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# 226 Brucellosis (*Brucella* Species)

*Hanefi C. Gul and Hakan Erdem*

## SHORT VIEW SUMMARY

**Diagnosis**
- Brucellosis is diagnosed by isolation of *Brucella* from blood, bone marrow, or tissue or by serology, although the polymerase chain reaction assay is available in a few laboratories. The preferred serology is the tube agglutination test, with a titer of at least 1:160. The rose-bengal card agglutination test is useful for screening. *Brucella canis* requires a separate serology.

**Microbiology**
- *Brucella melitensis, Brucella suis, Brucella abortus,* and, rarely, *B. canis* cause brucellosis and are nonmotile gram-negative coccobacilli.

**Epidemiology**
- Although uncommon in the United States, brucellosis is a worldwide zoonosis of wild and domestic animals, principally cattle, swine, goats, and sheep. Infection is acquired by contact with infected animals, their tissues, or ingestion of unpasteurized milk or dairy products from infected cows, goats, and sheep.

**Clinical Settings**
- Fever, sweating, myalgia, weight loss, headache, and malaise without localizing symptoms are evident. Lymphadenopathy or hepatosplenomegaly may occur. Onset can be acute or insidious and persist for weeks or months if untreated.
- Focal infections include osteomyelitis (particularly spondylitis or sacroiliitis), epididymo-orchitis, endocarditis, meningitis, meningoencephalitis, and myeloradiculitis.

**Treatment**
- Doxycycline with streptomycin is the standard regimen. For alternatives and treatment of neurobrucellosis, see Table 226.1 and text.

Brucellosis is the most frequently encountered worldwide zoonotic disease, which can be acquired from sheep, goat, cattle, swine, and other animals and transmitted to humans.[1] Each year half a million new human brucellosis cases cause serious consequences on health and socioeconomic issues, particularly in underdeveloped countries.[2] The disease is under control in developed countries.[3] The disease is frequently transmitted from unsterilized milk and dairy products, and outbreaks related to unpasteurized milk are reported.[4] Brucellosis is one of the most frequent laboratory-acquired infections.[5] In addition, brucellosis is classified as a category B biological weapon by the Centers for Disease Control and Prevention (CDC) owing to the ease of facilitated transmission.[6]

## HISTORY

Brucellosis is one of the earliest known diseases. Throughout history the disease has taken many different names, including Mediterranean, Maltese, or Crimean fever and Bang disease. It was named *undulant fever* after 1913 and then *brucellosis,* the name used today, from 1940 onward.[7,8]

David Bruce, a military surgeon, isolated brucellae from the spleens of the patients in Malta between 1886 and 1887. The bacterium was named *Micrococcus melitensis* after isolation. A Danish doctor, Bang, later proved that the principal cause of abortion in bovines was a miniscule bacillus that he named *Bacillus abortus.*[6] In 1914 Traum isolated the bacteria from the aborted fetuses of pigs and gave the name *Bacterium abortus suis.* In reference to Bruce's name, the bacterium was renamed under *Brucella* as *Brucella melitensis*, *Brucella abortus,* and *Brucella suis* in the 1920s. In 1953 van Drimmelen isolated *Brucella ovis* from sheep; Stoenner and Lackman recovered *Brucella neotomae* from rodents in 1957; and Carmichael and Bruner isolated *Brucella canis* from dogs in 1968.[6] In 1994 Ewalt and Ross isolated *Brucella pinnipidiae* and *Brucella cetaceae* from sea mammals, namely dolphins and seals for *B. pinnipidiae* and whales for *B. cetaceae*. These species were later named *Brucella pinnipedialis* and *Brucella ceti.*[9] Later, *Brucella microti* was identified in animals in 2008 and *Brucella inopinata* from a breast implant in 2010.[10,11]

## MICROBIOLOGY

*Brucella* spp. are small, gram-negative, nonmotile, non–spore-forming, intracellular-reproducing, aerobic coccobacilli with a size of 0.6 to 1.5 µm in length and 0.5 to 0.7 µm in width. Three species have been divided into biovars on the basis of biologic and serologic criteria. Four species are pathogenic for humans: *Brucella abortus* (seven biovars), *B. melitensis* (three biovars), *B. suis* (five biovars), and *B. canis*. Even though all the *Brucella* spp. are catalase positive, their oxidase and urease activity, as well as hydrogen sulfide production, varies. Although most *Brucella* spp. reproduce in aerobic conditions, *B. abortus* and *B. suis* are microaerophilic and require 5% to 10% carbon dioxide.[12] Although biochemical reactions have long been used to identify *Brucella* spp., matrix-assisted laser desorption/ionization time-of-flight mass spectrometry holds promise for use in reference laboratories.[13] Most *Brucella* spp. contain two annular chromosomes of approximately 2.1 and 1.2 mega–base pairs (Mbp).[14] *B. suis* does not follow this structure and has a 3.1 Mbp single annular chromosome.[15] The guanine-cytosine (GC) content of the chromosome is 57%. Genomes of different *Brucella* spp. have large areas of homology, leading to the suggestion that this genus should be given a single species name. Sequences of more than 30 *Brucella* genomes are available, including *B. suis*, *B. melitensis, B. canis,* and *B. abortus.*[14,15,16]

*Brucella* does not possess any defined endotoxin. The lipopolysaccharide layer on the cell wall shows endotoxic activity. The O-chain polysaccharide on the lipopolysaccharide layer has a significant role in the bacterial virulence. The three epitopes on the O-chain, named A, M, and C, can vary according to their species. The cytoplasmic, periplasmic, and outer membrane structural proteins bear antigenic features and are recognized by the immune system.[17] Changes in genes that affect the structure of the core oligosaccharide create attenuated mutants that interact more efficiently with the innate immune system (increase dendritic cell maturation and the production of inflammatory cytokines).[18] A phospholipase A1 specific for phosphatidylethanolamine, BveA, has been identified as a factor contributing to the resistance of *Brucella* to polymyxins, an activity which, in other bacteria, may provide protection from polymyxin-producing bacteria in the rhizosphere. The construction of mutants in the *bveA* gene, which encodes a predicted

esterase, modifies the lipid content of *B. melitensis* membranes and attenuates survival, replication, and persistence in mice.[19]

The bacterium survives for 6 weeks at 4°C in cream, 30 days in ice cream, and 15 to 100 days in fresh cheese.[20,21] Boiling and pasteurizing the milk kill the bacterium. Brucellae also die when the milk goes sour or lactic acid fermentation occurs. It is safe to consume cheese after 60 to 90 days. The bacterium is also sensitive to heating, ionized radiation, and disinfectants.[22,23]

## EPIDEMIOLOGY

The incidence of brucellosis across the world varies from less than 0.03 to 160 per 100,000 population.[2] Brucellosis is most commonly seen in the Mediterranean countries, the Balkans, the Persian Gulf, the Middle East, and Central and South America.[2] Since the disintegration of the former Soviet Union, the Asian continent has also emerged as a significant focus.[21] Thus brucellosis is observed more frequently in developing countries, and it is practically eradicated in developed countries.[2,7,24] As an example, Muslim and Druze populations in Israel suffered from outbreaks, with a report describing disease in children in the period 2005–11, noting that 50.8% were bacteremic.[25] Hence the disease should be suspected in returning febrile travellers.[26]

The most invasive and pathogenic type of human brucellosis is due to *B. melitensis,* followed by *B. abortus* and *B. suis*. The most common animal hosts for *B. melitensis* biovars 1 to 3 are sheep, goats, camels, and buffalo; *B. abortus* biovars 1 to 6 are seen mostly in bison and camels; *B. suis* biovars 1 to 5 are reported in pigs, reindeers, and rodents; and *B. canis* affects dogs.[6]

Contaminated sheep and goat milk with *B. melitensis* appears to be the leading source of human brucellosis worldwide.[7,25] Other routes of transmission to humans are direct contact with infected animals or their secretions through bruises and lacerations on the skin, inhalation of infected aerosols, and conjunctival inoculation.[27] A child with *B. canis* bacteremia led to a case investigation that found that the child's dog was the probable source.[25] Due to the high frequency of brucellosis among farmers, veterinarians, doctors, and laboratorians, it is recognized as an occupational disease. Laboratorians, in particular, are likely to acquire the microorganism through aerosols or direct contact,[5,25] and laboratory-induced outbreaks are also reported.[25] In a review of 71 reported laboratory-acquired cases, the median incubation period was 8 weeks.[25] Four of six pregnant laboratorians with brucellosis aborted their fetus. Antimicrobial prophylaxis in highly exposed laboratorians appeared to be effective.[25] Serologic conversion has occurred in a radiology technician whose exposure was assisting at the aspiration of a *Brucella*-infected prosthetic joint.[28] Although human-to-human transmission is quite rare, congenitally and sexually transmitted cases are reported as well.[27] Because infections related to blood transfusion and bone marrow transplantation are reported, questioning blood donors for symptoms of brucellosis and the use of diagnostic tests can be considered in endemic areas.[27]

## PATHOGENESIS

*Brucella* is an intracellular microorganism that can survive inside the macrophages, where it has specific survival mechanisms. The bacterium is protected through immune system—evading mechanisms such as blocking macrophage apoptosis, suppressing Th1-specific immune response, and inhibiting tumor necrosis factor-α (TNF-α) production.[22] Among the significant virulence factors are the lipopolysaccharide structure on the cell membrane, VirB type-IV secretion system (T4SS), the two-component BvrR/BvrS system, and the cyclic β-1,2 glucan system.[25,29,30] In addition, virulence factors of the bacterium include the adenine and guanine monophosphate systems, which inhibit the fusion of phagolysosomes, release of myeloperoxidase, and TNF production. A superoxide dismutase detoxifies reactive oxygen intermediates, and urease protects *B. abortus* and *B. suis* from gastric acid as they pass through the stomach.[31–33]

*Brucella* species taken into the body arrive at local lymph nodes either inside polymorphonuclear leukocytes and macrophages or extracellular.[31] The microorganisms reproducing intracellularly spread to the neighboring cells, local lymph nodes, or reticuloendothelial organs such as liver, spleen, and bone marrow.[34,35] Brucellae form granulomas made up of epithelioid cells, polymorphonuclear leukocytes, lymphocytes, and giant cells in tissues and organs. Granulomas are known to be more frequent in *B. abortus* infections.[36] Although toxemia is commonly observed in *B. melitensis,* abscess formation in joints and spleen is more often related to *B. suis.*[37]

## IMMUNE RESPONSE

Adaptive immune responses play a crucial role in controlling the infection. Cytokines such as interferon-γ (IFN-γ) and interleukin-2 (IL-2), secreted by $CD8^+$ T cells in particular, are the most significant agents in preventing the progression of the disease.[38] During infection an increase is observed in the number of γδ T cells carrying Vγ9δ T receptors. These cells not only increase bactericidal activities of macrophages but also eliminate the infected cells by cytotoxic effects.[36,39,40] Secreted cytokines, such as IL-12, IFN-γ, and TNF-α molecules, play an essential role in a natural and adaptive immune response.[39,31]

Cellular immunity has a fundamental role in controlling the disease. Although the presence of specific antibodies is of utmost importance in diagnosis, they play a limited role in the immune response.[6] The immunoglobulin M (IgM) antibodies increase in the first week and the IgG antibodies in the second.[37] After 4 weeks of rising, both immunoglobulin levels decrease rapidly after a successful treatment. Furthermore, IgG levels decrease faster than IgM levels with treatment. Even after eradication of active infection, IgM antibodies can remain positive in low titers for months or even years. A high level of IgG and IgA antibodies for longer than 6 months is a sign of chronic infection or relapse.[37,41]

## CLINICAL MANIFESTATIONS

Brucellosis can involve any organ or system in the body. Hence it mimics a myriad of human disorders and is known as a "great imitator."[12] The disease is a systemic infection with diverse clinical spectra, extending from asymptomatic disease to fatal illness.[7] Clinical history of living in endemic areas, international travel, unpasteurized milk or milk products consumption, hunting, working in a laboratory, vaccinating livestock, close contact with animals or animal tissues may be suggestive of the disease. The incubation period is usually 1 to 4 weeks, although it may extend beyond several months. Although clinical differences between species are difficult to determine, *B. melitensis* infections are reported to present more acutely compared with disease caused by *B. abortus.*[24] In addition, clinical presentation and complications of *B. suis* infection in humans are reported to be similar to *B. melitensis* or *B. abortus* infections.[42]

The disease presents as either acute febrile illness or chronic infection. The onset of symptoms may be either abrupt or insidious, developing over several days to weeks. The most frequent complaints are arthralgia, fever, and fatigue seen in up to 75% to 100% of the cases, followed by sweating, malodorous perspiration, lack of appetite, myalgia, chills, and back pain.[7,12,43] Brucellosis was formerly named *undulant fever* because fever waxes and wanes in due natural course of the disease.[8] The most common clinical findings are fever and hepatomegaly in one-third to one-half of patients, followed by splenomegaly, peripheral arthritis, sacroiliitis, scrotal swelling, neck stiffness, and lymphadenopathy.[44,45] Subclassifying the patients into "acute" (<8 weeks), "subacute" (8–52 weeks), and "chronic" (>52 weeks) categories according to the onset of the disease appears to be imprecise.[46] However, fever seems to be more frequent when the onset of the disease is within 1 month.[47]

Brucellosis is characterized by frequent organ-based complications, prolonged courses of the disease, treatment failures, and relapses. When a specific organ involvement is detected, the disease is defined as the focal form of brucellosis, for which different management strategies are required. Focal involvement in due course of brucellosis is seen in more than half of the patient population.[44,48] Owing to its subtle nature, the disease is one of the leading causes of fever of unknown origin[49] and is one of the reasons for febrile neutropenia in endemic areas.[50]

A relapse in brucellosis is defined by the reappearance of clinical signs and symptoms with or without a positive culture.[7] Relapse rates are frequently around 5% to 15%, depending on the regimen used.[7,44,51,52,53] It frequently occurs within 6 months after the discontinuation of therapy and tends to be milder than the original attack.[54] Relapse is not usually due to the emergence of antibiotic-resistant strains. Rather, it is frequently

due to poor compliance with therapy.[46] Other reasons may include inappropriate antibiotic use, microbial virulence factors, and focal infections.[7]

## COMPLICATIONS

### Skeletal System

Osteoarticular involvement is the most common complication seen in up to half of brucellosis cases. Sacroiliitis, spondylodiskitis, and peripheral arthritis are the common types of osteoarticular lesions.[44,48] The lumbar vertebrae are frequently affected spinal sites (Fig. 226.1),[55,56] and large joints of the lower limbs, such as hips, knees, and ankles, are the most often involved joints.[6,57] Compared with tuberculous spondylodiskitis, brucellar spinal disease is a significantly less suppurative infection, requiring surgical intervention, and is characterized with less frequent spinal and neurologic complications.[56] Sacroiliitis is common in younger patients (Fig. 226.2), whereas spondylitis and peripheral arthritis are common in older patients.[12,58,59] *Brucella* infection related to joint prostheses has also been reported.[60] Paravertebral, epidural, and psoas abscesses related to spondylitis and vertebral osteomyelitis can be difficult to treat.[55,56,61]

**FIG. 226.1 Sagittal fat-saturated T2-weighted magnetic resonance image of the lumbar spine showing increased bone marrow signal intensity in the L4 and L5 vertebral bodies with paravertebral soft tissue lesion.** *(Courtesy Dr. Tuba Sanal, Gülhane Askeri Tip Akademisi School of Medicine.)*

**FIG. 226.2 Fat-saturated T2-weighted oblique coronal image showing osteitis at the iliac side of the right sacroiliac joint *(arrow).*** *(Courtesy Dr. Tuba Sanal, Gülhane Askeri Tip Akademisi School of Medicine.)*

Plain radiographs should be reserved for spondylitis and advanced arthritis.[62] In addition, computed tomography (CT) and bone scintigraphy may have limitations owing to inadequate soft tissue resolution. Magnetic resonance imaging (MRI) appears to be the method of choice to assess the extent of osteoarticular brucellosis and in the follow-up of therapeutic responses.[62,63] The characteristic Pedro-Pons sign (a steplike erosion at the anterosuperior portion of a vertebral body) in spondylitis is noteworthy.[64]

### Nervous System

Neurologic involvement occurs in approximately 10% of cases and is a serious complication of brucellosis.[44,65] Brucellosis comprises 0.5% of all community-acquired central nervous system (CNS) infections.[66] The disease can be classified into categories: acute meningitis or meningoencephalitis, chronic peripheral form (radiculoneuropathy), and chronic CNS infection (meningoencephalitis, myelitis, cerebellar involvement, cranial nerve palsies).[65] Headache and fever are observed in slightly more than half of the cases as the predominant complaints, followed by sweating, weight loss, and back pain. In addition, meningeal irritation is reported only in one-third of the cases, followed by confusion, hepatomegaly, hypoesthesia, and splenomegaly.[65] An evaluation of 263 patients with MRI or CT for CNS neurobrucellosis found that 54.3% had normal scans, and diffuse leptomeningeal inflammation was detected most frequently in one-fourth of the cases.[67] Added to that, 1.7% of *Brucella* meningitis did not display CNS pleocytosis.[68] Under these circumstances the disease has a quite subtle presentation and has a predilection to present chronic courses.[66] Some reports relate brucellosis to Guillain-Barré syndrome in endemic areas.

According to a multinational study on neurobrucellosis, half of the neurobrucellosis patients present to hospitals with serious neurologic complications. Cranial nerve involvement, mostly affecting the sixth and eighth cranial nerves, complicates one-fifth of the cases. Moreover, polyneuropathy and radiculopathy, depression, paraplegia, stroke, and abscess formation are reported. Mortality is as low as less than 1% with suitable antibiotics, although one-fifth of treated patients experience permanent sequelae. Walking difficulty and hearing loss are predominant sequelae, followed by urinary incontinence, visual disturbances, and amnesia. Roughly 4% of the patients come to neurosurgery. Moreover, the cerebral abscesses are generally small, and only one-third of patients with abscess formation appear to require surgical drainage.[52] The Thwaites and the Lancet scoring systems used to detect CNS tuberculosis may erroneously identify neurobrucellosis as tuberculous meningitis. Hence caution is indicated in the differential diagnosis.[69] In conclusion, although mortality is rare, neurobrucellosis is a serious disease with frequent sequelae despite treatment.[52,65,70]

### Genitourinary Tract

Genitourinary complications are mostly seen in men and are reported in 5% to 10% of all brucellosis patients. Epididymo-orchitis is observed most frequently in males and pyelonephritis in females[48,51,57,71] Any patient with scrotal pain and swelling with coexistent arthritis and arthralgia should raise consideration for brucellar epididymo-orchitis.[51] Infection is usually unilateral, with fever or sweating in only about a third of patients.[72] The disease may simulate testicular lesions, such as tumors or tuberculosis.[73] Nephritis is mostly a complication of endocarditis. Renal, scrotal, and testicular abscesses, as well as prostatitis, caused by *Brucella* have been reported.[51,74]

### Cardiovascular System

*Brucella* cardiovascular involvement includes endocarditis,[75] myocarditis,[76] pericarditis,[77] endarteritis,[78] thrombophlebitis,[79] and mycotic aneurysms.[80] Pancarditis leading to heart failure is known to be a serious complication.[81] Endocarditis is the most common cardiovascular complication and is reported in approximately 1% of all brucellosis cases.[48,75] Mortality in brucellosis is mostly attributed to endocarditis.[12] Echocardiography and automated blood cultures contribute significantly to diagnosis of cardiovascular brucellosis.[75] In a multicenter *Brucella* endocarditis study, aortic and mitral valves were the most frequently involved sites in the heart. Underlying cardiac valvular disorders are reported in 60% of the

patients. Although mortality is reported to be 13% despite treatment, it is significantly higher when pericardial effusion or congestive heart failure develops.[75]

### Gastrointestinal System

Human brucellosis is one of the enteric fevers where systemic symptoms generally predominate over gastrointestinal (GI) complaints. GI involvement in a brucellosis patient should be considered if there are any related signs or symptoms, such as nausea and vomiting, diarrhea, constipation, and abdominal tenderness.[82] Diarrhea is reported in 3% to 6% of patients with brucellosis.[6,7,83] Although the precise reason is not known, it may be due to mucosal ulcerations in intestinal Peyer patches or other microorganisms present in contaminated raw milk.[83] Spontaneous bacterial peritonitis is one of the leading GI disorders, followed by splenic abscess and acute abdominal pain.[48] The majority of the patients with spontaneous bacterial peritonitis are reported to have underlying liver cirrhosis.[83] Acute and chronic pancreatitis,[84,85] colitis,[86] intestinal obstruction,[87] and ileitis[48] have been reported occasionally.

### Hepatobiliary System

Hepatic involvement in brucellosis covers a wide spectrum, ranging from mild elevation of aminotransferases to manifest hepatitis, including granulomatous forms, and to liver abscesses.[88] Increases in aminotransferases are noted in one-fourth to one-third of brucellosis cases and are more frequent in the acute stages.[44,45,47,71] Hepatic involvement in brucellosis can present with clinical hepatitis in 3% of the cases.[44,48] In most patients with hepatic involvement, the histopathologic analysis is interpreted as reactive hepatitis.[89] Although *B. abortus* tends to establish a granulomatous form of hepatitis, *B. melitensis* may cause both diffuse and granulomatous lesions in the liver. Even necrotizing hepatic lesions are reported in the literature.[90] Cholecystitis is a rare event,[48] except with coexistent gallstones.[91] *Brucella* hepatitis may lead to liver decompensation and cirrhosis if left untreated.[88]

### Respiratory System

Respiratory system involvement in brucellosis is reported in up to 1% of cases.[44,48] Bronchitis, pneumonia, and pleural effusion are the predominating pulmonary presentations.[48,92] Granulomas and solitary nodules in the lung parenchyma, as well as abscess and cavity formation, are also noted.[93,94] Miliary mottling can be present in pneumonias and may result in confusion with pulmonary tuberculosis.[95] Brucellosis is one of the most common laboratory-acquired infections, mostly due to airborne transmission.[5] Sand and airborne dust have been shown to have the potential to carry *Brucella* spp.[96] Although the interrelations between the development of pneumonia and airborne transmission are unclear, one of eight laboratory workers who experienced a brucellosis outbreak in a laboratory by airborne route had pneumonia.[97] Respiratory system brucellosis, which is usually indistinguishable from other community-acquired pneumonia forms, has a benign course and responds to treatment used for uncomplicated brucellosis.[94,98]

## HEMATOLOGIC COMPLICATIONS

A wide spectrum of laboratory abnormalities related to the hematologic system in brucellosis is reported. Anemia, leukopenia, leukocytosis, thrombocytopenia, thrombocytosis, and pancytopenia are relatively common.[6,12,44,48] Bone marrow involvement shows hypercellularity, hemophagocytosis, and granulomas.[99] Although hematologic abnormalities are usually mild, disseminated intravascular coagulation can occur.[100] Hypersplenism due to brucellosis may contribute to the severity of hematologic complications.[101] In rare instances massive bleeding[102] or capillary leak syndrome, defined as unexplained capillary hyperpermeability, can occur.[103] Nevertheless, the blood profile improves with treatment. The disease may also mimic lymphoma.[104]

## CUTANEOUS LESIONS

Nonspecific cutaneous and mucosal lesions are reported in 2% to 6% of brucellosis cases.[44,48,105] These manifestations are due to hypersensitivity, depositing immune complexes, or direct invasion by the organism.[106] Erythematous, papulonodular, and erythema nodosum-like lesions are the most frequent eruptions, and they usually appear at the initial stages of the infection.[105] Psoriatic lesions, palmar erythema, malar eruption, palmar eczema, petechiae, and purpura are reported in a diverse spectrum.[107,108] *Brucella* may trigger immunologic responses leading to leukocytoclastic vasculitis.[109,110]

## OCULAR LESIONS

Uveitis, the most frequent eye complication, followed by optic neuritis, papilledema, keratitis, conjunctivitis, and other diverse ocular complications are observed in brucellosis. Panuveitis, in which nearly all patients had lost their vision, is reported in one-fifth of all uveitis cases.[111] Direct invasion, septic emboli from the infected site, endocardium in particular, and formation of immune complexes are reported as the mechanisms of *Brucella* eye disease.[111,112] Isolation of the microorganism from the vitreous humor has been reported.[113] Alternatively, visual improvement after treatment with steroids can be interpreted as evidence of an immune basis for the inflammation.[114]

## PREGNANCY

Brucellosis manifests as a chronic infection resulting in sterility and abortion. Erythritol is considered as a contributor to the pathogenesis in animals.[115] Two reasons are known to be responsible for the lesser role of *Brucella* infection in human abortion: (1) Human placenta does not produce erythritol[7] and (2) the presence of anti-*Brucella* activity in human amniotic fluid may have protective effects.[116]

Brucellosis complicates pregnancy in one-third to one-half of women infected while pregnant, particularly in the first two trimesters. The disease is linked to intrauterine infection, fetal death, spontaneous abortion, premature delivery, and low birth weight in the neonate.[117] Congenital brucellosis is detected in 6.4% of the newborns, and the surviving newborn may experience serious sequelae.[118] Thus prompt initiation of antibiotic treatment may be lifesaving for both fetus and newborn.

## DIAGNOSIS

Diagnosis of brucellosis requires the assessment of medical history, clinical evaluation, and routine laboratory and radiologic tests, combined with culture, serology, or polymerase chain reaction (PCR) assay.[119,120] Brucellar disease may easily be confused with tuberculous meningitis or spondylodiskitis in nonendemic and developed countries.[56,69] The routine laboratory tests are complete blood count, erythrocyte sedimentation rate, C-reactive protein, and liver function tests, although they are not specific for the diagnosis, and wide patient variability is known to exist.[56,69,119]

Blood and bone marrow are the most suitable specimens used in the isolation of *Brucella.* In patients receiving antibiotics, as well as patients with a chronic form of brucellosis, bone marrow culture appears more sensitive.[121] In addition, automated culture systems have improved the speed and the efficacy of *Brucella* isolation, usually within 3 days.[122] Moreover, automated culture systems are much more often positive than conventional cultures for sterile body fluids including cerebrospinal fluid (CSF).[122,123]

Numerous serologic methods are used in the diagnosis of brucellosis. Although serum agglutination test (SAT or Wright reaction) is usually recognized as the reference technique, with a positive defined as a titer of at least 1:160.[119,124] It is labor intensive and time consuming. Thus rose-bengal slide agglutination test (RBT), which uses stained killed *B. abortus* bacterial cells, offers a simple and affordable card test. The RBT has been traditionally used as a feasible format of SAT for rapid screening in emergency departments.[125,126] The Coombs antiglobulin test is used to detect nonagglutinating antibodies against *Brucella* cells. Serial dilutions up to high titers may be necessary to get beyond prozones with this process.[119] Immunocapture enzyme-linked immunosorbent assay (ELISA, Brucellacapt [Vircell; Granada, Spain]) adds patient serum to a microwell, which is coated with antibodies against human IgG, IgM, and IgA. Stained killed *Brucella* cells are added, and agglutination is observed. This method yields results comparable to the Coombs test.[127,128] Likewise, ELISA appears to be comparable to conventional serologic tests in the diagnosis of the disease.[129] However, the CDC has warned that false-positive ELISA tests for brucellosis require that positive results should be confirmed by standard agglutination tests.[130]

Negative serology does not exclude the diagnosis in brucellosis, and using more than one test is recommended in probable cases.[123,131] In addition, *Brucella* antibodies can persist long after the patient's recovery, and thus it is not always possible to distinguish patients with active disease from those with past infection.[41,132] In these cases the IgG avidity test can be valuable because high IgG avidity would suggest immune memory, not a new-onset disease. On the other hand, patients with titers lower than the diagnostic threshold and low avidity would suggest primary infection.[133] However, the PCR assay seems satisfactory in symptomatic patients with negative serology, and it provides recognition of the disease as early as 10 days after inoculation, particularly during outbreaks or relapses.[6,134,135] Of note, standard serologic testing does not detect the rare human cases of *B. canis* because of antigenic differences.[136]

## TREATMENT

Antimicrobials with accumulation in phagocytes may be important for therapeutic success in brucellosis (Table 226.1).[137] Monotherapy is usually not recommended and is associated with frequent therapeutic failures or relapses.[7] However, the use of doxycycline or minocycline alone for 6 to 8 weeks can be considered in the absence of focal disease and may be cost-effective in countries with limited resources.[54] Currently, there are two recommended antibiotic combinations for the treatment of adults with uncomplicated brucellosis, both for a minimum of 6 weeks.[1,7,138] The first regimen is oral doxycycline 100 mg twice daily for 6 weeks plus intramuscular streptomycin 1 g once daily for 2 to 3 weeks. Seven days of gentamicin (5 mg/kg) combined with doxycycline appears to have similar efficacy to streptomycin plus doxycycline.[1,139,140] Although the use of aminoglycosides may produce ototoxicity and nephrotoxicity,[141] the adverse effects in due course of treatment are generally mild to moderate and rarely serious and requiring modification.[53] The second regimen is doxycycline added to 600 to 900 mg oral rifampin (15 mg/kg) once daily for 6 weeks. Although streptomycin combined with doxycycline is shown to be superior to doxycycline plus rifampin in terms of relapse and therapeutic failure,[53,142] there are clinicians who favor the use of the second combination because it is rather cheap, easy to use, and available in most countries.[1] Added to that, the use of doxycycline and an aminoglycoside showed more rapid normalization of liver aminotransferases in clinical brucellar hepatitis cases.[88]

Primary drug resistance to tetracyclines or aminoglycosides has long been known to be absent in *Brucella* isolates,[143,144] and relapses could not have been related to resistance.[143] Thus relapses usually responded well to a further course of the same antibiotics[7,143] However, decreased susceptibility or resistance to rifampin is described in some recent studies.[145–148] In addition, when patients are infected with *Brucella* RB51, a strain of *B. abortus* that is used to vaccinate cattle, rifampin should not be used because this particular strain is resistant to it.[149] Current data disclose that rifampicin combination therapy increased the risk of overall failure and relapse compared with the streptomycin-containing regimen.[149,150] Hence doxycycline combined with an aminoglycoside seems to be the first-line therapy in brucellosis. Although quinolones combined with rifampin yielded results similar to doxycycline plus rifampin,[150] quinolones are recommended only as second-line regimens, used with either rifampin or doxycycline.[1,7] They may be considered in case of antibiotic resistance or drug toxicity. Trimethoprim-sulfamethoxazole (TMP-SMX) is also associated with a high rate of relapses and should always be combined with another agent.[7] TMP-SMX is of particular importance in children because doxycycline should be avoided in children younger than 8 years due to dental complications. The relapse rates appear to be less than half with a three-drug regimen of aminoglycoside, doxycycline, and rifampin compared with doxycycline plus rifampin.[151,152] Three-drug combinations, including TMP-SMX (double-strength tablet twice daily) or an aminoglycoside, can be used to treat difficult cases, such as nonresponding or relapsing disease and focal brucellosis. There is a preliminary report on the efficacy of adding hydroxychloroquine to the standard regimen.[153]

Therapeutic strategies in focal brucellosis should be individualized according to the site involved. In addition, longer therapeutic courses are usually necessary in these cases. As an example, extension of infection through paravertebral and epidural spaces, psoas muscle, or nerve roots in due course of spondylodiskitis requires a longer treatment for a mean of 4 to 5 months.[55] In a series of 32 cases of *Brucella* spondylitis the median duration of treatment was 6 months.[63] In another report *Brucella* spondylitis appear to respond to doxycycline (for 3 months) plus streptomycin for 2 to 3 weeks.[154] The optimal treatment of brucellar meningitis seems to be 1 month of intravenous ceftriaxone 4 g daily in two divided doses combined with doxycycline and rifampin for 4 to 5 months. This regimen provides a low incidence of neurologic complications, requires shorter therapy, and is more successful when the negative outcome (relapse plus therapeutic failure) is considered.[52] The antibiotics should be continued depending on the clinical response[7] and until CSF parameters return to normal.[52] Although data are lacking on the optimal treatment of endocarditis, combinations including aminoglycosides seem rational choices.[75] Surgery contributes to survival in select endocarditis patients, such as those with congestive heart failure, valvular regurgitation, uncontrolled infection with antibiotics, embolic complications, or large vegetations.[75,155] Recurrent infection, unstable spinal column, marked kyphosis, uncontrollable pain related to spinal involvement, and focal abscesses are other probable indications for surgery in brucellosis.[52,56,156]

Early diagnosis and adequate treatment of brucellosis during pregnancy improves maternal and fetal outcomes.[157] Rifampin 900 mg once daily for at least 6 weeks is the treatment of choice during pregnancy. Adding TMP-SMX can be considered, but this option should probably be avoided preceding the 13th week and after the 36th week of gestation because of concern about teratogenicity and kernicterus.[54]

**TABLE 226.1 Treatment of Brucellosis**

| CLINICAL FORM | PREFERRED REGIMEN | ALTERNATIVE REGIMEN |
|---|---|---|
| Uncomplicated | Doxycycline, 100 mg PO twice daily for at least 6 wk<br>*Plus*<br>Streptomycin, 1 g IM daily for 2–3 wk | Doxycycline, 100 mg PO twice daily for at least 6 wk<br>*plus*<br>Gentamicin, 5 mg/kg IM daily for 1 wk<br>*or*<br>Doxycycline, 100 mg PO twice daily for at least 6 wk<br>*plus*<br>Rifampin, 600–900 mg (15 mg/kg) once daily for at least 6 wk |
| Neurobrucellosis | Ceftriaxone, 2 g IV twice daily for at least 1 mo<br>*Plus*<br>Doxycycline, 100 mg PO twice daily for 4–5 mo<br>*Plus*<br>Rifampin, 600–900 mg (15 mg/kg) PO once daily for 4–5 mo | Trimethoprim-sulfamethoxazole, 160/800 mg PO twice daily for 5–6 mo<br>*plus*<br>Doxycycline, 100 mg PO twice daily for 5–6 mo<br>*plus*<br>Rifampin, 600–900 mg (15 mg/kg) PO once daily for 5–6 mo |

[a]See text for additional information.
*IM,* Intramuscularly; *IV,* intravenously; *PO,* orally.

## PREVENTION

Although secure food supplies and safe laboratories are among the significant factors in prevention, controlling the disease is only possible by its eradication from domestic animals. Vaccination of domestic cattle, sheep, and goats with *B. abortus* S19, RB51, and 104M, and *B. melitensis* REV1 and vaccines plays a crucial role in control of brucellosis. No vaccine has yet been developed for *B. suis* or *B. canis*.[7,158] Nor is there a vaccine for humans.

There are limited data on prophylactic antibiotic usage subsequent to exposure to *Brucella*. In their review of laboratory-acquired brucellosis, Traxler and colleagues[159] reported that none of the 34 persons who received postexposure prophylaxis developed infection. Twenty-five of 34 received rifampin plus doxycycline. Brucellosis due to erroneous inoculation of animal attenuated-live vaccines to humans has been reported.[7] In cases of a high-risk contact, such as a laboratory exposure or accidental vaccine inoculation, rifampin plus doxycycline is recommended at standard dosages for 3 weeks. If there is a contraindication to doxycycline use, TMP-SMX prophylaxis is suggested.[160]

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*The complete reference list is available online at Expert Consult.*


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# 227 *Francisella tularensis* (Tularemia)

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## SHORT VIEW SUMMARY

### Definition
- Tularemia is the zoonotic disease caused by *Francisella tularensis.*

### Epidemiology
- Tularemia is widely distributed but is primarily a disease of the Northern Hemisphere.
- In the United States, the majority of cases reported in 2016 occurred in Arkansas, Colorado, Kansas, Missouri, Nebraska, Oklahoma, South Dakota, and Wyoming.
- Tularemia peaks in the late spring and summer in the United States.
- Lagomorphs and rodents are important animal reservoirs.
- Significant transmission vectors include ticks and biting flies in the United States and mosquitoes in Europe.
- Other routes of transmission include aerosol droplets, contaminated mud or water, skin contact with infected carcasses, and animal bites.
- Occupations that have an increased risk for tularemia include laboratory worker, farmer, landscaper, veterinarian, sheep worker, hunter or trapper, and cook or meat handler. Clinicians should warn laboratory personnel if tularemia is suspected.
- Tularemia spread by aerosol is a potential bioterrorist weapon.

### Microbiology
- *Francisella* organisms are small, aerobic, pleomorphic gram-negative coccobacilli.
- *F. tularensis* requires cysteine or cystine (or another sulfhydryl source) for growth and therefore will not grow well on most conventional solid media.
- The three *Francisella* species that are potential human pathogens are *F. tularensis, F. philomiragia,* and *F. hispaniensis* (see Table 227.1). However, only *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* are relatively common.
- Infections caused by *F. tularensis* subsp. *tularensis* are generally more severe than those caused by *F. tularensis* subsp. *holarctica.*
- The infectious dose in humans is 10 to 50 organisms when injected intradermally or when inhaled.
- *F. tularensis* is a facultative intracellular pathogen that survives within host macrophages by impairing phagosome-lysosome fusion, and it is capable of suppressing or avoiding many other humoral and cellular host defenses.
- Recovery from infection depends on the development of host cell–mediated immunity.

### Clinical Manifestations
- Tularemia starts abruptly with fever, chills, headache, anorexia, and fatigue after an average incubation period of 3 to 5 days.
- There are six significant illness patterns: ulceroglandular (see Fig. 227.3), glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic (see Fig. 227.5); pneumonic and typhoidal tularemia are expected to be the primary forms resulting from a bioterrorism event.
- Secondary rashes are relatively common.
- The most common complications of tularemia are lymph node suppuration and persistent debility.

### Diagnosis
- Diagnosis rests on clinical suspicion, and because of its potential danger, laboratory personnel should be notified whenever tularemia is suspected.
- *F. tularensis* is a Tier 1 select agent, and its possession and shipment are tightly restricted.
- Routine cultures and smears are often negative, and the diagnosis is frequently confirmed serologically; polymerase chain reaction tests are not widely available but offer a rapid diagnosis.

### Therapy
- Streptomycin and gentamicin are the drugs of choice for all forms of severe tularemia except meningitis (see Table 227.2).
- Selected adults and children with mild to moderate disease may be treated with oral agents (see Table 227.2).
- Surgical intervention is typically limited to drainage of suppurated nodes or of empyemas.

### Prevention
- The best prevention is avoiding exposure to the organism; a vaccine for tularemia is currently unavailable in the United States.
- Patients with tularemia do not need special isolation because person-to-person spread does not occur. Standard precautions for handling contaminated secretions are adequate.
- Antibiotic prophylaxis after potential exposures of unknown risk, such as tick bites, is not recommended.
- Either ciprofloxacin, 500 mg, or doxycycline, 100 mg, either given orally twice daily for 14 days is recommended for adults with suspected or proven high-risk exposure to *F. tularensis.*
- Observation without prophylactic antibiotics is appropriate for exposed children (except during a bioterrorist event) and adults with lower risk exposures.

*Francisella tularensis* is a gram-negative pathogen primarily of animals and occasionally of humans. The disease is now recognized as tularemia in most areas of the world, but it has been called rabbit fever, deer fly fever, Francis disease, and market men's disease in the United States; wild hare disease (yato-byo) and Ohara disease in Japan; and water-rat trappers' disease in Russia. Tularemia continues to be responsible for significant morbidity and mortality, despite the availability of numerous antibiotics active against the organism.[1]

*F. tularensis* infections have become a broader public health issue centering on concerns regarding potential military or terrorist uses in biological warfare. In 2000, tularemia returned to the list of reportable diseases in the United States after exclusion in 1995. With heightened surveillance and recognition of ongoing cases, attention is often quickly focused upon outbreaks of natural *F. tularensis* infection that do occur regularly throughout the world.

## HISTORY

Tularemia has been so intimately linked to investigators in the United States that it has been referred to as "the first American disease" or an "American achievement."[2,3] However, its history is notable for important contributions from other areas of the world, including Japan and the former Soviet Union. Hare-associated illness compatible with tularemia

has been known in Japan since 1818, and perhaps the earliest written description of a patient with unmistakable tularemia was provided by Soken Honma in 1837.[4]

Credit for recognizing the clinical syndromes and identifying the organism belongs to American workers. In 1911, while evaluating possible plague outbreaks after the San Francisco earthquake of 1906, McCoy described a plague-like illness then prevalent in ground squirrels. With Chapin, he successfully cultured the causative bacteria in 1912.[5] They named it *Bacterium tularense* because their discovery occurred in Tulare County, California. Although the cause was unknown at the time, Pearse first clinically described human tularemia following contact with biting flies in Utah in 1911, which he termed *deer fly fever*.[6] In 1914, the first case with bacteriologic confirmation was reported in a patient from Ohio with an ocular infection.[7,8] In 1919, Dr. Edward Francis linked the deer fly as the vector of disease while recovering an unusual organism in the blood of febrile patients from Millard County, Utah, who also had suppurative lymphadenopathy[9]; he subsequently coined the human disease tularemia to emphasize the frequently accompanying bacteremia.[10] By 1928, working for the U.S. Public Health Service, Francis had firmly established the actual cause of deer fly fever as *B. tularense,* more than a decade after the organism was found in squirrels.[11] Francis also contributed to improving cultivation methods for *B. tularense,* making a serologic diagnosis, identifying tick and other reservoirs as other vectors for transmission, clarifying the clinical syndromes associated with tularemia, and emphasizing the risk to laboratory workers and consumers from infected sources. In 1974, for this lifetime of achievements, the genus in which the organism is classified was renamed *Francisella* in his honor.

In Japan, Ohara had described a rabbit-associated febrile disease in 1926.[12] He subsequently, transmitted the illness to his wife by rubbing rabbit hearts over her hand, and he recovered an organism from her lymph nodes[13]; Francis and Moore later showed that this Japanese organism was identical to *B. tularense.*[14] In 1926, tularemia was recognized in Astrakhan, Russia, and scattered outbreaks of serious human disease occurred throughout the country during the subsequent decades.[15] Scientists in the former Soviet Union also have intensively studied the disease and its causative organism.

## MICROBIOLOGY

*Francisella* organisms are small, aerobic, catalase-positive, pleomorphic, gram-negative coccobacilli that are nonmotile and non–spore forming. They are more uniformly rod-shaped during logarithmic growth, during which they tend to exhibit bipolar staining with Gram or Giemsa methods; this staining pattern accentuates a coccoidal appearance, measuring 0.2 × 0.2 µm. The *F. tularensis* cell wall has an unusually high level of fatty acids with a unique profile. Wild-type strains possess an electron-transparent, lipid-rich capsule. Loss of the capsule may lead to loss of serum resistance and virulence, but may not diminish viability or survival within neutrophils.[13] The capsule had traditionally been thought to be directly neither toxic nor immunogenic, though recent studies have suggested that bacterial lipoproteins may interfere with neutrophil apoptosis by way of Toll-like receptor (TLR) mechanisms, and therefore foster local tissue destruction.[16]

*Francisella* spp. belong to the Gammaproteobacteria class in the family Francisellaceae and include the human pathogens *F. tularensis, F. philomiragia,* and *F. hispaniensis;* the fish pathogens *F. noatunensis* subsp. *orientalis* and *F. halioticida;* and environmental isolates of *F. guangzhouensis.* Other unnamed *Francisella* spp. also have been rare human pathogens. The first tick endosymbiont isolated, *Wolbachia persica,* has been reclassified as part of the genus *Francisella* based on sequencing with the new name *Francisella persica* comb. nov.[17] Molecular techniques have also defined clinical and environmental isolates, expanding by four new species designations within the genus: *Francisella opportunistica* sp. nov., *Francisella salina* sp. nov. *Francisella uliginis* sp. nov., and *Francisella frigiditurris* sp. nov.[18]

*Francisella* traditionally has been categorized by growth characteristics, biochemical reactions, and virulence properties (Table 227.1). The three *Francisella* spp. potentially pathogenic for humans are *F. tularensis, F. philomiragia,* and *F. hispaniensis.*[19–21] Although all three have been associated with human disease, only the *tularensis* and *holarctica* subspecies of *F. tularensis* are relatively common. *F. tularensis* subsp. *tularensis* (*nearctica*), also referred to as type A, is found almost exclusively in North America and is the most virulent species. Although previously thought to be restricted to North America, *F. tularensis* subsp. *tularensis* has been isolated in Europe and its identity verified using molecular techniques.[22]

*F. tularensis* subsp. *holarctica* (*palaearctica*), also referred to as type B, is found predominantly in Asia, Australia, and Europe but also in North America; it is less virulent in humans and of low virulence in rabbits. The *F. tularensis* live vaccine strains are derived from *F. tularensis* subsp. *holarctica* (see later under "Vaccination"). *F. tularensis* subsp. *novicida* is of low virulence and only rarely causes human infection, mainly in patients who are immunosuppressed.[23] Although previously classified as a separate species, its reclassification as a subspecies of *F. tularensis* was proposed based on a high degree of genetic similarity to *F. tularensis.*[19,24] However, this has been controversial, and some authorities feel it should remain a separate *Francisella* species based on phenotypic as well as genomic differences.[20,23] Strains isolated in Japan have

**TABLE 227.1 Characterization of *Francisella* Species**

| | *F. TULARENSIS* SUBSPECIES[a] | | | | |
|---|---|---|---|---|---|
| **FEATURE** | ***tularensis*** | ***holarctica*** | ***novicida*** | ***F. PHILOMIRAGIA*** | ***F. HISPANIENSIS*** |
| Cysteine growth requirement | + | + | – | – | – |
| Growth in broth plus 6% NaCl | – | – | +[b] | +[c] | NA |
| Motility | – | – | – | – | NA |
| Oxidase | – | – | – | +[c] | + |
| Nitrate reduction | – | – | – | – | NA |
| Acid from: | | | | | |
| Glucose | +[b] | +[b] | +[b] | +[b] | + |
| Glycerol | + | – | + | + | + |
| Gelatin hydrolysis | – | – | – | [b] | NA |
| Relative virulence | | | | | |
| Humans | High | Intermediate | Low | Low | Low |
| Rabbits | High | Low | Low | NA | NA |

[a]The fourth *F. tularensis* subspecies, *F. tularensis* subsp. *mediasiatica,* and the *japonica* biovar of *F. tularensis* subsp. *holarctica* are described in the text.
[b]Variable or delayed.
[c]Using Kovacs test; negative using cytochrome oxidase test.
*NA,* Not available; *NaCl,* sodium chloride.
*Data from references 19 through 21 and reference 33.*

been designated *F. tularensis* subsp. *holarctica* biovar *japonica,* but their differentiation from other *F. tularensis* subsp. *holarctica* strains by traditional phenotypic testing was not possible.[21]

A fourth subspecies, *F. tularensis* subsp. *mediasiatica,* has been found only in sparsely settled regions in Central Asia and Russia.[20,25,26] An absence of human infection reports suggests that if capable of infecting, it would have low virulence.

*F. philomiragia* was previously called *Yersinia philomiragia.* Reclassified because it shared the unique fatty acid profile of the *Francisella* spp. and substantial DNA relatedness to this genus, it does have some unique biochemical features (see Table 227.1) and DNA hybridization patterns that distinguish it from *F. tularensis. F. philomiragia* is of low pathogenicity for humans, although it has been isolated from muskrats and a dog.[20,27,28] It is naturally found in brackish and salt water, and in liquid media it may form biofilms and interact with free-living amebas.[29] All strains originally tested produced β-lactamase and were most susceptible to aminoglycosides, cefoxitin, cefotaxime, fluoroquinolones, tetracycline, and chloramphenicol. However, an infection caused by *F. philomiragia* resistant to cefazolin and cefotaxime has been reported.[30] The most active agents against six strains in a standardized broth microdilution assay were the aminoglycosides and the fluoroquinolones.[31] The complete genome of *F. philomiragia* has been sequenced, and it encodes a class A carbapenemase.[28,32] *F. hispaniensis* was isolated from the blood and urine of a septic patient in Spain 1 month after undergoing lithotripsy.[33] The organism was classified as a separate species of *Francisella* based on its phenotypic and genotypic properties (see Table 227.1).[19]

The taxonomy of *Francisella* has been complicated because biochemical reactions may be variable, weak, or delayed and also in part because of the different terms given to organisms isolated in different areas of the world. Classification of *Francisella* has been advanced by the sequencing of the whole genome from representative strains of *F. tularensis* subsp. *tularensis* (strain Schu S4), *F. tularensis* subsp. *holarctica* (including the live-attenuated vaccine strain), and *F. tularensis* subsp. *novicida,* along with the application of various molecular typing methods.[22,24,34–37] These have included 16S ribosomal DNA gene sequence analysis, microarray analysis of the whole genome, multiple-locus variable-number tandem repeat analysis, canonical insertion-deletion markers, and single nucleotide polymorphism analysis.[22,36] Reports using these techniques have supported the currently accepted taxonomy as outlined previously and in Table 227.1, demonstrated the utility of these methods for species and subspecies typing, and identified *F. tularensis* subsp. *holarctica* biovar *japonica* as a distinct group.[37,38] Molecular typing has identified three genotypes of *F. tularensis* subsp. *tularensis* (designated A1a, A1b, and A2) and at least 10 genotypes of *F. tularensis* subsp. *holarctica.*[39] Genomic analyses have indicated that there was a common *F. tularensis* ancestor for clonal subspecies evolution, that *F. tularensis* subsp. *novicida* is the oldest, and that *F. tularensis* subsp. *tularensis* appeared before *F. tularensis* subsp. *holarctica,* which is the youngest.[40]

*F. tularensis* requires cysteine or cystine (or another sulfhydryl source) for growth and therefore will not grow using most routine solid media or gram-negative selective media such as MacConkey or eosin methylene blue agars. It may be recovered with the use of glucose cysteine blood agar, thioglycollate broth, chocolate agar suitable for gonococcal growth, modified Thayer-Martin medium, buffered charcoal-yeast agar, or cysteine heart agar with 9% chocolatized sheep blood.[20,21] Blood agar may support the growth of some *F. tularensis* isolates on initial plating but not on subpassage.[41] Some strains of *F. tularensis* lack an overt requirement for cysteine or enriched medium for growth, and clinically significant strains of *Francisella* have been reported that do not show the expected fastidious growth characteristics.[21] A novel brain heart infusion supplemented with 2% Vitox, 10% Fildes, and 1% histidine (BVFH) broth has been described as facilitating the early growth of *F. tularensis* compared to customary media formulations.[42]

*Francisella* should be suspected, however, whenever a slowly growing, small, and poorly staining gram-negative coccobacillus is isolated on chocolate agar and grows poorly or not at all on blood agar. Visible colonies take 2 to 5 days to appear. Incubation at 35°C is optimal, with or without an atmosphere of increased carbon dioxide. The recovery of *F. tularensis* from contaminated specimens may be facilitated by plating onto antibiotic-containing media known to support the growth of *F. tularensis.*[20] Virtually all *F. tularensis* strains are positive for β-lactamase, and a weak class A β-lactamase native to *F. tularensis* has been identified.[43]

Differentiation of *Francisella* from other bacteria can be accomplished using direct fluorescent antibody (DFA) staining, slide agglutination, polymerase chain reaction (PCR) assay, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) sequencing, or cellular fatty acid composition analysis.[20,44] Antisera can distinguish between *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *novicida* but not between *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica;* strains within subspecies do not have antigenic differences detectable by antisera.

It is important to note that automated laboratory identification systems should not be used for the identification of *Francisella* because they may generate aerosols and commonly misidentify *F. tularensis* as *Haemophilus* or *Aggregatibacter* species.[41] Whenever *F. tularensis* is suspected, the state public health laboratory should be notified immediately, and any local microbiologic testing should be done using a biological safety cabinet and following Biosafety Level 3 procedures.[41] Isolates that cannot be excluded as *F. tularensis* should be sent to a reference laboratory in the Laboratory Response Network, typically the state public health laboratory. Federal regulations rigorously control possession and transport of *F. tularensis* and must be followed when sending an isolate to a referral laboratory for identification.

## Virulence

*F. tularensis* produces no known exotoxins, but many components of the *Francisella* envelope contribute to its pathogenicity, though questions remain about many putative virulence factors.[33,44,45] The lipopolysaccharide (LPS) from the live vaccine strain of *F. tularensis* possesses at least 1000-fold less endotoxin activity than the LPS from *Escherichia coli.*[46] This is in part because *F. tularensis* LPS has a different structure composed primarily of the O antigen that makes the capsule and lacks many of the traditional LPS incitatory endotoxin components. Unique features of this LPS include reduced acyl chains, longer fatty acid chains, and less lipid A phosphorylation.[47] The capsule can also block both immunoglobulin M (IgM) and complement binding with C3.[48] Many of these features are believed to contribute both to immune activation and evasion.

Unlike other gram-negative bacteria, it is less recognized by TLR4 and does not bind to LPS-binding protein.[33,45,49] However, purified *F. tularensis* LPS can stimulate human B cells and, when present together with the *F. tularensis* heat shock protein GroEL, can synergistically activate human macrophages.[50,51] The O-antigen side chains from *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* are identical but differ from that of *F. tularensis* subsp. *novicida.*[49]

Pili have been visualized on the surface of *F. tularensis* subsp. *holarctica* live vaccine strain and *F. tularensis* subsp. *novicida.* Genome sequencing has detected genes homologous to those from other bacteria that encode type IV pili in *F. tularensis* subspp. *tularensis, holarctica,* and *novicida;* and type IV pili assembly contributes to virulence in murine models.[52] Type IV pili and other bacterial surface proteins mediate adherence of *F. tularensis* to host cells, and a type IV pilus subunit of *F. tularensis* subsp. *holarctica* is a ligand for intercellular adhesion molecule 1 on rat vascular epithelial cells.[53,54]

Host immune responses are directed against numerous cell wall antigens, including membrane proteins, LPS, and carbohydrates, but previously it was not possible to identify dominant antigens. Proteomic analysis using serum from donors who have had tularemia or who have been immunized with the live vaccine strain has identified a large number of *F. tularensis* protein antigens and found possible immunodominant antigens.[55] These include many cytoplasmic and membrane proteins, as well as hypothetical proteins of unknown localization. Further proteomic analyses will be helpful to refine future diagnostic tests for tularemia and the construction of effective vaccines.[56,57]

Phenotypic correlates of virulence have included the capsule and citrulline ureidase activity. Wild encapsulated strains of *F. tularensis* are resistant to the bactericidal activity of normal serum, a capsule-deficient mutant of the live vaccine strain also is serum resistant, and the capsule-deficient mutant is less virulent in mice than its wild-type

parent.[58] The contribution of citrulline ureidase to virulence is unclear. There are pathogenic isolates that do not possess this activity. Plasmids have been found in isolates of *F. tularensis* subsp. *holarctica, F. tularensis* subsp. *novicida,* and *F. philomiragia,* but have not been found in the more virulent *F. tularensis* subsp. *tularensis* and thus are not essential for virulence. Several acid phosphatases (Acp) are present in *Francisella,* and they are important for its survival within macrophages. AcpA can inhibit the respiratory burst of neutrophils, expression of AcpA and histidine acid phosphatase (Hap) is induced by growth within macrophages, and AcpA is secreted into the cytosol of macrophages.[59,60] Although deletion of *acpA, acpB, acpC,* and *hap* from *F. tularensis* subsp. *novicida* results in a mutant strain that is impaired in its ability to survive within macrophages and to escape from the phagosome, deletion of *acpA* and the combination of *acpA, acpB,* and *acpC* deletions do not alter the virulence of *F. tularensis* type A strain Schu S4.[60,61] Mechanisms for iron acquisition are present in *F. tularensis,* and their disruption attenuates in vitro intracellular growth and organism virulence in a mouse model.[62]

Genomic and proteomic analyses have the potential to more specifically identify virulence factors in *F. tularensis.*[57,63] *F. tularensis* contains a cluster of genes involved in virulence, the *Francisella* pathogenicity island (FPI). There are two copies of the FPI in *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* and one copy in *F. tularensis* subsp. *novicida;* although largely identical, the FPI from *F. tularensis* subsp. *tularensis* differs from that in the other subspecies.[64,65] The FPI contains 19 genes required for murine virulence and intracellular growth in macrophages, including *iglABCD* and *pdpABCD.* FPI regulation involves at least six proteins that regulate many FPI genes, and some also regulate non-FPI genes.[66,67] Within macrophage cytosol the organism depends on host amino acids as the gluconeogenic sources for metabolism.[68]

Intracellular growth, iron limitation, and oxidative stress also regulate expression of these genes.[66,67] Disruption of many of these genes has been shown to impair the organism's survival within macrophages and significantly reduces virulence in animal models. Several proteins encoded by the FPI that have been linked to intracellular growth and virulence likely form part of a type VI secretion system.[66,69,70] Many other genes can also be identified that probably play a role in *Francisella* virulence.[71]

### Other *Francisella* Species

A number of newly appreciated *Francisella*-like organisms have been identified by molecular testing or by culture.[72] The same novel *Francisella* species was isolated from blood in a patient with pneumonia, and from cerebrospinal fluid in a different patient with meningitis.[73] A survey of soil throughout Houston, Texas, using PCR amplification of DNA extracts identified multiple *Francisella*-like organisms that were distinct from known species of *F. tularensis* and *F. philomiragia.*[74] *Francisella*-like organisms have been identified as a cause of granulomatous diseases in several fish species. They have been cultured, and 16S ribosomal RNA gene sequencing has shown them to be related most closely to *F. philomiragia.* However, they are currently believed to represent new *Francisella* species: *F. noatunensis* comb. nov. and *F. noatunensis* subsp. *orientalis.*[75] *F. halioticida* has caused mass mortality among farmed abalone in Japan.[76]

Several endosymbiotic bacteria of ticks have been classified within the Francisellaceae family on the basis of 16S ribosomal gene sequence data. These include *Wolbachia* (*Francisella*) *persica,* an endosymbiont found in Rocky Mountain wood ticks (termed *Dermacentor andersoni* symbiont), and symbiont B of the soft tick *Ornithodoros moubata.*[21] Similar organisms can be found in other hard and soft ticks, suggesting that they may be more widely distributed than previously observed. A *Francisella*-like organism has been found as an endosymbiont of a *Paramecium* species, and a related organism has been isolated from the waters off Hong Kong.[77,78]

## EPIDEMIOLOGY

### Distribution

Tularemia is widely distributed, but it is primarily a disease of the Northern Hemisphere, and is most common between 30° and 71° north latitudes. It has been remarkably absent from the United Kingdom, Ireland, and South America. A single South Sudan case of *F. tularensis* bacteremia is the only report to date from Africa.[79] Reports of increased incidence in Europe, including Austria, Norway, Sweden, and Spain, have been ascribed to climate change.[80,81,82,83] Whipp and coworkers reported the first case of tularemia from Australia in 2003, caused by an *F. tularensis* subsp. *novicida*–like organism.[84]

Tularemia was very common in the United States before World War II. However, its incidence has declined steadily since the 1950s and has remained at fewer than 0.15 cases per 100,000 population since 1965.[85,86] Because of its stable and low incidence, tularemia was removed from the list of nationally reportable diseases in 1995 but was added back in 2000 in part because of the concern about its use for bioterrorism. Since 2001, the case rates have been 0.05 per 100,000 population or less.[86] Missouri, Arkansas, Oklahoma, Massachusetts, South Dakota, and Kansas reported 59% of the total US cases from 2001 through 2010, with Dukes County in Massachusetts and Buffalo and Shannon Counties in South Dakota having the highest annual incidence rates (Fig. 227.1).[85] Groups with high incidence rates include Native Americans and Alaskan Natives. Subtle changes have occurred in the geographic distribution of cases in the United States between 1965 and 1999.[87] The southern border of tularemia has shifted northward so that fewer cases have been reported from south central states in recent years. The number of reported cases in Colorado, Nebraska, South Dakota, and Wyoming dramatically increased in 2015, and this is consistent with the predicted effects of climate change on tularemia's geographic distribution.[87,88]

Environmental data also have been used to develop a model to predict risk for tularemia in specific geographic regions.[89] Detailed investigation using molecular typing found that specific *F. tularensis* strains may be geographically limited, with strain variation yielding differences in virulence and disease severity.[39] *F. tularensis* subsp. *tularensis* group A1 was found primarily in the central United States, including the states reporting the highest numbers of tularemia cases, as well as in California. *F. tularensis* subsp. *tularensis* group A2 was found primarily in the western United States at higher elevations than the A1 isolates.[90] The distribution of group A1 isolates correlated with the distribution of *Amblyomma americanum* and *Dermacentor variabilis* ticks and the eastern cottontail rabbit (*Sylvilagus floridanus*).[91] The distribution of group A2 isolates correlated with the distribution of *D. andersoni* and the deer fly *Chrysops discalis* and also with the mountain cottontail rabbit (*Sylvilagus nuttalii*). There were only a few isolates of *F. tularensis* subsp. *novicida,* but most were seen in the southeastern United States.[90] In contrast, isolates of *F. tularensis* subsp. *holarctica* were widely dispersed.[90] Testing of additional US isolates has identified two A1 genotypes, A1a and A1b. Genotype A1b isolates were significantly more likely

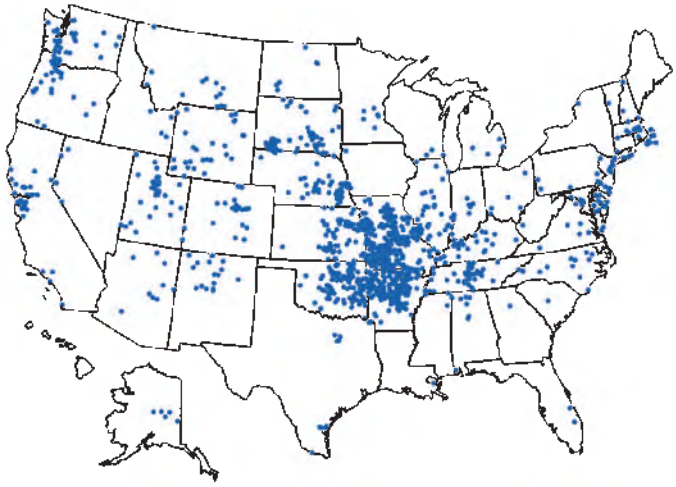

**FIG. 227.1 Total number of tularemia cases reported in the United States, 2001 to 2010.** One dot is placed randomly within county of residence for each reported case. Cases were reported from 47 states. *(From Centers for Disease Control and Prevention. Tularemia—United States, 2001–2010.* MMWR Morb Mortal Wkly Rep. *2013;62:963–966.)*

from invasive infections associated with significantly higher mortality in humans than A1a and A2 isolates.[92]

Geographic restriction of specific *F. tularensis* strains has been increasingly identified as newer molecular typing methods have been more widely applied.[39,93] Animal and human outbreaks within a single region may involve multiple *F. tularensis* types.[94,95]

Cases of *F. tularensis* subsp. *novicida* are uncommon. Almost avirulent in normal human hosts, these infections do not resemble customary tularemia, often are bacteremic, and appear to usually occur in patients with immunosuppression or significant comorbidities.[23,96] *F. tularensis* subsp. *mediasiatica* is among the least studied, found initially only in sparsely populated regions of Central Asia but more recently described in the Altai region of Russia.[25]

## Incidence

Historically, the incidence of tularemia in the United States has been most frequent in June through August as well as December. The summer peak corresponds to a higher number of tick-acquired cases, whereas the smaller peak in winter reflects an increased number of hunting-associated cases. However, in recent years the number of reported infections only peaks in the late spring and summer.[97] A review of 316 available *F. tularensis* human isolates from 39 states collected between 1964 and 2004 found that 208 (66%) were *F. tularensis* subsp. *tularensis* and 108 (34%) were *F. tularensis* subsp. *holarctica.*[98] Most isolates of both subspecies occurred between May and September; a very small increase in numbers in December was noted only for *F. tularensis* subsp. *tularensis* and not for *F. tularensis* subsp. *holarctica,* and only *F. tularensis* subsp. *tularensis* was associated with lagomorph exposure.[98] Males account for the majority of cases, perhaps because of greater exposure opportunities, though tularemia can occur in individuals of any age (Fig. 227.2). In the United States, the number of reported cases are highest in children ages 5 to 9 years and in adult men ages 55 to 59 years (see Fig. 227.2).[5,85,86,99] Occupations that have been associated with an increased risk for tularemia are laboratory worker, farmer, landscaper, veterinarian, sheep worker, hunter or trapper, cook, and meat handler.

In Europe, where most cases are from type B strains, the source and clinical presentation vary by country. Ticks and other insect bites predominate, causing ulceroglandular and glandular tularemia.[83] Oropharyngeal tularemia has caused outbreaks in places with unsafe food and water,[100–102] and during wartime, such as in Kosovo.[103] Pneumonic and typhoidal tularemia are uncommon in Europe.[82]

## Transmission

*F. tularensis* is capable of infecting hundreds of different vertebrates and invertebrates, but no more than a dozen mammalian species are important to its ecology in any geographic region. These include lagomorphs, particularly *Sylvilagus* and *Lepus* spp., and rodents such as voles, squirrels, muskrats, and beavers in North America. In Eurasia, infected animals include voles, hamsters, mice, and hares. Transmission of *F. tularensis* to humans occurs most often through the bite of an insect or contact with contaminated animal products. Other routes of transmission include aerosol droplets, contact with contaminated water or mud, and animal bites. Inoculation from freshwater fishhook injury has been described.[104] Illness may occur in families or friends because of shared activities and exposures. Nonetheless, human-to-human spread does not occur.

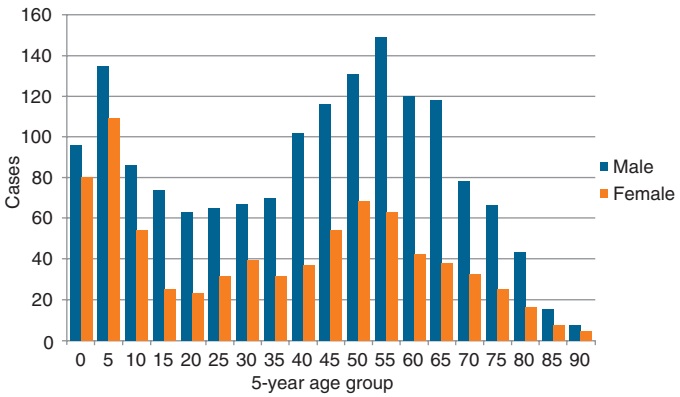


**FIG. 227.2 Tularemia cases by age group and sex—United States, 2001 to 2016.** Tularemia was more common in males, and occurred in persons of all ages but was most common in children. *(From Centers for Disease Control and Prevention. Tularemia: age and sex of reported cases, United States—2001–2016.* https://www.cdc.gov/tularemia/statistics/. *Accessed September 20, 2018.)*

Blood-feeding arthropods and flies are the most important vectors for tularemia in the United States. Ticks predominate in the Rocky Mountain states and eastward, whereas biting flies predominate in California, Nevada, and Utah. However, an increase in human tularemia noted in Wyoming between 2001 and 2003 was linked most often to transmission by biting flies and was associated with a simultaneous outbreak of tularemia in rabbits.[105] In contrast, mosquitoes are the most frequent insect vector in Sweden and Finland, and they are also important in the former Soviet Union. At least 13 species of ticks have been found to be naturally infected with *F. tularensis,* and transovarial passage may occur with some but not all vectors.[106] The dog tick (*D. variabilis*), wood tick (*D. andersoni*), and Lone Star tick (*A. americanum*) are commonly involved in North America. The organism may be present in tick saliva or feces inoculated either directly or indirectly into the bite wound. Tick transmission traditionally has been associated with *F. tularensis* subsp. *tularensis.* Several outbreaks of tick-borne tularemia have involved *F. tularensis* subsp. *holarctica,* although this organism is more often linked to water, rodents, and aquatic animals. Tularemia in children in the United States is now most often acquired through summertime tick exposures.

Animal contact is another important mode of acquiring tularemia. Skinning, dressing, and eating infected, undercooked animals, including rabbits, muskrats, beavers, squirrels, and birds, has transmitted tularemia, occasionally resulting in large outbreaks in hunters. Wild animals sold as pets are also potential vectors, as occurred in 2002 when infected prairie dogs were widely commercially distributed.[107] The first outbreak in Spain was associated with processing hare carcasses and hare meat, and was notable for a preponderance in women.[108] Airborne transmission has occurred from these activities, as well as from contact with water, contaminated dust, and hay.

An outbreak in 2000 of pneumonic tularemia on Martha's Vineyard was associated with mowing lawns and using a brush cutter, likely aerosolizing infected animal carcasses. Since then, cases of pneumonic tularemia have been regularly encountered on Martha's Vineyard during most summers.[109,110] An alternative explanation for pneumonic cases may rest with the finding of large numbers of *F. tularensis* bacteria within infected ticks, which may suggest that aerosolized ticks rather than animals may also contribute to cases.[91] In a highly endemic area, serologic evidence of *F. tularensis* infection among potential animal reservoirs on Martha's Vineyard found positive assays most frequently in raccoons (52.4%) and skunks (49.2%), although active infection in skunk and raccoon samples were not found by culture or PCR.[111] Domestic cats and other carnivorous animals may carry *F. tularensis* in the mouth or on claws after killing or feeding on infected prey, whether or not they are infected.[112] This is thought to be the mechanism by which occasionally domestic cats, and in one case a buzzard, transmit tularemia.[112,113] Similarly, a coyote bite caused a case of ulceroglandular tularemia in a child, and an infected dog was believed responsible for a case of pneumonic tularemia in Arizona.[114,115]

*F. tularensis* may survive for prolonged periods in water, mud, and animal carcasses even if frozen; however, cooking game meats thoroughly to the proper temperatures should minimize risk from ingestion. Nonetheless, contaminated food and water continue to be important environmental sources of tularemia. An outbreak of disease secondary to *F. novicida,* linked to contaminated ice, has been reported from a correctional institution in Louisiana.[96] Fresh crushed grapes were linked to an outbreak of oropharyngeal tularemia in Germany, probably contaminated with an infected rodent collected by a mechanical harvester.[116]

Disruptions of infrastructure caused by wars and natural disasters may also be significant contributing factors. In postwar Kosovo, an

epizootic increased rodent population contaminated ransacked homes and food with *F. tularensis* that led to foodborne and waterborne outbreaks among refugees returning to disrupted housing and sanitation.[117,118] Although tularemia was not reported in Kosovo before these outbreaks, it has remained endemic since.[117] The devastating effects of an earthquake in Turkey are believed to have resulted in water contamination and subsequent outbreaks of tularemia in the region.[119] During several outbreaks in Turkey, transmission from unchlorinated drinking water was confirmed using whole-genome sequencing.[120]

It has been demonstrated that *F. tularensis* subsp. *tularensis, F. tularensis* subsp. *novicida,* the live vaccine strain, and *F. philomiragia* will multiply intracellularly in *Acanthamoeba castellanii* and infect amebal cysts; *F. tularensis* subsp. *tularensis* is capable of surviving in amebal cysts for up to 21 days.[29,121,122] Such a relationship may prove relevant to natural aquatic reservoirs for *Francisella.*

## PATHOGENESIS

*F. tularensis* is a virulent organism for susceptible species, including the accidental human host. Although the organism is reported to penetrate intact skin, most investigators believe that penetration occurs through sites of skin disruption that may be inapparent if without known cause. Organisms may remain viable in carcasses or dust for up to 136 days, and culturable organisms have remained within infected frozen rabbit meat for more than 3 years.[123] The infectious dose in humans depends on the portal of entry: 10 to 50 organisms are sufficient when injected intradermally or when inhaled, though $10^6$ to $10^8$ organisms are required when ingested.[123] That low numbers of bacteria can cause infection through the skin, mucous membranes, and airways helps to explain in part the extreme risk that *F. tularensis* poses to laboratory workers. In general, *F. tularensis* subsp. *tularensis* causes more severe disease than either *F. tularensis* subsp. *holarctica* or *F. tularensis* subsp. *novicida.* The specific molecular reasons underlying these differences in virulence are unclear.

During the first 3 to 5 days after cutaneous inoculation, *F. tularensis* multiplies locally and produces a papule; ulceration may begin 2 to 4 days later.[4] Organisms spread from the site of entry to regional lymph nodes and may disseminate via a lymphohematogenous route to involve multiple organs. Bacteremia is probably common in this early phase, although it is only occasionally detected. Changes in draining regional lymph nodes appear after skin lesions develop.[4] An elegant description of the pathogenesis is offered by Geyer and colleagues.[124] Infection with *F. tularensis* is characterized by an acute inflammatory response that involves fibrin, neutrophils, macrophages, and T lymphocytes. Neutrophils and macrophages surround earlier inflammatory cells, stimulated by the initial inoculum, that haves become necrotic and degenerated. Eventually, lymphocytes, epithelioid cells, and giant cells migrate into the necrotic tissue. This extensive necrosis is noted in both lung tissue and lymph nodes. As the necrotic tissue expands, adjacent veins and arteries may thrombose. The organisms are usually present at the site of the necrotic tissue but are difficult to demonstrate on routine stains. Silver impregnation techniques (Steiner, Dieterle, and Warthin-Starry silver stains) enhance the visibility of the organisms, usually found in macrophages and epithelioid cells. Granulomas develop that occasionally may caseate; for this reason, specimens may be mistaken for tuberculosis. These changes can occur in any infected site and have been found at autopsy in lung, liver, spleen, lymph nodes, and bone marrow. Coalescence of necrotic foci may yield abscess formation. *F. tularensis* may remain viable in tissues for prolonged periods. The organism's intracellular residence within the liver and other sites may help to protect it from host defenses and permit early growth. *F. tularensis* is contained within granulomas in the livers of mice infected with the vaccine strain, and granuloma formation involves hepatic natural killer cells, interferon-γ (IFN-γ) production, and expression of inducible nitric oxide synthase.[125]

Most organisms recovered from blood in a mouse model of *F. tularensis* infection were free in plasma and not in leukocytes.[126] The organisms grew well in whole blood but not in plasma, implying a requirement for host cells. These observations suggest that circulating *F. tularensis* may be taken up by leukocytes where they survive and are protected from innate humoral defenses and potentially antibiotic effects, then escape into the plasma where they can begin a cycle of reinfection. *F. tularensis* also can infect erythrocytes and persist within them, where they are protected from killing by gentamicin; this has been proposed as one reason why tularemia may relapse.[127] Intraerythrocytic *F. tularensis* also are protected from the acidic pH within the tick gut, and this enhances tick colonization.[128]

*F. tularensis* is a facultative intracellular pathogen that is capable of growing within several different cell types, including macrophages, dendritic cells, hepatocytes, alveolar epithelial cells, and endothelial cells.[129,130] However, macrophages are the primary site of survival and replication. Entry into macrophages occurs through a unique mechanism involving engulfment by relatively capacious and asymmetrical pseudopod loops.[129] Direct transfer from infected cells to uninfected macrophages during a contact-dependent exchange of cytosolic material associated with trogocytosis also is possible.[131] Complement receptors, mannose receptors, class A scavenger receptors, Fcγ receptors, and surface nucleolin may be utilized for *F. tularensis* uptake, and the specific receptor pathway used may affect early suppression of innate defenses and intracellular trafficking of the organism.[129,132,133] Inside the macrophage, virulent *F. tularensis* strains impair maturation of the endosome, thus avoiding phagosome-lysosome fusion. Phagosomes containing virulent *F. tularensis* are transiently acidified by the vacuolar adenosine triphosphatase pump, and organisms quickly escape into the cytosol. Bacteria proliferate in the cytoplasm, induce apoptotic cell death, and are released to further spread the infection.[134]

### Immunity

Humoral immunity, directed against carbohydrate antigens, develops between the second and third weeks after infection, with the almost simultaneous appearance of IgM, immunoglobulin G (IgG), and immunoglobulin A (IgA) agglutinating antibodies. However, antibodies alone are insufficient to protect against virulent *F. tularensis* infection.[135] Opsonizing IgG and IgM antibodies are also produced, with the most efficient opsonization involving both immune serum and complement. *F. tularensis* binds activated complement C3 components to its bacterial surface, although LPS and cell wall prevent complement-mediated lysis of cells.[136] Nonetheless, the oxygen-dependent neutrophil killing of virulent wild-type strains is poor. Human neutrophils phagocytose opsonized *F. tularensis* organisms but do not kill them, as they escape into the neutrophil cytoplasm. Survival of *F. tularensis* within neutrophils is associated with inhibition of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by multiple mechanisms, failure of the respiratory burst, and impaired neutrophil responsiveness to other activating stimuli.[137] Neutrophil apoptosis is inhibited by *F. tularensis,* and survival of infected neutrophils is prolonged.[138]

Complete recovery from tularemia requires cell-mediated immunity, demonstrable approximately 1 week earlier than antibody responses and directed against protein antigens. This cell-mediated immunity is αβ T-cell dependent but may involve either $CD4^+$ or $CD8^+$ T cells. Attempts are underway to define better the critical molecular determinants that induce protective immunity. Current understanding of tularemia immunopathogenesis has largely derived from mice infected with *F. tularensis* subsp. *novicida* or other strains less virulent for humans. It is unknown how this relates to human tularemia caused by more virulent organisms. Furthermore, different routes of infection and different mouse strains may elicit dissimilar murine immune responses.[139] IFN-γ and tumor necrosis factor-α (TNF-α) activate macrophages to kill *F. tularensis* through the production of nitric oxide and other reactive nitrogen products, although alveolar macrophages may use other mechanisms to inhibit the organism.[140] Murine and human IFN-γ–activated macrophages also may inhibit growth of the virulent type A strain Schu S4 by mechanisms independent of reactive nitrogen and oxygen species.[141]

Several mechanisms are involved in the innate response that controls infection before the development of conventional cellular immunity. Early host defense against *F. tularensis* infection involves neutrophils, dendritic cells, macrophages, mast cells, TNF-α, IFN-γ, and interleukin-12, but these are not sufficient to resolve the infection. Although neutrophils are less important as a primary defense in the lung than in systemic murine infection, neutrophils recruited to the lung may contribute to lung damage.[142] Activation of natural killer T

cells in a murine model of pulmonary tularemia also contributes to the resultant inflammation and exacerbates disease.[143] The initial response to *F. tularensis* infection is dependent on membrane-associated TLR2 recognition, particularly in the lung, and cytosolic nucleotide oligomerization domain–like receptors.[139] In the mouse lung, virulent *F. tularensis* strains are capable of suppressing the expected early immune response.[144,145] TLR4 recognition of *F. tularensis* LPS is poor; interestingly, the resistance of mice to pulmonary infection with *F. tularensis* subsp. *novicida* is enhanced by pretreatment with a TLR4 agonist.[146]

For complete resolution of systemic infection, it is necessary that $\alpha\beta^+$ T cells be functional and present after the initial defenses provided by the macrophages, dendritic cells, cytokines, and neutrophils. $CD4^+$ and $CD8^+$ cells each contribute to the survival of mice infected with the vaccine strain or the virulent Schu S4 strain, and both are required to clear the infection fully.[140,147] The contribution of $\alpha\beta^+$ T cells involves TNF-α and IFN-γ production and is dependent on interleukin-12.[139] Also, it has been appreciated that both humoral and cellular immune mechanisms act together to achieve protective immunity.[148,149] Natural infection or vaccination in humans results in long-lasting memory $CD4^+$ and $CD8^+$ cells.[150] In mice, B cells contribute to protection against the attenuated and virulent strains that are not dependent on antibodies, but it is unknown if B cells play a similar role in human infection.[140,147]

*F. tularensis* is capable of suppressing or avoiding many aspects of humoral and cellular host defenses (see "Virulence" earlier).[151] It survives extracellularly in part because it is capable of avoiding complement-mediated lysis. It survives intracellularly by multiple mechanisms, including prevention of phagosome-lysosome fusion, escape from the phagosome, suppression of the inflammasome, alternative activation of macrophages, avoiding killing by cellular antimicrobial peptides, destabilization of host cell messenger RNA, impairment of proinflammatory cytokine production, inhibition of NADPH oxidase in neutrophils, and the induction of prostaglandin $E_2$ secretion, which can inhibit IFN-γ production.[137,151,152–156] It also is capable of inhibiting IFN-γ signaling and production. For example, impaired clearance of *F. tularensis* live vaccine strain from mice with pulmonary infection may result from increased local prostaglandin $E_2$ secretion and decreased numbers of IFN-γ–secreting T cells. Immunization of mice with *F. tularensis* may protect against subsequent challenge but not prevent persistent, low-level organ infection, and the essential determinants of sterilizing immunity remain unknown.[147]

Expansion of circulating γδ T cells has been documented in patients with acute tularemia. These cells respond to phosphoantigens from many different pathogens, including *F. tularensis*. The observed increase in the levels of $V\gamma9V\delta2$ cells occurs after the first week of illness and may persist for longer than 1 year after infection.[157] However, 10 to 30 years after natural infection, long-lived memory cells responsive to *F. tularensis* heat shock proteins are αβ T cells and not γδ T cells.[158] Similarly, lymphocyte responsiveness to the live vaccine strain persists for up to 34 years after vaccination, and IFN-γ expression involves both $CD4^+$ and $CD8^+$ cell populations.[150]

## CLINICAL MANIFESTATIONS

The clinical consequences of *F. tularensis* infection depend on the virulence of the particular organism, the portal of entry, the extent of systemic involvement, and the immune status of the host. The result can range from asymptomatic or inconsequential illness to acute sepsis and rapid death. Patients who seek medical attention usually present with at least one of six classic forms of tularemia: ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic. This somewhat artificial classification emphasizes only the predominant manifestations commonly encountered, and overlapping forms may be present in many patients.

The incubation period averages 3 to 5 days, but it ranges from 1 to 21 days. Tularemia usually starts abruptly, with the onset of fever that can range to 104°F, chills, headache, malaise, anorexia, and fatigue. Other prominent symptoms may include cough, myalgias, chest discomfort, vomiting, sore throat, abdominal pain, and diarrhea. A pulse-temperature deficit was noted in up to 42% of evaluable patients in the United States, although this was found in only 5% of patients infected with *F. tularensis* subsp. *holarctica* infection in Sweden.[159,160] Fever (usually >38.3°C [101°F]) classically lasts for several days, remits for a short interval, and then recurs along with other symptoms. Without treatment, fever lasts an average of 32 days while chronic debility, weight loss, and lymphadenopathy may persist for many months longer.[161] Less virulent strains cause a milder, self-limited illness that may resolve without therapy. Systemic symptoms may abate by the time medical help is sought so that the clinical picture is dominated by one or more of the six patterns listed; this may lead to confusion regarding the correct diagnosis, particularly in the 25% to 50% of patients without an evident source of infection.

### Ulceroglandular and Glandular Tularemia

Ulceroglandular tularemia has presented in the majority of cases, including in recent years those in Colorado, Nebraska, South Dakota, and Wyoming.[88] Tick bites and animal contacts are the usually recalled exposures. This form is most quickly recognized as tularemia compared to others. The initial specific complaint is often of enlarged and tender localized lymphadenopathy (Fig. 227.3). The inciting skin lesion may appear before, simultaneously with, or from one to several days after the adenopathy. It starts as a red, painful papule in a region draining into the involved lymph nodes. Vesicles also may be seen, and these may be mistaken for herpes simplex or varicella infection.[162] The papule then undergoes necrosis, leaving a tender ulcer with a raised border (see Fig. 227.3). If untreated, the ulcer may take weeks to heal and leave a residual scar. Multiple lesions may occur, particularly in those cases with animal sources.[160]

The location of the ulcer usually reflects the mode of acquisition; animal contacts tend to yield ulcers on the hands and forearms, while tick bites cause ulcers on the trunk, the perineum, the lower extremities, and the head and neck (see Fig. 227.3). The distribution of lymphadenopathy also reflects the exposure history, as illustrated in Fig. 227.4. Overall, cervical and occipital adenopathy are most common in children and inguinal adenopathy is most common in adults. Skin changes over the involved nodes occurred in 19.1% of patients in a series from Sweden,[159] which suggests underlying suppuration. Some patients have a sporotrichoid presentation with ascending subcutaneous nodules (see Fig. 227.3). Lymphangitis is rare unless there is another bacterial superinfection of the ulcer.

Glandular tularemia occurs when patients present with tender regional lymphadenopathy but without a visible cutaneous lesion. This form has traditionally accounted for one-fifth or fewer of cases in the United States. However, glandular tularemia was the most common primary clinical form in children (44%) and the second most common presentation overall among classifiable cases reported in Missouri between 2000 and 2007.[163] Glandular tularemia represents essentially the same process as ulceroglandular disease, except that a skin lesion either healed before presentation or was minimal, atypical, or overlooked.

Enlarged lymph nodes may persist for prolonged periods. In some patients, an exposure or prior febrile illness will be forgotten. For this reason, tularemia may not be considered in the initial differential diagnosis of some patients whose primary presentation is lymphadenopathy. In either ulceroglandular or glandular tularemia, the lymph nodes may suppurate (see Fig. 227.3). More than 20% will suppurate if left untreated or if treatment is delayed longer than 2 weeks.[3,164] When fluctuant, they should be needle aspirated or surgically drained. The differential diagnosis of ulceroglandular and glandular tularemia includes pyogenic bacterial infections, cat-scratch disease, syphilis, chancroid, lymphogranuloma venereum, tuberculosis, nontuberculous mycobacterial infection, toxoplasmosis, sporotrichosis, *Spirillum minus* rat-bite fever, anthrax, plague, and herpes simplex virus infection.

### Oculoglandular Tularemia

Oculoglandular tularemia represents only a minority of cases. In this form, organisms have gained entry through the conjunctiva, from contaminated fingers, splashes, or aerosols. The disease may be bilateral, but this is uncommon. Early complaints may include photophobia and excessive lacrimation. Examination shows lid edema and painful conjunctivitis, with injection, chemosis, and small, yellowish conjunctival ulcers or papules in some patients. Associated tender lymphadenopathy

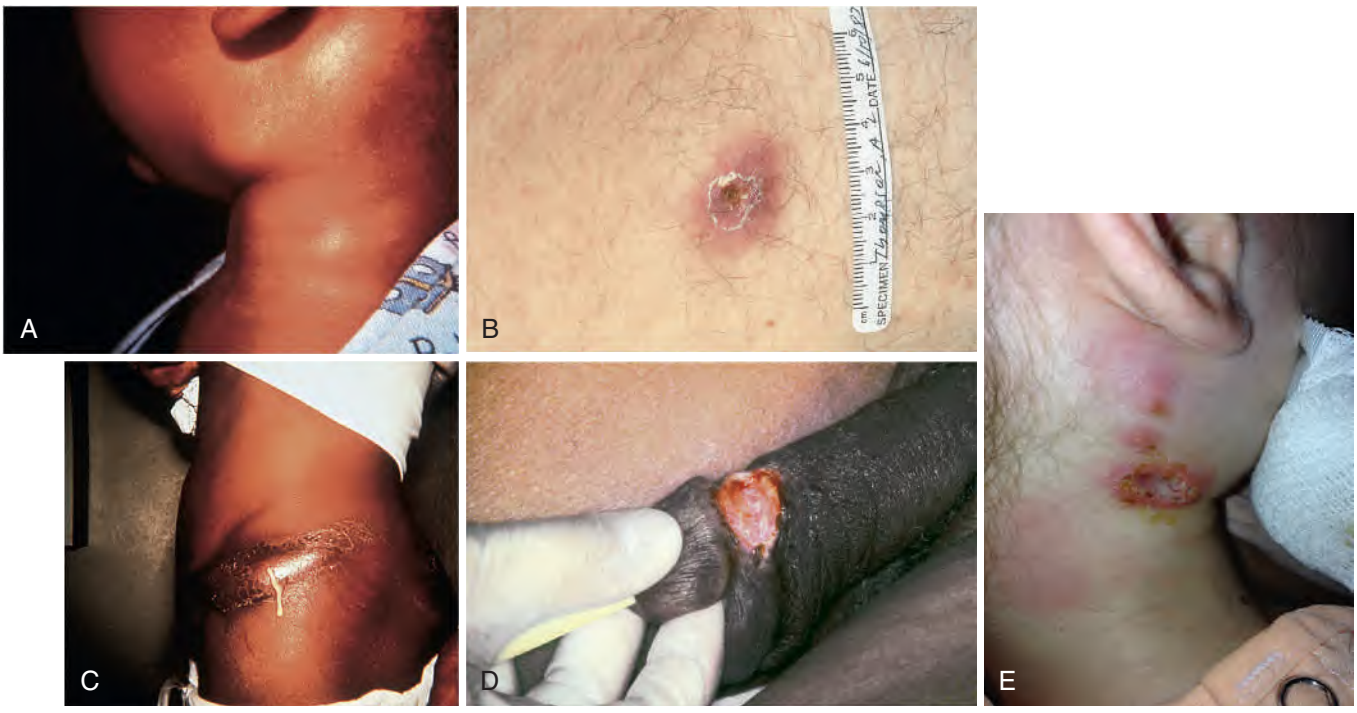


**FIG. 227.3 Examples of primary lesions seen in ulceroglandular tularemia.** (A) Large cervical and submandibular lymph nodes in a young child; an ulcer was found under the hairline on her forehead at the site of a tick bite. (B) Papule undergoing central necrosis with desquamation on the thigh of a middle-aged man. (C) Inguinal adenopathy and suppurative mass in a young hunter who had carried a dead hare at his side. (D) Penile ulcer that was suspected of being syphilis or another sexually transmitted disease until the history of a recent tick bite was obtained by the infectious diseases consultant. (E) Cervical ulcer and nodular adenopathy with a sporotrichoid appearance in a 6-year-old girl who had a tick removed from the area of the ulcer 2 weeks before presentation. The nodes coalesced, suppurated, and required drainage after 3 days of gentamicin therapy. Cultures of the ulcer and node drainage both grew *F. tularensis. (A and C, Courtesy Dr. Joseph A. Bocchini, Louisiana State University Health Sciences Center, Shreveport, LA. D, Courtesy Dr. John W. King, Louisiana State University Health Sciences Center, Shreveport, LA. E, Courtesy Dr. Robin Trotman, CoxHealth Infectious Diseases Specialty Clinic, Springfield, MO.)*

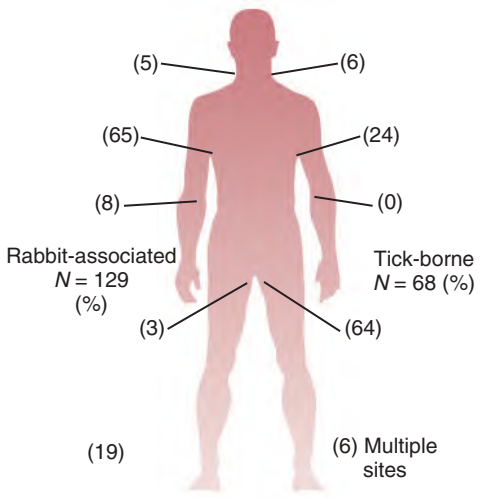


**FIG. 227.4 Distribution of lymphadenopathy in rabbit-associated and tick-borne tularemia.**

may occur in the preauricular, postauricular, submandibular, and cervical regions. If the adenopathy is extensive and more prominent than the eye findings, then this syndrome may be mistaken for mumps.[160]

Visual loss is rare, but complications include corneal ulceration, dacryocystitis, and nodal suppuration. Other ocular manifestations of tularemia also have been described, including cases of uveitis.[165,166] Tularemia may be an overlooked cause of Parinaud oculoglandular syndrome (conjunctivitis with ipsilateral preauricular adenopathy).[167] The differential diagnosis of oculoglandular tularemia includes pyogenic bacterial infections, adenoviral infection, syphilis, cat-scratch disease, and herpes simplex virus infection.

## Pharyngeal Tularemia

Pharyngeal tularemia, another variant of ulceroglandular disease, is the result of primary invasion through the oropharynx. The source may be contaminated food, water, or droplets. This form represents few cases overall in the United States, though it has been increasingly described in other countries with outbreaks.[168–170] In a large retrospective series from Turkey, pharyngeal tularemia accounted for 85% of cases.[168] Children appear to acquire this form more often than adults, and several family members may be affected simultaneously, especially if drinking from contaminated wells or other unchlorinated water sources.[102,168]

This form must be distinguished from a sore throat that may accompany any of the other major clinical forms of tularemia. In pharyngeal tularemia, the predominant complaints are fever, severe throat pain, and a neck mass representing lymphadenopathy. Exudative pharyngitis or tonsillitis is the rule, and one or more ulcers may be seen. A pharyngeal membrane has been described in some patients that is similar to a diphtheritic membrane.[161] Cervical, pre-parotid, and retropharyngeal adenopathy may be present, occasionally with bilateral involvement or abscess formation. When there is a delay in seeking care, the dominant manifestation may be cervical adenopathy without prominent fever or pharyngotonsillitis.

The differential diagnosis includes streptococcal pharyngitis, infectious mononucleosis, adenoviral infection, diphtheria, and tuberculosis; the latter especially may be confused with tularemia when granulomatous lymphadenitis is identified on biopsy.[171] Tularemia should be suspected in an endemic area whenever a severe sore throat is unresponsive to penicillin therapy and routine diagnostic tests have been unrewarding.

## Typhoidal Tularemia

Typhoidal tularemia refers to a febrile illness caused by *F. tularensis* unassociated with prominent lymphadenopathy and not fitting into any of the other primary forms. Typhoidal disease was among the most common forms recently reported in Arkansas, and is also frequently identified in states with increasing frequency of tularemia, including Colorado, Nebraska, South Dakota, and Wyoming.[88,172] The typhoidal form may result from any mode of acquisition, and it is the most difficult to diagnose. Because the portal of entry is usually inapparent clinically, a history of outdoor activities with tick, insect, or animal exposure should be pursued. Many patients have underlying severe chronic medical disorders and their presentation can be dramatic, with acute prostration and rapid death, or protracted illness.

Prominent symptoms of typhoidal tularemia may include any combination of fever with chills, headache, myalgia, sore throat, anorexia, nausea, vomiting, diarrhea, abdominal pain, and cough. The examination may reveal dehydration, hypotension, mild pharyngitis and cervical adenopathy, meningismus, and diffuse abdominal tenderness. Hepatomegaly and splenomegaly are found uncommonly in the acute stages and become more likely the longer the duration of illness. Severe disease may cause jaundice produced by cholestasis. Diarrhea is a major manifestation only in typhoidal tularemia. Bowel movements are loose and watery but only rarely bloody. Children may have more severe intestinal involvement, including focal areas of bowel necrosis.[161] Rare gastrointestinal manifestations include cholangitis, granulomatous hepatitis, and liver abscess.

Secondary pleuropulmonary involvement is fairly frequent in this form. Pulmonary infiltrates, pleural effusions, or even pulmonary nodules are described in up to 45% of subacute to chronic typhoidal cases[173,174,175]; it is even more frequent in laboratory-acquired infections. Additional findings in severely ill patients may include hyponatremia, elevated creatine phosphokinase level, myoglobinuria, pyuria, renal failure, and positive blood cultures. The differential diagnosis of typhoidal tularemia would be extensive and includes typhoid fever caused by *Salmonella* spp., brucellosis, *Legionella* infection, Q fever, disseminated mycobacterial or fungal infection, rickettsioses, malaria, endocarditis, and any other cause of prolonged fever without localizing signs.

## Pneumonic Tularemia

Pneumonic tularemia refers to an illness whose initial presentation is dominated by pulmonary infection. Pneumonic tularemia was the primary clinical presentation in 39% of adults and 24% of patients overall among classifiable cases reported in Missouri between 2000 and 2007, and as common as ulceroglandular disease in Colorado, Nebraska, South Dakota, and Wyoming during 2015 when the number of reported tularemia cases in these states significantly increased.[88,163] It may result from direct inhalation of the organism or secondary hematogenous spread to the lung.

Primary pneumonic tularemia is a risk for certain occupations, including sheep shearers, farmers, landscapers, and laboratory workers. Five cases of tularemic pneumonia in Martha's Vineyard were attributed to lawn mowing and brush cutting, possibly thereby aerosolizing infected carcasses, excreta, or ticks.[176] Cases also have been described as resulting from common exposure in a more casual setting, such as a converted mill building for holiday stays.[177] This case was linked to infected dog dander exposure, as was a recent case in a patient with sarcoidosis who had no other likely exposure.[115] Pneumonia with *F. tularensis* subsp. *holarctica* bacteremia also has been described after freshwater near drowning.[178] Secondary pneumonia may occur early or after a delay of weeks to months in the course of tularemia.[3] Although secondary pneumonia may complicate any of the syndromes already discussed, Evans and colleagues[160] found pneumonia to be most frequent in typhoidal (83%) and ulceroglandular (31%) diseases. Scofield and associates[179] reported that patients with pneumonic involvement were more likely to be older, recall no exposure risk, present with typhoidal illness, have positive cultures, stay hospitalized longer, and have a higher mortality rate. From 25% to 30% of patients have infiltrates on radiographic examination without any clinical findings of pneumonia.[160] A series of 58 patients with pulmonary tularemia in Finland found 47% lacking respiratory symptoms and 7% having a normal chest radiograph, though variable findings of chest computed tomography secured a pneumonic designation.[180] Pneumonia from *F. tularensis* subsp. *tularensis* is typically a more severe disease than that caused by *F. tularensis* subsp. *holarctica,* but pneumonia caused by *F. tularensis* subsp. *holarctica* may be severe in immunocompromised patients.[181] The illness may be prolonged with either subspecies.

Common symptoms include fever, cough, no or minimal sputum production, substernal tightness, and pleuritic chest pain.[109] Hemoptysis may occur but is uncommon. Physical examination may be nonspecific or may reveal rales, consolidation, and a friction rub or signs of effusion. Some patients need mechanical ventilation, and adult respiratory distress syndrome may complicate the course of any form of tularemia. Routine examination of sputum does not help to suggest the diagnosis. However, a false-positive DFA stain for *Legionella* on bronchoscopy specimens has been reported and may be easily confused with *Legionella* pneumonia.[182,183] Infected pleural fluid is exudative and negative on Gram stain, and usually contains more than 1000 leukocytes/mm$^3$; cells are predominantly lymphocytes, but neutrophilic effusions may occur. Pleural effusions seen with tularemia frequently mimic those of tuberculosis. Similar findings for both include a lymphocyte-rich exudative pleural effusion and a high adenosine deaminase concentration.[184] Granulomas may be found on pleural biopsy, prompting understandable confusion with tuberculosis.

Acute radiographic changes may include subsegmental or lobar infiltrates (Fig. 227.5), hilar adenopathy, pleural effusion, and apical or miliary infiltrates; less common changes include ovoid densities, cavitation, and bronchopleural fistula. However, in some patients the initial chest radiographs are normal. Secondary pneumonias are more likely to involve the lower lobes and be bilateral, perhaps because of their hematogenous origin. Healing usually occurs without residual changes, but fibrosis and calcifications may result. Therefore tularemia may manifest as enigmatic community-acquired pneumonia that does not respond to routine therapies. The differential diagnosis of pneumonic tularemia includes *Mycoplasma* pneumonia, *Legionella* infection, *Chlamydia pneumoniae* infection, Q fever, psittacosis, tuberculosis, the deep mycoses, and many other causes of atypical or chronic pneumonias.

Patients in four consecutive cases of pneumonic tularemia reported from Switzerland presented with fever of 1 to 8 weeks' duration and were found to have one or more dense nodular pulmonary infiltrates suspicious of cancer and hilar or mediastinal lymphadenopathy.[173] $^{18}$F-Fluorodeoxyglucose–positron emission tomography/computed tomography scans were highly positive in all four patients. Diagnosis was suspected by finding necrotizing granulomas in the nodes by using

**FIG. 227.5 Chest radiograph of untreated tularemia pneumonia.** This patient remained symptomatic for more than 3 months. The diagnosis was established serologically when poorly developed granulomas were found in a transbronchial biopsy specimen, other causes were excluded, and the exposure history was finally obtained. *(From Penn RL, Kinasewitz GT. Factors associated with a poor outcome in tularemia.* Arch Intern Med. *1987;147:265–268.)*

transbronchial needle aspiration or thoracoscopy. Node tissue was PCR positive for *F. tularensis* in three patients; subspecies were not determined. Tularemia agglutination titers were positive in all cases, with titers of 1:320 to 1:5120. Three patients responded to ciprofloxacin. One relapsed after doxycycline and responded to gentamicin plus ciprofloxacin. A similar patient from France also responded to ciprofloxacin.[185]

### Secondary Skin Manifestations

Primary skin rashes due to tularemia have been described, including on the face and elsewhere.[186] Secondary rashes are an underappreciated part of tularemia and may be found in up to 52% of cases, especially with oropharyngeal presentations.[159,187,188,189] They usually appear within the first 2 weeks of symptoms, but in a minority of cases they are delayed. The rash is more common in women than in men. Cutaneous changes may include diffuse maculopapular and vesiculopapular eruptions, pustules, erythema nodosum, erythema multiforme, acneiform lesions, and urticarial and vasculitis-like eruptions.[189] Sweet syndrome has also been reported in association with tularemia.[190] Although any secondary rash may be part of any form of tularemia, erythema nodosum has been found to occur most commonly with pneumonic tularemia, while erythema multiforme was described in 11.3% of patients in a large Turkish series, most of whom presented with oropharyngeal or glandular forms.[191]

### *Francisella tularensis* subsp. *novicida* and *Francisella philomiragia* Infections

The clinical manifestations of infections caused by *F. tularensis* subsp. *novicida* are less well characterized than for the other subspecies. Heightened surveillance for *F. tularensis* and the availability of molecular methods for subspecies identification have led to several recent reports of *F. tularensis* subsp. *novicida* involvement in human disease. A *F. tularensis* subsp. *novicida*–like organism was isolated from an adult with a toe abscess and regional adenopathy that complicated a cut sustained in brackish water in Australia, and *F. tularensis* subsp. *novicida* bacteremia was documented in a patient from Thailand with terminal ovarian cancer.[84,192] Brett and coworkers[193] identified *F. tularensis* subsp. *novicida* bacteremia in a patient who nearly drowned while body surfing along the South Carolina coast.[193] An outbreak of three symptomatic cases of *F. tularensis* subsp. *novicida* bacteremia occurred in a Louisiana prison, and one patient died.[96] *F. tularensis* subsp. *novicida* was isolated from one of the facility ice machines, believed to be the most likely vehicle for its transmission. These worldwide reports and the molecular identification of older *F. tularensis* isolates as *F. tularensis* subsp. *novicida* after being previously classified as other subspecies suggest that infections caused by *F. tularensis* subsp. *novicida* may be underappreciated.

*F. philomiragia* infection has caused a skin vesicle, pneumonia, empyema, sepsis, peritonitis, splenic microabscesses, and meningitis. This organism predominantly infects patients with host defenses impaired by chronic granulomatous disease, near drowning in salt water or estuaries, myeloproliferative disorders, or renal transplantation.[194,195,196] Only a small number of cases have been documented, more from North America than Europe, and the organism has been found in tissues, blood, cerebrospinal fluid, and other body fluids.

### Clinical Recognition of a Bioterrorism Event

The 2001 Tularemia Consensus Statement developed by the Working Group on Civilian Biodefense concluded that aerosolization would be the most likely method for dispersing *F. tularensis,* because inhalation of bacilli would affect the highest number of people with the most devastating manifestations of the disease.[197] Children are theorized to be more vulnerable than adults to an aerosolized agent because of their higher respiratory rate, more permeable skin, and higher skin-to-mass ratio.[198] Secondary cases may also arise following contact with contaminated environmental sources or animals.[199]

After an aerosol release and an incubation period of 3 to 5 days, patients may present with the acute onset of fever (38°–40°C [100.4°–104°F]), malaise, headache, rigors, coryza, and sore throat. The subsequent clinical syndrome depends on the immune status of the host, the inhaled inoculum, and the virulence of the released agent. It is anticipated that most patients would develop either primary pneumonic or typhoidal forms.[197] A significant number of patients may develop respiratory failure or have signs and symptoms heralding severe sepsis. Because airborne organisms also may invade through extrapulmonary sites and food and water may be contaminated, less common presentations could include oculoglandular, pharyngeal, ulceroglandular, or glandular disease.[200] Rapid recognition and reporting of a possible bioterrorist event due to tularemia is a difficult clinical challenge. It should be suggested by clustered cases of pneumonic or typhoidal disease, particularly in urban areas in patients without the usual exposure history.

## COMPLICATIONS AND OUTCOME

Suppuration of involved lymph nodes is currently the most common complication of tularemia (see Fig. 227.3), and this may occur even after directed antibiotic therapy. Among the tularemia patients from Missouri with lymphadenopathy reported between 2000 and 2007, 19% required drainage of suppurative nodes. Lymph node suppuration was associated with a longer delay in starting effective antibiotic therapy.[164] Nodes that suppurate after appropriate therapy are often sterile but benefit from drainage.[201] Patients with severe disease may manifest disseminated intravascular coagulation, renal failure, rhabdomyolysis, jaundice, and hepatitis. Meningitis, encephalitis, pericarditis, peritonitis, osteomyelitis, splenic rupture, and thrombophlebitis have become very rare since antibiotic therapy has become available. Guillain-Barré syndrome rarely has been described complicating cases of ulceroglandular tularemia.[202]

Rare cases of otitis media,[203] otomastoiditis,[204] endocarditis,[205,206] aortitis,[207] prosthetic joint infection with and without bacteremia,[208,209] and peritonitis[210] caused by *F. tularensis* have been reported. Endocarditis complicated typhoidal disease in all four patients reported in a recent series.[206] Meningitis occurs rarely with ulceroglandular and typhoidal disease, and the cerebrospinal fluid in these patients almost always shows a mononuclear cell pleocytosis, with a high protein concentration and hypoglycorrhachia.[211] Brain abscesses may be seen as a complication of meningitis.[212]

Tularemia may lead to months of debility in some patients, usually associated with late lymph node suppuration or persistent fatigue. A recent outbreak of waterborne oropharyngeal tularemia in the Republic of Georgia was marked by delayed diagnosis and treatment, the frequent occurrence of neuropsychiatric symptoms, and slow resolution of adenopathy. Neuropsychiatric symptoms in this cohort included headaches, chronic fatigue, difficulty concentrating, and sleep disturbances.[213] Features that are associated with a worse prognosis include increasing age, serious coexisting medical conditions, symptoms lasting 1 month or longer before treatment, significant pleuropulmonary disease, typhoidal illness, renal failure, a delay in the diagnosis, and inappropriate antibiotic therapy.[1,179] Overall death rates in the modern antibiotic era have been 2% to 4% but may range up to 24% depending on the strain.[196] Mortality had been as high as 60% before the introduction of streptomycin as treatment.[214]

## DIAGNOSIS

The diagnosis of tularemia ultimately rests on clinical suspicion. Results of routine laboratory testing are nonspecific. The leukocyte count and sedimentation rate may be normal or elevated. Thrombocytopenia, hyponatremia, elevated serum aminotransferase values, increased creatine phosphokinase level, myoglobinuria, and sterile pyuria are occasionally found.[1]

### Routine Cultures and Pathology

Because of its potential danger to laboratory personnel, individuals working in the area or who may come in contact with patient specimens should be notified if tularemia is suspected. The organism is rarely seen on Gram-stained smears or in tissue biopsy specimens and it does not grow in routinely plated cultures. However, *F. tularensis* may be recovered from blood, pleural fluid, lymph nodes, wounds, sputum, and gastric aspirates when processed on supportive media. Isolations by blood culture have included the less virulent *F. tularensis* subsp. *holarctica* as well as the more virulent *F. tularensis* subsp. *tularensis.*

Guidelines for sentinel-level clinical laboratories were recently issued for *F. tularesis*.[41] For clinicians concerned about this infection, Biosafety Level 2 is sufficient for laboratory handling of routine clinical specimens, but Biosafety Level 3 should be used to process isolates suspected of being *F. tularensis*.[20] Because *F. tularensis* is a Tier 1 select agent, its possession and shipment are tightly restricted.[215] Isolates thought to be *F. tularensis* must be shipped to the state public health laboratory or a specialized registered laboratory in the Laboratory Response Network, and the remainder destroyed within 1 week. These laboratories also may provide reliable, rapid diagnostic tests. Misidentification of *F. tularensis* may occur when commonly available automated laboratory identification systems are used; it is most often reported as *Haemophilus influenzae* (satellite test or X and V factor test positive) or *Aggregatibacter* spp.

Many methods for the rapid diagnosis of tularemia have been reported, including DFA and immunohistochemical staining of smears and tissues, antigen detection in urine, detection using specific monoclonal antibodies, RNA hybridization with a 16S ribosomal probe, and various PCR techniques. DFA and PCR tests are usually available in specialized Laboratory Response Network laboratories. Nonculture methods for directly detecting *Francisella* species in clinical specimens are being explored, including real-time PCR, recombinase polymerase amplification, and direct deep DNA sequencing.[199,216–220] Ongoing research is focused on the development of field-stable PCR, recombinase polymerase amplification, proteomic, and immunologic methods for the rapid and simultaneous detection of multiple potential bioterrorism agents.[222] Newer methods that may limit the need for laboratory manipulation of unknown culture isolates and that can identify the organism to the subspecies level include real-time PCR and MALDI-TOF.[223–226]

The use of PCR analysis for diagnosis is appealing in that smears and cultures are usually negative, standard microbiologic isolation may be hazardous to laboratory personnel, serologic diagnosis may take several weeks to confirm, and the basic methodology is widely available in many clinical laboratories. Although very sensitive in artificial media, PCR assays are less sensitive when applied to biologic specimens and false-negative results may occur. A review of three reports from Sweden found that PCR assay using primers for the 17-kDa lipoprotein *F. tularensis* gene *tul4* was 77% sensitive and culture was 63% sensitive for the diagnosis of ulceroglandular tularemia.[214] PCR analysis may prove useful for diagnosing tularemia in patients several weeks into their illness and in those already receiving suppressive empirical antibiotic therapy. Real-time PCR assays with multiple targets offer promise for improved sensitivity and specificity, including for field use.[219] An automated cartridge-based PCR assay can detect *F. tularensis* bacteremia in a primate model, but is not yet commercially available.[227] However, one commercially available multiplex PCR system does include *F. tularensis* in two of its special panels.[228]

### Serologic Diagnosis

Serologic studies are the most common way the diagnosis of tularemia is confirmed. Antibodies to *F. tularensis* may be demonstrated by tube agglutination, microagglutination, hemagglutination, enzyme-linked immunosorbent assay, and immunochromatographic assay; the tube agglutination and microagglutination tests are the standard methods in the United States, and enzyme-linked immunosorbent assays also are used in Europe.[20,229,230] Standard serologic tests detect infections with *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* equally well. They are usually negative with *F. tularensis* subsp. *novicida* and with *F. philomiragia*. Standard tube agglutination titers are usually negative in the first week of illness, are positive in most patients after 2 weeks, and peak after 4 or 5 weeks. The microagglutination assay is up to 100-fold more sensitive than tube agglutination.

IgM and IgG antibodies appear together, and high titers of both may persist for longer than a decade after infection, limiting the value of a single positive result.[231] A presumptive diagnosis is supported by an acute tube agglutination titer of 1:160 or more, or an acute microagglutination titer of 1:128 or more, in the presence of compatible disease, but this may also reflect remote infection. Definitive serologic diagnosis requires a fourfold or greater change in titer between acute and convalescent specimens, and at least one test must be positive.[20] Serologic studies may need to be repeated at 7- to 10-day intervals before a rise is demonstrated. Antibodies may cross react with *Brucella* spp., *Proteus* OX19, *Legionella* spp., and *Yersinia* spp., but titers to *F. tularensis* are almost always higher. False-positive heterophil agglutinins also rarely occur during tularemia.

### Investigational Diagnostic Assays

Tests for cell-mediated immunity, such as whole-blood IFN-γ release assay in response to tularemia antigens, are promising and may be positive earlier than serologic studies, but they are not commercially available.[229] Gene profiling of cells in whole-blood samples obtained from patients with tularemia offers promise as a future rapid diagnostic tool.[232]

## THERAPY

### Antibiotic Treatment

Because tularemia circulates within zoonotic reservoirs and environmentally, not within humans, antibiotic resistance has not been reported clinically and has not evolved over time.[233] However, antibiotic-resistant organisms have been produced within laboratories and could be introduced in a bioterrorism scenario.[234,235] Antimicrobials with good in vitro activity include aminoglycosides, tetracyclines, and fluoroquinolones, though only aminoglycosides and tetracyclines are approved by the US Food and Drug Administration for treatment of tularemia. As an intracellular organism and a β-lactamase producer, not unexpectedly, there is resistance to the β-lactam antibiotic class.[43] The macrolide class also tends to have poor activity, although different susceptibilities occur between A and B strains.[236,237]

The Centers for Disease Control and Prevention and the World Health Organization recommend aminoglycosides as the drugs of choice for the treatment of severe tularemia.[238,239] Streptomycin is preferred in adults because it is efficacious with minimal relapses and has been approved in the United States for tularemia treatment by the US Food and Drug Administration. Gentamicin is an acceptable substitute that may be given intravenously, is often more readily available, and has less vestibular toxicity than streptomycin; in addition, gentamicin blood levels are usually easier to obtain in a timely fashion (Table 227.2). The recommended dosage of streptomycin that is effective therapy for tularemia is 7.5 to 10 mg/kg intramuscularly every 12 hours for 7 to 14 days. An alternative regimen is 15 mg/kg intramuscularly every 12 hours for the first 3 days, followed by half this dose to complete treatment. For patients who are very ill, 15 mg/kg every 12 hours may be given throughout a 7- to 10-day course. Doses greater than 2 g/day of streptomycin in adults do not increase efficacy and should not be given. The pediatric weight-based regimens for streptomycin are up to a maximum of the adult dose (see Table 227.2). The first few days of streptomycin rarely may induce a Jarisch-Herxheimer–like reaction, with an increase in symptoms and a transient decrease of the serum agglutination titer.

Gentamicin is preferred for children because it may be given intravenously, may be more readily available than streptomycin, and may have fewer adverse effects (see Table 227.2).[240] However, Kaya and coworkers[241] retrospectively identified 11 children with oropharyngeal tularemia who had a poor response to 7 to 10 days of gentamicin treatment; 7 patients had persistent adenopathy that improved after therapy was changed to streptomycin, and the other 4 patients developed suppurative nodes that responded to streptomycin and surgical drainage. The desired gentamicin peak serum level is at least 5 μg/mL. The efficacy of single daily gentamicin dosing has been reported for small numbers of adult cases but has not been rigorously studied.[109,242] Nonetheless, it is efficacious, and some practitioners consider once-daily gentamicin an acceptable alternative for adults that facilitates completing treatment as an outpatient.[109] However, divided dosing of gentamicin is recommended for children (see Table 227.2).[240] The doses of both streptomycin and gentamicin need to be adjusted for renal insufficiency.

Penetration of the aminoglycosides into the cerebrospinal fluid is poor and erratic, and it may be inadequate in tularemic meningitis. Pittman and colleagues[243] reported a central nervous system shunt infection caused by *F. tularensis* that was successfully treated with intrathecal gentamicin. Contentin and colleagues[244] reported a case of

**TABLE 227.2 Antibiotic Therapy for Tularemia**

| INDICATION AND PATIENT GROUP | RECOMMENDED ANTIBIOTICS AND DOSAGES |
|---|---|
| **Serious Disease** | |
| Adults | Streptomycin,[a] 10 mg/kg IM q12h for 7–10 d (not to exceed 2 g/d), *or* Gentamicin,[a] 5 mg/kg/d IM or IV divided q8h for 7–10 d |
| Children | Streptomycin,[a] 15–20 mg/kg IM q12h for 7–10 d (not to exceed 2 g/d), *or* Gentamicin,[a] 5 mg/kg/d IM or IV divided q8–12h for 7–10 d |
| **Mild to Moderate Disease** | |
| Adults | Ciprofloxacin,[b] 500 mg orally twice daily for 10–14 d, *or* Doxycycline, 100 mg orally twice daily for 14–21 d |
| Children[c] | Gentamicin,[a] 5 mg/kg/d IM or IV divided q8–12h for 7–10 d, *or* Ciprofloxacin,[c] 20–40 mg/kg/d orally divided twice daily for 10–14 d (not to exceed 1500 mg/d) |
| **Meningitis** | |
| Adults | Streptomycin or gentamicin in the doses given for moderate to serious disease plus either ciprofloxacin, 400 mg IV every 8–12 h, doxycycline, 100 mg IV every 12 h, or chloramphenicol, 15–25 mg/kg IV q6h (not to exceed 4 g/d), for 14–21 d |
| Children | Gentamicin in the doses for moderate to serious disease plus either ciprofloxacin, 20–30 mg/kg/d IV divided every 8 or 12 h (not to exceed 1.2 g/d), or doxycycline, 2.2–4.4 mg/kg/d IV divided q12h (not to exceed 200 mg/d), for 14–21 d |

[a]The streptomycin and gentamicin doses listed are for patients with normal renal function, and they need to be adjusted for renal impairment. See text for an alternative streptomycin regimen for very ill patients. In adults with normal renal function, once-daily administration of gentamicin also is acceptable.
[b]For adults an oral ciprofloxacin dose of 750 mg twice daily also has been used in some reports.
[c]Gentamicin is preferred for children with moderate disease; ciprofloxacin is an alternative for children with mild disease.

*F. tularensis* subsp. *holarctica* meningitis that was cured after 4 weeks of high-dose ciprofloxacin combined with 18 days of gentamicin and 6 days of thiamphenicol. Additional cases of tularemic meningitis have been documented, and successful treatment has included a combination of streptomycin with chloramphenicol or a combination of doxycycline with either streptomycin or gentamicin (see Table 227.2).[211,245] Chloramphenicol may be unavailable or in short supply in the United States; it should not be chosen to treat other forms of tularemia because of its potentially serious toxicity and the availability of more effective alternatives with less dangerous potential side effects.

Selected children with mild disease and adults with mild to moderate disease may be treated with one of the oral antibiotics listed in Table 227.2, either alone or after initial treatment with an aminoglycoside.[240] Doxycycline and tetracycline are bacteriostatic for *F. tularensis.* Historically, use of this class led to higher rate of relapse after treatment, which is why they are recommended to be given for at least 14 days (see Table 227.2). A recent retrospective case series from Missouri reported no relapses among 17 patients receiving either doxycycline (16 patients) or tetracyclines (1 patient) as their tularemia therapy (median treatment duration of 21 days, range 10–42 days), suggesting that selected patients treated with a tetracycline for the recommended duration may have better outcomes than previously reported failure rates of 15% to 48%.[246] Of the 16 patients treated with doxycycline, 9 had drainage of suppurative lymph nodes prior to starting therapy and 3 required a repeat drainage procedure while receiving doxycycline (one patient after 1 week and two patients after 2 weeks of treatment).[246] Doxycycline is preferred over tetracycline because it is better tolerated and provides the convenience of twice-daily dosing. Tetracycline should not be used in children younger than 8 years of age, during pregnancy, or during lactation unless the benefits clearly outweigh the risks. Doxycycline is not recommended for the treatment of tularemia in children, although it is thought not to cause tooth enamel staining under the age of 8, and is now recommended for other tick-borne infections by the American Academy of Pediatrics.[240,247,248] Relapses may follow any regimen but are more common when tetracyclines are used for less than 14 days. Although a relapse after initial treatment with doxycycline may be re-treated with doxycycline given for at least 14 to 21 days, re-treatment with a different agent such as streptomycin or gentamicin may be preferred.

In vitro susceptibility studies have found that the fluoroquinolones are active against *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica,* as well as *F. tularensis* subsp. *mediasiatica* and *F. tularensis* subsp. *novicida.*[31,249,250] Clinical experience with the fluoroquinolones as therapy for tularemia caused by *F. tularensis* subsp. *holarctica* has been favorable, even in immunocompromised hosts. Some consider fluoroquinolones to be the drugs of choice for adults with mild to moderate *F. tularensis* subsp. *holarctica* infections,[251] and Eliasson and coworkers[252] have recommended the use of gentamicin combined with a fluoroquinolone for patients with severe tularemia. However, there is less published experience using these agents in the United States or for infections caused by the more virulent *F. tularensis* subsp. *tularensis.* Among tularemia cases reported in Missouri between 2000 and 2007, 9 of 10 patients given ciprofloxacin alone or combined with ineffective agents were cured.[164] Fluoroquinolones are preferred over doxycycline as oral treatment of tularemia for appropriate adults by some authorities because of efficacy with a reported lower likelihood of relapse.[251,252] Ciprofloxacin has also been effective therapy in children with tularemia, and is a recommended alternative for children with mild disease (see Table 227.2).[187,240,253] However, the outcome with fluoroquinolone therapy may be suboptimal in some children as well as in adults.[174,254] Importantly, acquired fluoroquinolone resistance has not been documented in natural tularemia, even among patients who failed fluoroquinolone treatment.[255]

Ciprofloxacin was active in vitro against the few isolates of *F. philomiragia* tested. It was used successfully to treat a child with chronic granulomatous disease and *F. philomiragia* adenitis and lung infection, but it was part of an unsuccessful regimen in a young man with *F. philomiragia* sepsis.[31,194,256]

Drugs with well-established clinical efficacy (see Table 227.2) have exhibited achievable minimal inhibitory concentrations against *F. tularensis* on standardized in vitro susceptibility tests.[31,249,250] Other agents with relatively low minimal inhibitory concentrations have included erythromycin, rifampin, cefoxitin, cefotaxime, ceftriaxone, and ceftazidime. The effectiveness of these drugs in treating tularemia is not fully established, and ceftriaxone has failed in several patients treated as outpatients.[257] Ceftriaxone exhibited poor intracellular inhibitory activity against a strain of *F. tularensis* subsp. *holarctica* grown in macrophage-like cell monolayers, whereas aminoglycosides, doxycycline, telithromycin, fluoroquinolones, and rifampin were active in this assay.[236] However, rifampin is not recommended to treat tularemia because of its potential to induce the emergence of resistance.[238] Although erythromycin has been used successfully in a few patients who were believed to have *Legionella* infections, resistance to erythromycin is prevalent in many areas in Europe and Russia and in general the drug is considered unreliable as therapy.[31,182,238,258,259] *F. tularensis* subsp. *tularensis* and biovar II strains of *F. tularensis* subsp. *holarctica* are considered resistant to macrolides, but macrolide minimal inhibitory concentrations are lower to biovar I strains of *F. tularensis* subsp. *holarctica.*[260,261] Ikäheimo and associates[262] found that all 38 type B clinical isolates they tested were resistant to imipenem in vitro.

## Pregnant and Immunosuppressed Patients

Tularemia cases occurring during pregnancy or in immunosuppressed patients have been reported only rarely, but they may be more frequently

encountered during a bioterrorism event, affecting large numbers of people without the usual risk factors.[263] Treatment of pregnant or immunocompromised patients with tularemia is challenging, and optimal antibiotic regimens are unknown. Aminoglycosides, tetracyclines, and fluoroquinolones have potential risks to the fetus when used during pregnancy; immunocompromised patients with tularemia may have an increased risk for relapse or treatment failure.

The risk to the fetus of tularemia during pregnancy is unknown; although prematurity and fetal loss may occur, healthy newborns also have been reported after maternal tularemia.[264,265] Before the availability of effective antibiotics, Pullen and Stuart[266] reported three patients with tularemia during pregnancy and all gave birth to normal children. Dienst[161] reported two pregnant patients with tularemia; one was successfully treated with streptomycin in the fifth month of pregnancy and had a normal birth, and the other spontaneously aborted a 6-week-old fetus after 35 days of illness. Four pregnant patients with tularemia and suppurative adenopathy were treated successfully with gentamicin followed by oral ciprofloxacin and lymph node drainage; all the patients had otherwise normal pregnancies, and the infants were normal at 18 months of follow-up.[267] Among three pregnant women with tularemia in Turkey, one patient was treated with gentamicin and two patients were untreated; all three patients had uncomplicated pregnancies and delivered healthy babies.[268] Tularemia in a pregnant woman in France, where macrolide-sensitive strains of *F. tularensis* subsp. *holarctica* predominate, was successfully treated with a 6-week course of azithromycin.[269] However, a review of 101 patients with tularemia in France identified two pregnant patients, and both were unsuccessfully treated with macrolides.[174]

Potentially immunocompromising illnesses were present in another 8 of the 101 French patients, but their treatments and outcomes were not specifically identified.[174] Case reports of tularemia in patients with hematopoietic stem cell transplantation, renal transplantation, acquired immunodeficiency syndrome, chronic graft-versus-host disease, and TNF inhibitor therapy have described successful treatment with at times prolonged courses of gentamicin, fluoroquinolones, or doxycycline, alone or in various combinations.[270–273,274,275,276]

### Surgical Treatment

Surgical therapies are limited to drainage of abscessed lymph nodes and chest tube drainage of empyemas. Multiple nonprospective series reported fewer treatment failures when antibiotic therapy is combined with surgical drainage when feasible.[201,277]

### Immunotherapy

Antibodies to *F. tularensis* and serum from vaccinated people are effective passive therapies for murine infection.[135,278,279] Proteomic analysis has been used to identify target antigens for the development of monoclonal antibodies that are effective in protecting mice from pulmonary intranasal challenge when given either intranasally or intraperitoneally.[280] Together, these results offer the future hope of immunotherapeutic agents for tularemia.

### Therapy for Tularemia After a Bioterrorism Event

Recommendations for therapy for tularemia in the context of a bioterrorism event were presented in the Working Group for Civilian Biodefense Consensus Statement.[197] Specific treatment options depend on whether there are contained or mass casualties; smaller numbers of infected people permit individual medical care that would not be possible with larger numbers of patients. Thus treatment options for contained casualties are similar to those listed in Table 227.2 and as discussed earlier; in addition, the Working Group also included chloramphenicol alone as an option for both adults and children or ciprofloxacin as an option for children. For contained casualties, the Working Group recommended that streptomycin, gentamicin, or ciprofloxacin be given for 10 days and that doxycycline or chloramphenicol be given for 14 to 21 days.[197] In mass casualty situations, only oral ciprofloxacin or doxycycline for 14 days is recommended by the Working Group for both children and adults.[197] Susceptibility testing of the *F. tularensis* strain used as a bioweapon will be important because it may have been modified to select for resistance to standard antibiotic treatments. Molecular assays capable of rapidly and reliably detecting ciprofloxacin resistance in *F. tularensis* have been reported.[281]

The Working Group preferred gentamicin for the treatment of pregnant patients among contained casualties and preferred oral ciprofloxacin for pregnant patients among mass casualties.[197] The Working Group recommended treatment with either streptomycin or gentamicin for immunocompromised patients in both contained and mass casualty situations.[197]

## PREVENTION

Avoiding exposure to the organism is the best prevention of tularemia. Wild animals should not be skinned or dressed using bare hands, and bare hands should not be used to handle an animal that appears ill. Gloves, masks, and protective eye covers should be worn when performing such tasks and when disposing of dead animals brought home by household pets. Wild game should be cooked thoroughly before ingestion. Wells or other waters that are contaminated by dead animals should not be used. Before mowing, checking property for animal carcasses will avoid aerosolizing potentially infected animal tissue. Treatment of community water supplies with standard chlorination protects against waterborne tularemia.[197,282,283] The most important measure to avoid tick bites in infested areas is wearing clothing that is tight at the wrists and ankles and that covers most of the body. Chemical tick repellents may also be of benefit. Frequent checks should be made for attached ticks so that they may be removed promptly; this must not be done with bare hands, and care should be taken not to crush the tick.

Hospitalized patients with tularemia do not need special isolation because person-to-person spread does not occur, and even in the preantibiotic era, secondary cases were not found. Standard universal precautions for contaminated secretions are adequate when handling drainage from wounds or eyes.

### Vaccination

Vaccines prepared from killed *F. tularensis* are ineffective, in part because they only induce an antibody response. A live vaccine based on an attenuated strain of *F. tularensis* subsp. *holarctica* (LVS vaccine), originally obtained from the former Soviet Union, was developed in the United States. The LVS vaccine is an attenuated *F. tularensis* strain that occurs in two colony phenotypes, only one of which is immunogenic. This vaccine does not spread from the inoculation site, induces cell-mediated and humoral immunity, is effective in preventing typhoidal disease, and reduces the severity of ulceroglandular disease but does not prevent it.[284,285] However, the live vaccine strain of *F. tularensis* cannot provide high immune response in the presence of antimicrobial agents such as doxycycline. Importantly, the LVS vaccine has been ineffective in protecting against pulmonary challenge with virulent *F. tularensis* subsp. *tularensis*.[286] LVS vaccination was considered in the past for people who worked with *F. tularensis* and for anyone else with repeated occupational exposures. However, the LVS vaccine is not approved and is no longer available for general use in the United States. Removal was prompted by questions about its stability, basis of attenuation, efficacy after challenge with other *Francisella* strains by varied routes of exposure, usefulness in immunocompromised patients, and the need to administer it by scarification.[284,287]

A new tularemia vaccine is actively being sought using several different strategies, including developing inactivated or subunit vaccines, finding different attenuated strains with defined mutations that are immunogenic, and improving the LVS vaccine.[286,288–290] Genomic and proteomic analyses are being used to identify candidate *F. tularensis* proteins that are recognized by patients who have had tularemia for inclusion in potential subunit vaccines.[55,291] Analysis of differences in immune responses to vaccines among protected and unprotected animals may help identify human correlates of protection.[286,292] In humans and animal models, protection against pulmonary challenge is enhanced when the LVS vaccine is given by aerosol or intranasally. This and other observations suggest that mucosal immunity may contribute to vaccine-induced protection against respiratory tularemia. An LVS-based, multivectored vaccine has successfully protected mice against respiratory

challenge with virulent *F. tularensis, Bacillus anthracis,* and *Yersinia pestis*.[293]

An improved LVS vaccine was produced using accepted good manufacturing practices that had limited toxicity in rabbits; this vaccine induced IgG, IgM, and IgA antibodies that cross reacted with *F. tularensis* subsp. *tularensis* strain Schu S4. A phase I dose-escalating trial of this new LVS vaccine in humans found it to be safe and immunogenic, eliciting both antibody and IFN-γ responses.[294] A phase II double-blind, placebo-controlled trial comparing the new DVC-LVS vaccine in humans to the existing vaccine (USAMRIID-LVS) demonstrated equivalent safety and seroconversion rates with earlier antibody production at 14 days.[295] However, there are many biologic and regulatory issues that will need to be addressed before this or another vaccine is available for human use.[113,296]

## Antibiotic Prophylaxis

Antibiotic prophylaxis after potential exposures of unknown risk, such as tick bites, is not recommended. In the past, intramuscular streptomycin was given for preemptive treatment of documented exposures from laboratory accidents because streptomycin successfully aborts illness when given in the incubation period after experimental inoculation. Gentamicin should be effective for this purpose as well, but this has not been confirmed. Currently, either ciprofloxacin, 500 mg, or doxycycline, 100 mg, given orally twice daily for 14 days is recommended for adults with suspected or proven high-risk exposure to *F. tularensis*.[107,197] Exposed children may be observed for fever or other signs of illness without antibiotics, except during a bioterrorist event.[107] Observation without antibiotics is also appropriate for individuals with lower-risk exposures and in vaccinated individuals. No therapy is needed for someone whose only exposure is to a patient with tularemia because human-to-human transmission does not occur.

Recovery from tularemia is believed to confer protective immunity for life, although a few recurrent infections have been documented.[297,298] Most recurrences have been clinically mild ulceroglandular disease, and systemic symptoms have been uncommon. Therefore previously infected individuals are not likely candidates for vaccination or preemptive antibiotic therapy after a known exposure.

## Antibiotic Prophylaxis After a Tularemia Bioterrorism Event

All people, regardless of age or history of past tularemia, identified early in the incubation period after exposure to an *F. tularensis* bioterrorist event are candidates for antibiotic prophylaxis. The Working Group for Civilian Biodefense recommended either doxycycline or ciprofloxacin orally for 14 days for all exposed people except immunocompromised patients.[197] Mice are significantly protected from lethal *F. tularensis* subsp. *tularensis* respiratory infection by fluoroquinolones, and this is true even when the fluoroquinolone is delayed up to 72 hours after infection; however, delay of doxycycline beyond 24 hours is ineffective.[299,300] These results suggest that a fluoroquinolone may be preferred for oral prophylaxis after exposure to an *F. tularensis* bioweapon.

The doxycycline and ciprofloxacin regimens for adults are the same as those discussed previously for prophylaxis after other high-risk exposures. The Working Group believed that 14 days of either oral doxycycline or oral ciprofloxacin is an appropriate choice for children exposed to *F. tularensis* as a result of bioterrorism.[197] The doxycycline dosing recommended by the Working Group is 2.2 mg/kg twice daily for children weighing less than 45 kg and 100 mg twice daily for children weighing 45 kg or more.[197] The dosing of ciprofloxacin recommended by the Working Group for children exposed to an *F. tularensis* bioweapon is 15 mg/kg orally twice daily, not to exceed 1 g daily.

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*The complete reference list is available online at Expert Consult.*


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