Tularemia as a Biological Weapon

Medical and Public Health Management

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Objective The Working Group on Civilian Biodefense has developed consensus-based recommendations for measures to be taken by medical and public health professionals if tularemia is used as a biological weapon against a civilian population.

Participants The working group included 25 representatives from academic medical centers, civilian and military governmental agencies, and other public health and emergency management institutions and agencies.

Evidence MEDLINE databases were searched from January 1966 to October 2000, using the Medical Subject Headings *Francisella tularensis*, *Pasteurella tularensis*, *biological weapon*, *biological terrorism*, *bioterrorism*, *biological warfare*, and *biowarfare*. Review of these references led to identification of relevant materials published prior to 1966. In addition, participants identified other references and sources.

Consensus Process Three formal drafts of the statement that synthesized information obtained in the formal evidence-gathering process were reviewed by members of the working group. Consensus was achieved on the final draft.

Conclusions A weapon using airborne tularemia would likely result 3 to 5 days later in an outbreak of acute, undifferentiated febrile illness with incipient pneumonia, pleuritis, and hilar lymphadenopathy. Specific epidemiological, clinical, and microbiological findings should lead to early suspicion of intentional tularemia in an alert health system; laboratory confirmation of agent could be delayed. Without treatment, the clinical course could progress to respiratory failure, shock, and death. Prompt treatment with streptomycin, gentamicin, doxycycline, or ciprofloxacin is recommended. Prophylactic use of doxycycline or ciprofloxacin may be useful in the early postexposure period.

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> *I know of no other infection of animals communicable to man that can be acquired from sources so numerous and so diverse. In short, one can but feel that the status of tularemia, both as a disease in nature and of man, is one of potentiality.*—R. R. Parker[1]

Tularemia, a bacterial zoonosis, is the subject of this fifth article in a series providing recommendations for medical and public health management following use of various agents as biological weapons of terrorism.[2-5] The causative agent of tularemia, *Francisella tularensis*, is one of the most infectious pathogenic bacteria known, requiring inoculation or inhalation of as few as 10 organisms to cause disease.[6, 7] Humans become incidentally infected through diverse environmental exposures and can develop severe and sometimes fatal illness but do not transmit infection to others. The Working Group on Civilian Biodefense considers *F tularensis* to be a dangerous potential biological weapon because of its extreme infectivity, ease of dissemination, and substantial capacity to cause illness and death.[8-11]

CONSENSUS METHODS

The working group comprised 25 representatives from academic medical centers, civilian and military governmental agencies, and other public health and emergency management institutions. This group followed a specified process in developing a consensus statement. MEDLINE databases from January 1966 to October 2000 were searched using the Medical Subject Headings *Francisella tularensis*, *Pasteurella tularensis*, *biological weapon*, *biological terrorism*, *bioterrorism*, *biological warfare*, and *biowarfare*. Review of the bibliographies of these references led to identification of relevant materials published prior to 1966. In addition, participants identified other published and unpublished references and sources for review.

The first draft of the consensus statement was a synthesis of information obtained in the formal evidence-gathering process. Members of the working group were asked to make written comments on this first draft in May 1999. Subsequent revised drafts were reviewed and edited until full consensus of the working group was achieved.

HISTORY AND POTENTIAL AS A BIOLOGICAL WEAPON

Tularemia was first described as a plaguelike disease of rodents in 1911 and, shortly thereafter, was recognized as a potentially severe and fatal illness in humans.[12] Tularemia's epidemic potential became apparent in the 1930s and 1940s, when large waterborne outbreaks occurred in Europe and the Soviet Union[13-15] and epizootic-associated cases occurred in the United States.[16, 17] As well, *F tularensis* quickly gained notoriety as a virulent laboratory hazard.[18, 19] Public health concerns impelled substantial early investigations into tularemia's ecology, microbiology, pathogenicity, and prevention.[19-22]

Francisella tularensis has long been considered a potential biological weapon. It was one of a number of agents studied at Japanese germ warfare research units operating in Manchuria between 1932 and 1945[23]; it was also examined for military purposes in the West. A former Soviet Union biological weapons scientist, Ken Alibeck, has suggested that tularemia outbreaks affecting tens of thousands of Soviet and German soldiers on the eastern European front during World War II may have been the result of intentional use.[24] Following the war, there were continuing military studies of tularemia. In the 1950s and 1960s, the US military developed weapons that would disseminate *F tularensis* aerosols[10]; concurrently, it conducted research to better understand the pathophysiology of tularemia and to develop vaccines and antibiotic prophylaxis and treatment regimens. In some studies, volunteers were infected with *F tularensis* by direct aerosol delivery systems and by exposures in an aerosol chamber.[10] A live attenuated vaccine was developed that partially protected against respiratory and intracutaneous challenges with the virulent SCHU S-4 strain of *F tularensis*,[6, 7] and various regimens of streptomycin, tetracyclines, and chloramphenicol were found to be effective in prophylaxis and treatment.[25-27] By the late 1960s, *F tularensis* was one of several biological weapons stockpiled by the US military.[10] According to Alibeck, a large parallel effort by the Soviet Union continued into the early 1990s and resulted in weapons production of *F tularensis* strains engineered to be resistant to antibiotics and vaccines.[24]

In 1969, a World Health Organization expert committee estimated that an aerosol dispersal of 50 kg of virulent *F tularensis* over a metropolitan area with 5 million inhabitants would result in 250 000 incapacitating casualties, including 19 000 deaths.[28] Illness would be expected to persist for several weeks and disease relapses to occur during the ensuing weeks or months. It was assumed that vaccinated individuals would be only partially protected against an aerosol exposure. Referring to this model, the Centers for Disease Control and Prevention (CDC) recently examined the expected economic impact of bioterrorist attacks and estimated the total base costs to society of an *F tularensis* aerosol attack to be $5.4 billion for every 100 000 persons exposed.[9]

The United States terminated its biological weapons development program by executive order in 1970 and, by 1973, had destroyed its entire biological arsenal.[10] Since then, the US Army Medical Research Institute of Infectious Diseases has been responsible for defensive medical research on *F tularensis* and other potential biological warfare agents to better protect the US military, including protocols on decontamination, prophylaxis, clinical recognition, laboratory diagnosis, and medical management.[29] The CDC operates a national program for bioterrorism preparedness and response that incorporates a broad range of public health partnerships.[30, 31]

EPIDEMIOLOGY

Geographic Distribution and Human Exposures

Tularemia occurs throughout much of North America and Eurasia.[15, 21, 22, 32] In the United States, human cases have been reported from every state except Hawaii; however, most cases occur in south-central and western states (especially Missouri, Arkansas, Oklahoma, South Dakota, and Montana).[33-35] In Eurasia, the disease is also widely endemic, although the greatest numbers of human cases are reported from northern and central Europe, especially Scandinavian countries and those of the former Soviet Union.[36, 37] Tularemia is almost entirely a rural disease, although urban and suburban exposures occasionally do occur.[38-41]

Throughout its range, *F tularensis* is found in widely diverse animal hosts and habitats and can be recovered from contaminated water, soil, and vegetation.[15, 20-22, 32] A variety of small mammals, including voles, mice, water rats, squirrels, rabbits, and hares, are natural reservoirs of infection. They acquire infection through bites by ticks, flies, and mosquitoes, and by contact with contaminated environments. Although enzootic cycles of *F tularensis* typically occur without notice, epizootics with sometimes extensive die-offs of animal hosts may herald outbreaks of tularemia in humans.[16, 22, 42, 43] Humans become infected with *F tularensis* by various modes, including bites by infective arthropods,[42, 44-47] handling infectious animal tissues or fluids,[17, 48, 49] direct contact with or ingestion of contaminated water, food, or soil,[13, 20, 40, 50, 51] and inhalation of infective aerosols.[43, 52-56] Persons of all ages and both sexes appear to be equally susceptible to tularemia. Certain activities, such as hunting, trapping, butchering, and farming, are most likely to expose adult men. Laboratory workers are especially vulnerable to infection, either by accidentally inoculating themselves or by inhaling aerosolized organisms.[18, 22, 56-58] Ordinary exposures during examination of an open culture plate can cause infection. Although *F tularensis* is highly infectious and pathogenic, its transmission from person to person has not been documented.

Incidence

The worldwide incidence of tularemia is not known, and the disease is probably greatly underrecognized and underreported. In the United States, reported cases have dropped sharply from several thousand per year prior to 1950 to less than 200 per year in the 1990s.[33-35] Between 1985 and 1992, 1409 cases and 20 deaths were reported in the United States, for a mean of 171 cases per year and a case-fatality rate of 1.4%.[34] Persons in all age groups were affected, but most were children

younger than 10 years and adults aged 50 years or older. Of 1298 cases for which information on sex was available, 942 (72.6%) occurred in males, and males outnumbered females in all age groups. Most cases occur in June through September, when arthropod-borne transmission is most common.[17, 35, 59] Cases in winter usually occur among hunters and trappers who handle infected animal carcasses.[17, 35, 48] In the United States, cases are mostly sporadic or occur in small clusters[34, 35, 49]; in Eurasia, waterborne, arthropod-borne, and airborne outbreaks involving hundreds of persons have been reported.[40, 43, 44, 51, 53-55]

Natural Occurrences of Inhalational Tularemia

The largest recorded airborne tularemia outbreak occurred in 1966-1967 in an extensive farming area of Sweden.[43] This outbreak involved more than 600 patients infected with strains of the milder European biovar of *F tularensis* (*F tularensis* biovar palaearctica) [type B]), most of whom acquired infection while doing farm work that created contaminated aerosols. Case exposures and disease onsets occurred during a period of months but peaked during the winter, when rodent-infested hay was being sorted and moved from field storage sites to barns. Among 140 serologically confirmed cases thought to have been infected by inhalation, most had typical acute symptoms of fever, fatigue, chills, headache, and malaise; only 14 (10%) of confirmed patients had symptoms of pneumonia, such as dyspnea and chest pains. Patients generally responded well to tetracycline, and no deaths were reported. Inhalational tularemia in the United States has involved only single cases or small clusters of cases, variously resulting from laboratory exposures,[18, 56, 57] disturbance of contaminated animal carcasses,[38, 39, 41] and suspected infective environmental aerosols.[41, 52] Cases of inhalational tularemia in the United States are thought to be due mostly to the more virulent *F tularensis* biovar tularensis (type A) and usually follow an acute and severe course, with prominent pneumonitis. Some cases, however, have radiographic evidence of pleuropneumonia with minimal or absent respiratory signs on physical examination.[39, 41, 52]

Although airborne *F tularensis* would be expected to principally cause primary pleuropneumonic infection, some exposures might contaminate the eye, resulting in ocular tularemia; penetrate broken skin, resulting in ulceroglandular or glandular disease; or cause oropharyngeal disease with cervical lymphadenitis. In the aforementioned Swedish outbreak, conjunctivitis was reported in 26% of 140 confirmed cases and an infected ulcer of the skin was reported in nearly 12%; pharyngitis was reported in 31% and oral ulcers in about 9% of the cases; and 32% of these patients had various exanthemas, such as erythema multiforme and erythema nodosum.[43] Tularemia outbreaks arising from similar agricultural exposures have been reported from Finland,[53] mostly presenting with general constitutional symptoms rather than specific manifestations of pneumonia; enlargement of hilar nodes was the principal radiographic finding in these cases.[54]

Inhalational Tularemia Following Use as a Biological Weapon

Although *F tularensis* could be used as a weapon in a number of ways, the working group believes that an aerosol release would have the greatest adverse medical and public health consequences. Release in a densely populated area would be expected to result in an abrupt onset of large numbers of cases of acute, nonspecific febrile illness beginning 3 to 5 days later (incubation range, 1-14 days), with pleuropneumonitis developing in a significant proportion of cases during the ensuing days and weeks. Public health authorities would most likely become aware of an outbreak of unusual respiratory disease in its early stages, but this could be difficult to distinguish from a natural outbreak of community-acquired infection, especially influenza or various atypical pneumonias. The abrupt onset of large numbers of acutely ill persons, the rapid progression in a relatively high proportion of cases from upper respiratory symptoms and bronchitis to life-threatening pleuropneumonitis and systemic infection affecting, among others, young, previously healthy adults and children should, however, quickly alert medical professionals and public health authorities to a critical and unexpected public health event and to bioterrorism as a possible cause (Table 1). Until the etiology became clear, clinicians would need to work closely with epidemiologists and diagnostic laboratories to differentiate the illness from various community-acquired pneumonias and to determine if it could have resulted from use of one of several potential bioterrorism weapons agents, such as those causing tularemia, plague, anthrax, or Q fever.[2, 4, 29]

In general, tularemia would be expected to have a slower progression of illness and a lower case-fatality rate than either inhalational plague or anthrax. Plague would most likely progress very rapidly

to severe pneumonia, with copious watery or purulent sputum production, hemoptysis, respiratory insufficiency, sepsis, and shock.[4] Inhalational anthrax would be differentiated by its characteristic radiological findings of prominent symmetric mediastinal widening and absence of bronchopneumonia.[2] As well, anthrax patients would be expected to develop fulminating, toxic, and fatal illness despite antibiotic treatment.[29] Milder forms of inhalational tularemia could be clinically indistinguishable from Q fever; establishing a diagnosis of either would be problematic without reference laboratory testing. Presumptive laboratory diagnoses of plague or anthrax would be expected to be made relatively quickly, although microbiological confirmation could take days. Isolation and identification of *F tularensis* using routine laboratory procedures could take several weeks.

Once a substantial cluster of cases of inhalational tularemia had been identified, epidemiological findings should suggest a bioterrorist event. The abrupt onset and single peak of cases would implicate a point-source exposure without secondary transmission. Among exposed persons, attack rates would likely be similar across sex and age groups, and risk would be related to degree of exposure to the point source (Table 1). An outbreak of inhalational tularemia in an urban setting should trigger a high level of suspicion of an intentional event, since all reported inhalational tularemia outbreaks have occurred in rural areas.

MICROBIOLOGY AND VIRULENCE FACTORS

Francisella tularensis is a small, nonmotile, aerobic, gram-negative coccobacillus. It has a thin lipopolysaccharide-containing envelope and is a hardy non–spore-forming organism that survives for weeks at low temperatures in water, moist soil, hay, straw, and decaying animal carcasses.[21, 22, 60, 61] *Francisella tularensis* has been divided into 2 major subspecies (biovars) by virulence testing, biochemical reactions, and epidemiological features.[62] *Francisella tularensis* biovar tularensis (type A) may be highly virulent in humans and animals, produces acid from glycerol, demonstrates citrulline ureidase activity, and is the most common biovar isolated in North America.[22, 60] *Francisella tularensis* biovar palaearctica (type B) is relatively avirulent, does not produce acid from glycerol, and does not demonstrate citrulline ureidase activity. In Europe and Asia, all human tularemia is thought to be caused by the milder type B strains, although recent studies there have identified naturally occurring *F tularensis* related to *F tularensis* biovar tularensis.[63, 64] A few rapidly growing strains of *F tularensis* have been recovered from the blood of immunocompromised patients not showing seroreactivity to *F tularensis*.[65]

Transformed plasmids have been engineered to express chloramphenicol and tetracycline resistance in *F tularensis*.[66] Virulent, streptomycin-resistant *F tularensis* strains have been examined in biowarfare agent studies both in the United States and the Soviet Union.[24, 27, 56] Although *F tularensis* virulence factors are poorly understood and characterized,[67, 68] it is possible that strain virulence could be enhanced through laboratory manipulation.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Pathogenesis

Francisella tularensis can infect humans through the skin, mucous membranes, gastrointestinal tract, and lungs. It is a facultative intracellular bacterium that multiplies within macrophages.[68, 69] The major target organs are the lymph nodes, lungs and pleura, spleen, liver, and kidney.[19, 20, 49, 70-72] Untreated, bacilli inoculated into skin or mucous membranes multiply, spread to the regional lymph nodes and further multiply, and may then disseminate to organs throughout the body. Bacteremia may be common in the early phase of infection. The initial tissue reaction to infection is a focal, intensely suppurative necrosis consisting largely of accumulations of polymorphonuclear leukocytes, followed by invasion of macrophages, epithelioid cells, and lymphocytes. Suppurative lesions become granulomatous, and histopathological examination of the granulomas shows a central necrotic, sometimes caseating zone surrounded by a layer of epithelioid cells, multinucleated giant cells, and fibroblasts in a radial arrangement, typical of other granulomatous conditions, such as tuberculosis and sarcoidosis.[20, 70, 71]

Monkeys that inhaled the virulent SCHU S-4 strain of *F tularensis* (type A) developed acute bronchiolitis within 24 hours of exposure to 1-µm particles and within 48 hours of exposure to 8-µm particles.[73] By 72 hours following challenge, inflammation was present in peribronchial tissues and alveolar septa. Bronchopneumonia was most pronounced in animals exposed to the smaller particles and was characterized by tracheobronchial lymph node enlargement and reddish, firm, 0.2- to 0.5-cm-diameter discrete inflammatory lesions scattered throughout the lungs. In the absence of treatment, the disease progressed to pneumonic consolidation and organization, granuloma formation, and eventual chronic interstitial fibrosis.

Humans with inhalational exposures also develop hemorrhagic inflammation of the airways early in the course of illness, which may progress to bronchopneumonia.[54] Histopathological examination of affected lungs shows alveolar spaces filled with an exudate of mononuclear cells. Pleuritis with adhesions and effusion and hilar lymphadenopathy are common radiological and pathological findings.[70, 72]

Clinical Manifestations

The primary clinical forms of tularemia vary in severity and presentation according to virulence of the infecting organism, dose, and site of inoculum. Primary disease presentations include ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, typhoidal, and septic forms.[19, 20, 49, 70, 72, 74, 75] The term *typhoidal tularemia* has been used to describe illness in tularemia patients with systemic infections manifesting as fever and other constitutional signs without cutaneous or mucosal membrane lesions or regional lymphadenitis. Sometimes, these patients present with prominent gastrointestinal manifestations, such as diarrhea and pain. Confusion is created when *typhoidal tularemia* is used to describe the illness in patients infected by inhalation, especially when there are signs of pleuropneumonic disease; this usage can be misleading and has been discouraged.[54, 75]

The onset of tularemia is usually abrupt, with fever (38°C-40°C), headache, chills and rigors, generalized body aches (often prominent in the low back), coryza, and sore throat. A pulse-temperature dissociation has been noted in as many as 42% of patients.[49] A dry or slightly productive cough and substernal pain or tightness frequently occur with or without objective signs of pneumonia, such as purulent sputum, dyspnea, tachypnea, pleuritic pain, or hemoptysis.[7, 19, 26, 70, 74] Nausea, vomiting, and diarrhea sometimes occur. Sweats, fever and chills, progressive weakness, malaise, anorexia, and weight loss characterize the continuing illness. Studies of volunteers have shown that *F tularensis* aerosol exposures can incapacitate some persons in the first 1 or 2 days of illness, and significant impairment in performing tasks can continue for days after antibiotic treatment is begun.[76] In untreated tularemia, symptoms often persist for several weeks and, sometimes, for months, usually with progressive debility. Any form of tularemia may be complicated by hematogenous spread, resulting in secondary pleuropneumonia, sepsis, and, rarely, meningitis.[74, 77]

Prior to the advent of antibiotics, the overall mortality from infections with the more severe type A strains was in the range of 5% to 15%, and fatality rates as high as 30% to 60% were reported for untreated pneumonic and severe systemic forms of disease.[72, 78] Currently, the overall case-fatality rate of reported cases in the United States is less than 2%.[34, 49] Type B infections are rarely fatal.

In ulceroglandular tularemia, the form that typically arises from handling a contaminated carcass or following an infective arthropod bite, a local cutaneous papule appears at the inoculation site at about the time of onset of generalized symptoms, becomes pustular, and ulcerates within a few days of its first appearance. The ulcer is tender, generally has an indolent character, and may be covered by an eschar. Typically, one or more regional afferent lymph nodes may become enlarged and tender within several days of the appearance of the papule. Even with antibiotic treatment, the affected nodes may become fluctuant and rupture. In oculoglandular tularemia, which follows direct contamination of the eye, ulceration occurs on the conjunctiva, accompanied by pronounced chemosis, vasculitis, and regional lymphadenitis. Glandular tularemia is characterized by lymphadenopathy without an ulcer.

Oropharyngeal tularemia is acquired by drinking contaminated water, ingesting contaminated food, and, sometimes, by inhaling contaminated droplets or aerosols.[14, 20, 36, 43, 50, 51, 79] Affected persons may develop stomatitis but more commonly develop exudative pharyngitis or tonsillitis, sometimes with ulceration. Pronounced cervical or retropharyngeal lymphadenopathy may occur (Figure 1).[74, 79]

Tularemia pneumonia can be the direct result of inhaling contaminated aerosols or be secondary to hematogenous spread from a distal site. An aerosol release of *F tularensis* would be expected to result in acute illness with signs and symptoms of 1 or more of pharyngitis, bronchiolitis, pleuropneumonitis, and hilar lymphadenitis, accompanied by various manifestations of systemic illness. Inhalational exposures, however, commonly result in an initial clinical picture of systemic illness without prominent signs of respiratory disease.[7, 43, 53, 56] The earliest pulmonary radiographic findings of inhalational tularemia may be peribronchial infiltrates, typically advancing to bronchopneumonia in 1 or more lobes, and often accompanied by pleural effusions and hilar lymphadenopathy (Figure 2).[72, 75] Signs may, however, be minimal or absent, and some patients will show only 1 or several small, discrete pulmonary infiltrates or scattered granulomatous lesions of lung parenchyma or pleura. Although volunteers challenged with aerosols of virulent *F tularensis* (type A) regularly developed systemic symptoms of acute illness 3 to 5 days following exposure, only 25% to 50% of participants had radiological evidence of pneumonia in the early stages of infection.[7, 26] On the other hand, pulmonary infection can sometimes rapidly progress to severe pneumonia, respiratory failure, and death.[72, 80] Lung abscesses occur infrequently.[75]

Typhoidal tularemia is used to describe systemic illness in the absence of signs indicating either site of inoculation or anatomic localization of infection. This should be differentiated from inhalational tularemia with pleuropneumonic disease.[54, 75]

Tularemia sepsis is potentially severe and fatal. As in typhoidal tularemia, nonspecific findings of fever, abdominal pain, diarrhea, and vomiting may be prominent early in the course of illness. The patient typically appears toxic and may develop confusion and coma. Unless treated promptly, septic shock and other complications of systemic inflammatory response syndrome may ensue, including disseminated intravascular coagulation and bleeding, acute respiratory distress syndrome, and organ failure.[80]

DIAGNOSIS

Tularemia in humans occurs infrequently, resulting in a low index of diagnostic suspicion among clinicians and laboratorians. Since rapid diagnostic testing for tularemia is not widely available, the first indication of intentional tularemia might follow recognition by public health authorities of a clustering of acute, severe respiratory illness with unusual epidemiological features (Table 1). Suspicion of tularemia might be triggered in alert clinicians encountering patients with findings of atypical pneumonia, pleuritis, and hilar lymphadenopathy. Identification of *F tularensis* in clinical specimens may be missed or delayed for days or weeks when procedures for routine microbiological screening of

bacterial pathogens are followed, and it is unlikely that a serendipitous laboratory identification would be the sentinel event that alerted authorities to a major bioterrorism action.

Physicians who suspect inhalational tularemia should promptly collect specimens of respiratory secretions and blood and alert the laboratory to the need for special diagnostic and safety procedures. *Francisella tularensis* may be identified by direct examination of secretions, exudates, or biopsy specimens using direct fluorescent antibody or immunohistochemical stains.[81-83] By light microscopy, the organism is characterized by its small size (0.2 μm ✕ 0.2-0.7 μm), pleomorphism, and faint staining. It does not show the bipolar staining characteristics of *Yersinia pestis*,[4] the agent of plague, and is easily distinguished from the large gram-positive rods characteristic of vegetative forms of *Bacillus anthracis* (Figure 3).[2] Microscopic demonstration of *F tularensis* using fluorescent-labeled antibodies is a rapid diagnostic procedure performed in designated reference laboratories in the National Public Health Laboratory Network; test results can be made available within several hours of receiving the appropriate specimens if the laboratory is alerted and prepared. Suspicion of inhalational tularemia must be promptly reported to local or state public health authorities so timely epidemiological and environmental investigations can be made (BOX).

Growth of *F tularensis* in culture is the definitive means of confirming the diagnosis of tularemia.[60, 81] *Francisella tularensis* can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalational tularemia.[56] It is only occasionally isolated from the blood. *Francisella tularensis* grows best in cysteine-enriched broth and thioglycollate broth and on cysteine heart blood agar, buffered charcoal-yeast agar, and chocolate agar. Selective agar (such as chocolate agar selective for *Neisseria gonorrhea* isolation) may be useful when culturing materials from nonsterile sites, such as sputum. Inoculated media should be incubated at 37°C. Although growth may be visible as early as 24 to 48 hours after inoculation, growth may be delayed and cultures should be held for at least 10 days before discarding. Under ideal conditions, bacterial colonies on cysteine-enriched agar are typically 1 mm in diameter after 24 to 48 hours of incubation and 3 to 5 mm in diameter by 96 hours.[60, 81] On cysteine heart agar, *F tularensis* colonies are characteristically opalescent and do not discolor the medium (Figure 4).

Antigen detection assays, polymerase chain reaction, enzyme-linked immunoassays, immunoblotting, pulsed-field gel electrophoresis, and other specialized techniques may be used to identify *F tularensis* and to characterize strains.[84-87] These procedures are usually performed only in research and reference laboratories, however. In laboratories where advanced methods are established, results of antigen detection and polymerase chain reaction analyses can be obtained within several hours of receipt of isolates. Typically, serum antibody titers do not attain diagnostic levels until 10 or more days after onset of illness, and serology would provide minimal useful information for managing an outbreak. Serological confirmation of cases, however, may be of value for forensic or epidemiological purposes. Most laboratories use tube agglutination or microagglutination tests that detect combined immunoglobulin M and immunoglobulin G.[84, 85] A 4-fold change in titer between acute and convalescent serum specimens, a single titer of at least 1:160 for tube agglutination or 1:128 for microagglutination is diagnostic for *F tularensis* infection. Information on reference diagnostic testing and shipping/handling of specimens can be obtained from state public health laboratories and from the Division of Vector-Borne Infectious Diseases, CDC, Fort Collins, Colo (telephone: [970] 221-6400; e-mail: dvbid@cdc.gov).

VACCINATION

Beginning in the 1930s, the Soviet Union used a live attenuated vaccine to immunize tens of millions of persons living in tularemia-endemic areas.[88] In the United States, a live attenuated vaccine derived from the avirulent live vaccine strain has been used to protect laboratorians routinely working with *F tularensis*; until recently, this vaccine was available as an investigational new drug.[89] It is currently

under review by the US Food and Drug Administration (FDA), and its future availability is undetermined.

In a retrospective study of civilians working with *F tularensis* at a US Army research facility, the incidence of accidental acute inhalational tularemia among laboratorians declined from 5.70 cases per 1000 person-years of risk at a time when a killed vaccine was in use to 0.27 cases per 1000 person-years of risk after introduction of the live vaccine.[58] Although the incidence of ulceroglandular disease remained unchanged in the 2 periods, signs and symptoms were considered milder among those who received the live vaccine. In volunteer studies, the live attenuated vaccine did not protect all recipients against aerosol challenges with virulent *F tularensis.*[7, 26]

Correlates of protective immunity appear about 2 weeks following natural infection or vaccination. Given the short incubation period of tularemia and incomplete protection of current vaccines against inhalational tularemia, vaccination is not recommended for postexposure prophylaxis. The working group recommends use of the live vaccine strain only for laboratory personnel routinely working with *F tularensis*.

TREATMENT

Contained Casualty Situation

Adults
In a contained casualty situation, in which logistics permit individual medical management, the working group recommends parenteral antimicrobial therapy for tularemia (Table 2). Streptomycin is the drug of choice.[49, 74, 90, 91] Gentamicin, which is more widely available and may be used intravenously, is an acceptable alternative.[49, 74, 90-93] Treatment with aminoglycosides should be continued for 10 days. Tetracyclines and chloramphenicol are also used to treat tularemia[49, 74, 90]; however, relapses and primary treatment failures occur at a higher rate with these bacteriostatic agents than with aminoglycosides, and they should be given for at least 14 days to reduce chance of relapse.[27, 74, 90] Fluoroquinolones, which have intracellular activity, are promising candidates for treating tularemia. Ciprofloxacin, which is not labeled for use in tularemia, has been shown to be active against *F tularensis* in vitro[94] and in animals[95] and has been used to successfully treat tularemia in both adults and children.[90, 94, 96, 97] Treatment with ciprofloxacin should be continued for 10 days. In persons beginning treatment with parenteral doxycycline, ciprofloxacin, or chloramphenicol, therapy can be switched to oral antibiotic administration when clinically indicated. Very limited experiences in treating tularemia patients with β-lactam and macrolide antibiotics have been reported, and treatment failures have occurred.[98] Use of β-lactam and macrolide antibiotics in treating tularemia is neither FDA-approved nor recommended by the working group.

Children
In children, streptomycin or gentamicin is recommended by the working group as first-line treatment in a contained casualty situation (Table 2). Doxycycline, ciprofloxacin ($\leq$1 g/d), and chloramphenicol can be used as alternatives to aminoglycosides. Fluoroquinolones have been reported to cause cartilage damage in immature animals and are not FDA-approved for use in children. However, short courses of these agents have not been associated with arthropathy in pediatric patients, and the potential risks of their use must be weighed against their benefits in treating serious infections.[96, 99, 100]

Mass Casualty Situation

Doxycycline and ciprofloxacin, administered orally, are the preferred choices for treatment in the mass casualty setting, for both adults and children (Table 3). The ciprofloxacin dosage for children should

not exceed 1 g/d. In a mass casualty situation, the working group believes the benefits to children from short courses of doxycycline or fluoroquinolones (Table 3) outweigh the risks of their use.

Since it is unknown whether drug-resistant organisms might be used in a bioterrorist event, antimicrobial susceptibility testing of isolates should be conducted quickly and treatments altered according to test results and clinical responses.

Antibiotics for treating patients infected with tularemia in a bioterrorism scenario are included in a national pharmaceutical stockpile maintained by the CDC, as are ventilators and other emergency equipment needed to respond to situations of large numbers of critically ill persons that strip local and state resources.[30]

Management of Special Groups

Pregnant Women
In a contained casualty situation, short courses of gentamicin are likely to pose a low risk to fetuses when used to treat tularemia in pregnant women (Table 2). Rare cases of fetal nerve deafness and renal damage have been reported with other aminoglycosides but have not been reported with gentamicin. The benefits of gentamicin in treating pregnant women with tularemia are expected to outweigh any potential risk to fetuses. In a mass casualty situation, oral ciprofloxacin is considered the best alternative to gentamicin for pregnant women (Table 3).

Immunosuppressed Persons
There is scant experience in treating tularemia in immunocompromised patients. However, considering the greater occurrence in immunocompetent patients of tularemia relapses and treatment failures following use of bacteriostatic antimicrobial agents compared with aminoglycosides, streptomycin or gentamicin should be used when possible to treat patients with known immune dysfunction in either contained casualty or mass casualty situations (Table 2).

POSTEXPOSURE ANTIBIOTIC RECOMMENDATIONS

Persons beginning treatment with streptomycin, gentamicin, doxycycline, or ciprofloxacin in the incubation period of tularemia and continuing treatment daily for 14 days might be protected against symptomatic infection. In studies of aerosol challenge with infective doses of the virulent SCHU S-4 strain of *F tularensis*, each of 8 volunteers given oral dosages of tetracycline, 1 g/d for 28 days, and each of 8 volunteers given tetracycline, 2 g/d for 14 days, were fully protected when treatment was begun 24 hours following challenge.[27] Two of 10 volunteers given tetracycline, 1 g/d for only 5 days, developed symptomatic tularemia after antibiotic treatment was stopped.

In the unlikely event that authorities quickly become aware that an *F tularensis* biological weapon has been used and are able to identify and reach exposed persons during the early incubation period, the working group recommends that exposed persons be prophylactically treated with 14 days of oral doxycycline or ciprofloxacin (Table 3). In a circumstance in which the weapon attack has been covert and the event is discovered only after persons start to become ill, persons potentially exposed should be instructed to begin a fever watch. Persons who develop an otherwise unexplained fever or flulike illness within 14 days of presumed exposure should begin treatment as outlined in Table 2 and Table 3.

In the laboratory, persons who have had potentially infective exposures to *F tularensis* should be administered oral postexposure antibiotic prophylaxis if the risk of infection is high (eg, spill, centrifuge accident, or needlestick). If the risk is low, exposed persons can be placed on a fever watch and treated if they develop symptoms.

Postexposure prophylactic antibiotic treatment of close contacts of tularemia patients is not recommended since human-to-human transmission of *F tularensis* is not known to occur.

INFECTION CONTROL

Isolation is not recommended for tularemia patients, given the lack of human-to-human transmission. In hospitals, standard precautions[101] are recommended by the working group for treatment of patients with tularemia.

Microbiology laboratory personnel should be alerted when tularemia is clinically suspected. Routine diagnostic procedures can be performed in biological safety level 2 (BSL-2) conditions. Examination of cultures in which *F tularensis* is suspected should be carried out in a biological safety cabinet. Manipulation of cultures and other activities involving infectious materials with a potential for aerosol or droplet production (centrifuging, grinding, vigorous shaking, growing cultures in volume, animal studies) require BSL-3 conditions.[102] When *F tularensis* is presumptively identified in a routine BSL-2 clinical laboratory (level A), specimens should be forwarded to a BSL-3 laboratory (level B) (eg, a state public health laboratory) for confirmation of agent and other studies, such as antimicrobial susceptibility testing.[11] Bodies of patients who die of tularemia should be handled using standard precautions. Autopsy procedures likely to cause aerosols, such as bone sawing, should be avoided. Clothing or linens contaminated with body fluids of patients infected with *F tularensis* should be disinfected per standard precautions protocols.[101]

ENVIRONMENTAL DECONTAMINATION AND PROTECTION

Under natural conditions, *F tularensis* may survive for extended periods in a cold, moist environment. The working group lacks information on survival of intentionally dispersed particles but would expect a short half-life due to desiccation, solar radiation, oxidation and other environmental factors, and a very limited risk from secondary dispersal. In circumstances of a laboratory spill or intentional use in which authorities are concerned about an environmental risk (eg, inanimate surfaces wet with material thought to contain *F tularensis*), decontamination can be achieved by spraying the suspected contaminant with a 10% bleach solution (1 part household bleach and 9 parts water). After 10 minutes, a 70% solution of alcohol can be used to further clean the area and reduce the corrosive action of the bleach. Soap water can be used to flush away less hazardous contaminations. Persons with direct exposure to powder or liquid aerosols containing *F tularensis* should wash body surfaces and clothing with soap water. Standard levels of chlorine in municipal water sources should protect against waterborne infection.[60] Following an urban release, the risk to humans of acquiring tularemia from infected animals or arthropod bites is considered minimal and could be reduced by educating the public on simple avoidance of sick or dead animals and on personal protective measures against biting arthropods.

ADDITIONAL RESEARCH

Simple, rapid, and reliable diagnostic tests that could be used to identify persons infected with *F tularensis* in the mass exposure setting need to be developed. Further methods should be designed to rapidly define the molecular genetic characteristics of organisms, especially as they may relate to engineered attributes, such as enhanced virulence and resistance to antimicrobial agents or normally lethal environmental conditions. Complete sequencing and analysis of the genome of natural strains of *F tularensis* would provide an archival base for understanding genetic variants, functions of genes, and mechanisms of action useful in developing means to protect against *F tularensis*. Research is also needed to develop accurate and reliable procedures to rapidly detect *F tularensis* in environmental samples.

New technologies should be explored for developing active (eg, DNA-based) or passive (eg, monoclonal antibody–based) vaccines for rapid preexposure or postexposure protection.

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Box. Clinicians Caring for Patients With Suspected Tularemia Should Immediately Contact Their:

(1) Hospital epidemiologist or infection control practitioner and

(2) Local or state health departments

Consult your local telephone operator, the telephone directory under "governmental listings," or the Internet at http://www.cdc.gov/other.htm#states or http://www.astho.org/state.html

If the local and state health departments are unavailable, contact the Centers for Disease Control and Prevention at (970) 221-6400 or http://www.cdc.gov/ncidod/dvbid/dvbid.htm

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