> More than half of all cases reported came from Arkansas, Missouri, South Dakota, and Oklahoma, where the foci of infection are well established. Every state except Hawaii has reported cases of tularemia.

Human outbreaks, which are often preceded by animal outbreaks, are seasonal, with the highest incidence in late spring, summer, and autumn.<sup>28,29</sup> *F tularensis* has been detected in more than 100 mammalian species and several arthropods.<sup>30</sup> *F tularensis* can be transmitted to humans 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. However, human-to-human transmission has not been described. *F tularensis* is unique in its ability to adapt to a wide range of environmental, host, and vector conditions, and it can be categorized into two distinct transmission cycles involving different hosts and arthropod vectors. The cycle of disease is commonly associated with a subspecies, with type A commonly associated with the terrestrial cycle and type B commonly associated with the aquatic cycle.<sup>23,27,31,32</sup> The human clinical syndromes can be classified by the portal of entry.

### 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.<sup>8</sup> 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.<sup>9</sup> 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.<sup>33</sup>

### Food and Water Ingestion

Tularemia can also be contracted by eating meat from infected animals<sup>9</sup> or food contaminated by infected animals.<sup>34</sup> Water can also become contaminated from animals infected with tularemia and cause human infection. From March through April 1982, 49 cases of oropharyngeal tularemia were identified in Sansepolcro, Italy.<sup>3</sup> 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.<sup>35</sup> Waterborne transmission of ulceroglandular tularemia also occurred during a Spanish outbreak among 19 persons who had contact with river-caught crayfish.<sup>36</sup> The crayfish appear to have served as mechanical vectors, but some evidence suggests a potential role as hosts.<sup>23,36</sup> Contaminated water may have also contributed to recent outbreaks of oropharyngeal tularemia in Turkey<sup>37</sup> and Bulgaria.<sup>34</sup> 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*.<sup>38</sup>

### Mammalian Bites and Arthropod Vectors

Mammalian bites are another source of *F tularensis* transmission to humans. Instances of transmission from the bites or scratch of a cat, coyote, ground squirrel, and a hog to humans were documented more than 80 years ago.<sup>9</sup> 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.<sup>39</sup> Transmission of *F tularensis* by the bites of ticks and flies is also well documented.<sup>11</sup> *Dermacentor* species ticks (dog ticks) are important vectors in areas where enzootic transmission occurs in North America<sup>40</sup> and Europe.<sup>41</sup> *Ixodes* species ticks may also contribute to *F tularensis* transmission.<sup>42</sup> In Utah during the summer of 1971, 28 of 39 tularemia cases were contracted from deerfly (*Chrysops discalis*) bites.<sup>43</sup> 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*).<sup>44</sup>

### Aerosol Transmission

The largest recorded pneumonic tularemia outbreak occurred in Sweden during the winter of 1966 through 1967, when 676 cases were reported.<sup>45</sup> 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.<sup>46</sup> 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.<sup>47</sup> 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.<sup>47</sup> The strong epidemiological link with grass cutting adds plausibility to this explanation.<sup>48</sup> 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.<sup>47</sup>

The only other previously reported outbreak of pneumonic tularemia in the United States occurred in Martha's Vineyard during the summer of 1978.<sup>49</sup> 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,<sup>49</sup> and pneumonic tularemia was initially reported in Massachusetts in 1947.<sup>50</sup>

### 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.<sup>51</sup> 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 more than 100,000 tularemia cases occurred in the former Soviet Union,<sup>4</sup> and outbreaks with hundreds of cases after the war occurred in Austria and France.<sup>52</sup> 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.<sup>5</sup> However, after a decade of warfare, an outbreak of more than 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.<sup>56</sup> The Kosovo outbreak and subsequent investigation are described in detail in chapter 2, 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.<sup>9</sup> Six tularemia cases occurred during US Public Health Service laboratory investigations of tularemia outbreaks from 1919 through 1921.<sup>53</sup> Tularemia is the third most commonly acquired laboratory infection,<sup>54</sup> and recent laboratory-acquired infections of tularemia emphasize the laboratory hazard that this organism presents.<sup>55</sup> 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

One of the remarkable attributes of *F tularensis* is its low infectious dose. As few as 10 organisms can produce clinical disease in healthy human volunteers when administered by either subcutaneous injection or by aerosol exposure.<sup>56,57</sup> Research aimed at elucidating the unique characteristics that permit this organism to cause disease at such low numbers revealed that *F tularensis* boasts a variety of mechanisms to not only evade host defenses, but also to modulate them to survive and proliferate within its host.

*F tularensis*, which is an intracellular pathogen, is known to survive and replicate within a wide variety of cells including professional phagocytic cells, such as macrophages. To gain entry into these cells, *F tularensis* can efficiently use multiple receptors including the mannose receptor, FcγR, and complement receptor 3. Interestingly, a recent study using a fully virulent type A strain showed that entry of opsonized bacteria into human macrophages via complement receptor 3 suppressed the Toll-like receptor 2-dependent proinflammatory responses.<sup>58</sup> Bacterial entry through the mannose receptor resulted in rapid phagosomal escape and prolific cytosolic replication.<sup>59</sup> These findings

indicate that *Francisella* has evolved to use multiple entry pathways to enhance its ability to replicate in the intracellular environment.

Once inside the macrophage, *Francisella* can avoid the bactericidal activity of reactive oxygen species and nitrogen species through expression of enzymes including bacterial acid phosphatases (Acp), superoxide dismutases (Sod), and catalase enzymes (Kat).<sup>60-65</sup> Inhibition of these host defense mechanisms promotes bacterial virulence, as *F tularensis* live vaccine strain (LVS) mutants deficient in expression of SodB, SodC, or KatG are highly attenuated in mouse models of tularemia.<sup>60,61,63</sup> Phagosomal acidification is another host defense mechanism designed to restrict growth of bacterial pathogens. However, both *F tularensis* type A and B stains can inhibit acidification of the phagosome and subsequently escape from the phagosome, and reside in the macrophage cytoplasm.<sup>66,67</sup> The ability of *Francisella* to escape into the cytosol is in part dependent on proteins encoded on the *Francisella* pathogenicity island (FPI). Nano et al first described the FPI in 2004 and subsequently most genes contained within the FPI have been linked to virulence.<sup>68</sup> The FPI also contains genes that encode for a putative type VI secretion system that is required for phagosomal escape and virulence.<sup>69,70</sup> *IglC*, a 23-kDa protein, is believed to be both a core component and secreted effector of the T6SS. *IglC* has been implicated not only in phagosomal escape but also in influencing Toll-like receptor-4 signal transduction.<sup>71-75</sup> Regulation of the FPI is controlled by the *MglA* transcriptional regulator, which responds to various cues and in turn influences expression of more than 100 genes, including several other virulence factors.<sup>76</sup>

Once *Francisella* reaches the cytoplasm, replication begins slowly, but eventually large numbers of organisms can be found within a single macrophage.<sup>73,77,78</sup> 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.<sup>79</sup> This strategy to escape the macrophage may help shield *Francisella* from host immune surveillance mechanisms.

Another survival mechanism of *F tularensis* is the inhibition of Toll-like receptor signaling and cytokine secretion, as demonstrated in experiments with murine macrophages and the LVS of *F tularensis*.<sup>75</sup> Avoidance of Toll-like receptor signaling inhibits the typical robust innate immune response that is active in eliminating typical bacterial pathogens. 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- $\gamma$ -deficient mice die from sublethal doses of LVS,<sup>80</sup> and tumor necrosis factor- $\alpha$  is at least as important as interferon- $\gamma$  for control of *F tularensis* infection.<sup>81,82</sup> The host defense within macrophages appears to be crucial at controlling infection by *F tularensis*. In human monocytes/macrophages, the LVS strain and *F novicida* induced the processing and release of interleukin (IL)-1 $\beta$ , an essential component of the inflammatory immune response.<sup>83</sup> However, killed bacteria did not induce this response, but did induce the early phases required for IL-1 $\beta$ , such as mRNA transcription. This suggests that only live *Francisella* can escape from the phagosome, and thus trigger the function of caspase-1, which converts the precursor of IL-1 $\beta$  to its active form. In mice deficient in caspase-1 as well as ASC, an adaptor protein involved in the assembly of inflammasome complexes, substantially higher bacterial loads were observed, as well as early mortality, compared to normal mice.<sup>84</sup> Neutrophils perform an important function in limiting the spread of *F tularensis* after inoculation. Experiments have demonstrated that neutrophils can kill *F tularensis*,<sup>85</sup> and mice depleted of neutrophils appear more susceptible to infection with *F tularensis* LVS.<sup>86</sup>

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.<sup>87</sup> 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 may be required to produce a sufficient response.<sup>88</sup> Vaccination with *F tularensis* LVS appears to produce a long-term memory T-cell response (as measured by lymphocyte stimulation),<sup>89</sup> but it is unclear what degree of long-term protection is conferred by this response. Both CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes are required for an effective cell-mediated response to *F tularensis*.<sup>80</sup> The protective memory response is dependent on a robust proinflammatory cellular response, because administration of anti-interferon- $\gamma$  and antitumor necrosis factor- $\alpha$  antibodies to previously vaccinated mice dramatically lowers the lethal infective intradermal dose of *F tularensis*.<sup>82</sup> This memory response initially appears 2 to 4 weeks after initial infection,<sup>90-92</sup> and it can remain detectable for many years.<sup>89,93</sup>

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.<sup>94</sup> When laboratory workers received a formalin-killed whole-cell vaccine developed by Foshay et al,<sup>95</sup> a strong humoral

response was elicited but was not protective against cutaneous<sup>58</sup> or respiratory<sup>57</sup> 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.<sup>90</sup> Investigations focused on identifying protective antigens are ongoing, particularly in animal models.<sup>12</sup> 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.<sup>94</sup> 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.

The lipopolysaccharide structure of many gram-negative pathogens elicits a profound proinflammatory immune response, which can lead to the clinical manifestations of septic shock.<sup>96</sup> However, although *F tularensis* lipopolysaccharide can elicit a strong humoral response, it does not induce significant tumor necrosis factor- $\alpha$  and nitric oxide production in macrophages or IL-1 from polymorphonuclear cells,<sup>97</sup> in contrast to lipopolysaccharide from other gram-negative pathogens. Additionally, the lipopolysaccharide of *F tularensis* is structurally different in composition than typical gram-negative pathogens, which is believed to result in the poor Toll-like receptor 4 stimulation observed in type A and type B strains.<sup>98</sup> The O-antigen of *Francisella* has also been shown to be required for virulence. The ability of *Francisella* to avoid complement mediated killing is dependent on the presence of O-antigen as *F tularensis* mutants deficient in O-antigen expression are more sensitive to complement.<sup>98</sup> O-antigen was required for virulence of *F tularensis* in mice<sup>99</sup> and also played a role in cytosolic survival by avoiding autophagy.<sup>100</sup>

## 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.<sup>101</sup> The disease manifestations of tularemia have been ascribed to at least 10 different clinical categories (ulceroglandular, glandular, oculoglandular, oropharyngeal, enteric, gastrointestinal, typhoidal, respiratory, pneumonic, and septic). Symptoms overlap among these categories.<sup>102</sup> Alternatively,

Evans' review of 88 tularemia cases more than 30 years ago describes two syndromes based on clinical signs (ulceroglandular or typhoidal), portal of entry, and disease prognosis. This categorization simplifies this often confusing nomenclature, while emphasizing the obscure but potentially fatal typhoidal presentation, and may also reflect differences in host response to *F tularensis* infection.<sup>103</sup> With ulceroglandular tularemia, there is a vigorous inflammatory reaction, pneumonia is uncommon, and the patient rarely succumbs from infection. Typhoidal tularemia presents with few localizing manifestations, pneumonia is common, and mortality is higher in the absence of antimicrobial therapy.<sup>11,104</sup>

Typhoidal tularemia (15%–25% of cases) primarily occurs after infectious aerosol inhalation, but may also result from an intradermal or gastrointestinal infection.<sup>11,104</sup> The disease presents as a nonspecific syndrome with an abrupt onset of fever (38°C to 40°C), headache, malaise, myalgias, and prostration, but without lymphadenopathy.<sup>11</sup> Lymph nodes are less than 1 cm in diameter, and no skin or mucous membrane lesions are present. Nausea, vomiting, diarrhea, and abdominal pain may also occur. Mortality is greater with pneumonia.<sup>11</sup> Case fatality rates are approximately 35% in untreated naturally acquired typhoidal tularemia.<sup>102</sup> Untreated tularemia survivors may have persistent symptoms for weeks or months with progressive debilitation.<sup>102</sup>

Ulceroglandular tularemia (75%–85% of naturally occurring disease) most often occurs through mucous membrane or skin inoculation with blood or tissue fluids of infected animals.<sup>104</sup> Clinical symptoms in cases of ulceroglandular tularemia typically appear after an incubation period of 3 to 6 days. The lesions present on the skin or mucous membranes (including conjunctiva, oropharynx, etc) and lymph nodes are greater than 1 cm in diameter.<sup>11</sup> This form of the disease is characterized by sudden onset of fever (85% of cases), chills (52% of cases), headache (45% of cases), cough (38% of cases), and myalgias (31% of cases), along with the emergence of a painful papule at the inoculation site.<sup>104</sup> The fever may be associated with pulse-temperature disassociation (42% of cases in one series)<sup>104</sup> where the pulse increases fewer than 10 beats per minute per 1°F increase in temperature above normal. However, 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.<sup>102,104</sup> A rapid progression occurs at the site of inoculation in the untreated patient, with pustule formation leading to a painful ulcer, accompanied by regional painful lymphadenopathy. 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.<sup>104</sup> Lesions are typically associated with regional lymphadenopathy, and a lack of lymphadenopathy may suggest another etiologic agent.<sup>104</sup> 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.<sup>102</sup> Enlarged lymph nodes may become fluctuant and spontaneously drain. If untreated, these fluctuant lymph nodes may persist for months or years.<sup>102</sup> The ulceroglandular form in the eye (oculoglandular) presents 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.<sup>105</sup> Food and water contamination can also lead to oculoglandular infection.<sup>34</sup> In one series pharyngitis was observed in 24% of patients with tularemia.<sup>104</sup> Possible findings on examination include erythema, exudates, petechiae, hemorrhage, or ulceration. Other findings may include retropharyngeal abscess or suppuration of the regional lymph nodes. 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)<sup>37</sup> and Bulgaria (89% of cases),<sup>34</sup> 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%.<sup>52,104</sup> 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 tularemia through hematogenous spread, with an onset ranging from a few days to months after the appearance of initial nonpulmonary symptoms.<sup>52</sup> Approximately 30% of patients with ulceroglandular disease and 80% of patients with typhoidal tularemia also have pulmonary signs and symptoms consistent with pneumonia.<sup>104</sup> Pneumonic tularemia can also occur from direct inhalation of the organism, which has been demonstrated in human experimental models.<sup>56,106</sup> 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.<sup>106</sup> Chest radiographic findings in pneumonic tularemia are highly variable and nonspecific<sup>107</sup> because they can mimic findings associated with other clinical syndromes such as bacterial pneumonias, tuberculosis, lymphoma, or lung carcinoma.<sup>108</sup> 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, cavitory lesions, and bronchopleural fistulas.

A 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 more than a month later while searching for the cause of another pneumonic summer illness.<sup>46,109</sup> 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.<sup>110</sup> A decade earlier, of the 96 patients with tularemia presenting to a Veteran's Hospital in Arkansas, five had pneumonic tularemia.<sup>111</sup>

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.<sup>108</sup> 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.<sup>101,104,112</sup>

The laboratory findings with tularemia are non-specific. Hemoglobin and platelet counts are typically normal, but anemia has been associated with disease. White blood cell counts are usually only mildly elevated, with no alteration in the normal cell differential.<sup>104</sup> Microscopic pyuria may be observed<sup>104</sup> and nonspecific inflammatory markers such as erythrocyte sedimentation rate and C-reactive protein can be elevated. One case series described tularemia associated with skeletal muscle abscesses, elevated creatine kinase, and rhabdomyolysis.<sup>113</sup> 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.<sup>104</sup>

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%.<sup>102</sup> No specific infection control practices are recommended for tularemia, other than universal precautions, because no documented cases of human-to-human transmission exist.<sup>1</sup> However, special precautions are needed for clinical microbiology laboratory workers because of the high incidence of laboratory-acquired infections<sup>114</sup> (see Issues for Laboratory Workers).

## Diagnosis

The diagnosis of tularemia is difficult because the clinical presentations of the various forms are not specific and are consistent with several other syndromes. This nonspecific presentation combined with a low incidence rate may have the unintended consequence of excluding tularemia as a differential diagnosis. This situation is exemplified by a review of cases in Missouri, a known focal point of infection in the United States, where more than half of the documented tularemia infections were misdiagnosed as other infectious diseases.<sup>115</sup> Additionally, the diagnostic modalities available for isolation and identification of *F tularensis* 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<sup>116</sup> or epidemiological syndromic surveillance<sup>117</sup> 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,<sup>118–120</sup> blood cultures are typically negative, even in cases of severe disease.<sup>15</sup> Similarly, cultures from ulcer sites, sputum, gastric washings, and pharyngeal and conjunctival exudates are also usually negative.<sup>11</sup> Occasionally, positive blood cultures have been observed in immunocompromised persons (infected with the less virulent subspecies *holarctica*), and recovery may be improved when blind subculture is conducted.<sup>120</sup>

*F tularensis* is difficult to grow using standard media and requires media supplemented with cysteine or other sources of sulfhydryl groups.<sup>121</sup> Chocolate agar, charcoal yeast extract agar, and Thayer-Martin agar support the growth of *F tularensis*. *F tularensis* colonies appear gray-white on chocolate or Thayer-Martin agar (Figure 11-2). The organism is optimally grown in a CO<sub>2</sub> incubator and tends to grow more slowly than bacteria routinely encountered in clinical practice typically taking 48 to 72 hours to discern. 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* (ie, *novicida* subspecies) do not have these fastidious growth requirements.<sup>122</sup>



**Figure 11-2.** 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, Image 1912.

When recovered from clinical specimens, the organism may be presumptively identified with traditional microbiology techniques and biochemical testing. Automated identification systems in microbiology laboratories should be avoided because they may create aerosols and often misidentify the pathogen. Presumptive or suspected *F tularensis* isolates should be referred to a specialized laboratory for definitive testing.

### 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.<sup>104</sup>

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 non-specific presentation.<sup>123</sup> 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.<sup>1</sup>

### 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<sup>124,125</sup> and the genus-specific *tul4* gene encoding a 17-kd membrane lipoprotein.<sup>125–128</sup> Other PCR assays have been designed to target *fopA*, a locus encoding an outer membrane protein.<sup>126,129,130</sup> PCR testing of tissue specimens has been performed with mouse models,<sup>131</sup> rabbit tissue,<sup>132</sup> and humans with ulceroglandular tularemia.<sup>125,133</sup> 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<sup>11</sup> or other unknown con-

founding factors. Antigen-detection techniques have also been developed for *F tularensis*,<sup>132,134</sup> 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. Other assays to detect *F tularensis* have been studied, including capture enzyme-linked immunosorbent assays based on monoclonal antibodies specific for lipopolysaccharide and fluorescent antibody tests for detection in pathological samples.<sup>31,132</sup>

**Treatment**

Antibiotics usually provide curative therapy for tularemia, with resulting mortality rates of only 1% to 2.5%.<sup>1,104</sup> 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.<sup>1,11,135</sup> Relapses with streptomycin rarely occur. Gentamicin or other aminoglycosides are thought to be as effective as strepto-

mycin,<sup>136,137</sup> but no controlled trials have been reported. Beta-lactam antibiotics such as ceftriaxone<sup>136</sup> 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.<sup>1,123,136</sup> Chloramphenicol is another alternative,<sup>1</sup> but it is rarely used in the United States. The fluoroquinolones offer an additional treatment option,<sup>138-140</sup> especially with the high bioavailability of oral preparations. Although extensive clinical data are lacking for the fluoroquinolones, one report of a tularemia outbreak resulting from *F tularensis* subspecies *holoartica* in Spain noted a 5% failure rate for ciprofloxacin, compared to a 23% failure rate for streptomycin and 43% failure rate for doxycycline.<sup>123</sup> However, the number of patients treated with streptomycin in this study was 94, compared to only 22 being treated with ciprofloxacin. Although the clinical effectiveness with fluoroquinolones has been demonstrated in mild to moderate cases resulting from *F tularensis* subspecies *holoartica*, in severe cases a combination with gentamicin has been recommended.<sup>141</sup>

However, there is limited experience using fluoroquinolones to treat tularemia disease due to the more virulent *F tularensis* subspecies *tularensis*, but

**TABLE 11-1**  
**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 <sup>†</sup>	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.

<sup>†</sup>Usage is not approved by the Food and Drug Administration.

IM: intramuscular.

IV: intravenous.

Data source: Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA*. 2001;285:2763-2773.

it has been used successfully in a case that relapsed after doxycycline.<sup>142</sup> 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 11-1.

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.<sup>1</sup> For doxycycline and chloramphenicol, a longer course of 14 to 21 days is indicated.<sup>1</sup>

## PROPHYLAXIS

### Postexposure Prophylaxis

Recent consensus recommendations have addressed the issue of postexposure prophylaxis after the use of *F tularensis* in a biological attack.<sup>1</sup> 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.<sup>143</sup> 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.<sup>1</sup> Individuals who develop these symptoms should be presumptively treated as if they had tularemia. Consensus statements for postexposure prophylaxis are described in Table 11-2.

### 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.<sup>144</sup> This vaccine, developed from a type B strain, was transferred in 1956 to the United States,<sup>145</sup> where researchers Eigelsbach and Downs further characterized the strain designating it as the LVS of *F tularensis*. 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 thousands 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 to moderate-dose respiratory challenge and partially protected against high-dose respiratory challenge with virulent type A strains.<sup>56,106</sup> 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.<sup>81</sup> Intradermal administration of LVS can also protect mice from a lethal challenge dose

**TABLE 11-2**  
**ANTIBIOTICS FOR POSTEXPOSURE**  
**PROPHYLAXIS\***

Type of Patient	Preferred Antibiotic	Therapy
Adult	Doxycycline	100 mg orally twice daily
	Ciprofloxacin <sup>†</sup>	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 <sup>†</sup>	15 mg/kg orally twice daily
Pregnant women	Ciprofloxacin <sup>†</sup>	500 mg orally twice daily
	Doxycycline	100 mg orally twice daily

\*Recommendations are from the Working Group on Civilian Bio-defense.

<sup>†</sup>Usage is not approved by the Food and Drug Administration. Data source: Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA*. 2001;285:2763–2773.

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.<sup>146</sup> Injections of bacterial DNA (as unmethylated CpG motifs) can also confer a similar early protective response.<sup>147</sup> The prompt development of immunity after vaccination in mice suggests that the protective mechanisms are attributable to innate immunity<sup>80</sup> because an adaptive response requires 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.

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.<sup>95</sup> 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.<sup>94</sup>

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 is efficacious, as documented in a human challenge model; however, this protection is not 100%, particularly at high-dose aerosol challenges.<sup>56,106</sup> In addition, an epidemiological study showed that the incidence of typhoidal tularemia in laboratory workers decreased significantly after the introduction of vaccination with LVS.<sup>148</sup> The primary disadvantages are the potential hazards associated with a live vaccine (such as 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.<sup>11,12,149</sup> Promising vaccine candidates are being explored.<sup>12,149,150</sup>

### ISSUES FOR LABORATORY WORKERS

Tularemia is considered a significant hazard for laboratory workers.<sup>114</sup> All experiments that involve using *F tularensis* subspecies *tularensis* and fully virulent *F tularensis* subspecies *holarctica* strains should be conducted under biosafety level 3 conditions. Additionally, vaccination of at-risk personnel may diminish clinical manifestations of laboratory-acquired infections. A retrospective review of tularemia cases at USAMRIID was conducted. This study documented that typhoidal tularemia incidence dropped substantially after the LVS was offered to at-risk

laboratory workers. Incidence rates decreased from 5.70 to 0.27 cases per 1,000 at-risk employee-years.<sup>148</sup> 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.<sup>148</sup> 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.<sup>151</sup>

### USE OF TULAREMIA AS A BIOLOGICAL WEAPON

*F tularensis* could be used as a biological weapon in many 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—was used.<sup>2</sup>

Researchers have estimated that a large-scale aerosol release of 50 kg over a large metropolitan area could cause 250,000 incapacitating casualties.<sup>29</sup> 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.<sup>1</sup> However, because of the aforementioned difficulties in tularemia

diagnosis and the nonspecific clinical presentation, the determination of *F tularensis* 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.<sup>1</sup>

*F tularensis* may also be confused with another biological weapon. Epidemiological clues to distinguish tularemia from plague or anthrax are the clinical course of disease (slower with tularemia), case fatality rate (higher with plague<sup>152</sup> or anthrax<sup>153</sup>), and possibly the pattern of pulmonary manifestations observed on chest radiograph, such as the large pleural effusions and mediastinal widening characteristic of inhalational anthrax.<sup>154</sup> Pulmonary tularemia may be difficult to distinguish from Q fever, another potential biological weapon agent.

## SUMMARY

*F tularensis* constitutes a substantial threat as a biological weapon. The variety of clinical manifestations of *F tularensis* 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 health-

care professionals to minimize the impact of its use. Although the current LVS vaccine provides some protection against clinical disease associated with *F tularensis*, 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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