Tularemia is an acute febrile zoonotic disease caused by the bacterium *Francisella tularensis*. Shortly after the initial isolation of the organism in Tulare County, Calif. (McCoy & Chapin 1912), the first case of a human infection was reported from Cincinnati, Ohio (Wherry & Lamb 1914). The first documented waterborne outbreak occurred in Russia (Karpoff & Antonoff 1936). Drinking water has since been implicated as the source of infection in numerous published case reports and outbreak investigations. *F. tularensis* is considered a bioterrorism agent (Dennis et al. 2001); thus, from the water supply perspective, the organism has the potential to be associated with naturally occurring waterborne outbreaks or intentional acts of contamination (Khan et al. 2001, Burrows & Renner 1999). This review focuses on tularemia acquired from contaminated drinking water and the adequacy of control measures for preventing waterborne transmission.

**THE ORGANISM**

*F. tularensis* (formerly classified as *Bacterium tularense* and *Pasturella tularensis*) is a Gram-negative, small (0.2 × 0.2 × 0.7 μm), nonmotile, coccobacillus that requires cysteine and bioavailable iron for in vitro cultivation. A member of the taxonomic class Gammaproteobacteria, *Francisella* is the only genus in the family *Francisellaceae*. The organism grows as greenish–white colonies of butyrous consistency on chocolate agar media (see the photograph on this page). In the United States, it is classified as a Tier 1 select agent pathogen by the Federal Select Agent Program (www.selectagents.gov/SelectAgentsandToxinsList.html). Because of its highly infectious nature and strict biosafety requirements, studies with virulent strains of *F. tularensis* must be conducted in biosafety-level 3 laboratory facilities.

The three widely accepted subspecies (subsp.) are *F. tularensis* subsp. *tularensis* (type A), *F. tularensis* subsp. *bolarctica* (type B), and *F. tularensis* subsp. *mediasiatica* (Larson et al. 2011). *F. tularensis* subsp. *tularensis* is the more virulent subspecies and, with few exceptions (Johansson et al. 2000, Guryćová 1998), it has been reported only from North America. *F. tularensis* subsp. *bolarctica* is found throughout the northern hemisphere and is the predominant subspecies associated with water. Mortality associated with infection of *F. tularensis* subsp. *bolarctica* is low. This subspecies has also recently been reported from Tasmania, Australia (Jackson et al. 2012). The third subspecies, *F. tularensis* subsp. *mediasiatica*, is restricted to Central Asia. Genotyping procedures have been used to further divide these subspecies into various taxonomic clades. *F. novicida* is another member of this genus, but there is contention over whether it should be considered as a separate species or transferred to the rank of subspecies (Johansson et al. 2010). *F. tularensis* is genetically monomorphic with a >99% sequence similarity between subspecies *tularensis* and *bolarctica* (Larson et al. 2011).
The organisms *F. novicida* and *F. philomiragia* have also been associated with water, but rarely cause human illness. Studies have reported on the occurrence of a wide diversity of *Francisella*-like organisms from environmental samples; however, more information will be needed to determine whether any of these novel species have the potential to serve as human pathogens (Whitehouse et al. 2012, Barns et al. 2005).

**THE DISEASE**

Tularemia occurs predominantly in the northern hemisphere between latitude 30 and 70°N (Dlugaczyk et al. 2010). The etiologic agent can be transmitted by a variety of means, but transmission from person to person has not been documented (Dennis et al. 2001). Common modes of transmission include direct contact with infected animals, bites from blood-feeding arthropods, and ingestion of contaminated food and water. There are several clinical forms of the disease, and these are often dictated by the mode of transmission. The six widely accepted forms or clinical presentations are glandular, ocular, oropharyngeal, pneumatic, typhoidal, and ulceroglandular. The oropharyngeal form is most commonly observed for infections acquired by ingestion of contaminated water. Severe sore throat/tonsillitis, exudative pharyngitis, fever, and swollen cervical lymph nodes (see the photograph on this page), which may exhibit suppuration or require surgical intervention, are symptoms of oropharyngeal tularemia. This clinical presentation is rather unusual for a pathogenic organism, typhoidal, and ulceroglandular. The oropharyngeal form is most commonly observed for infections acquired by ingestion of contaminated water. Severe sore throat/tonsillitis, exudative pharyngitis, fever, and swollen cervical lymph nodes (see the photograph on this page), which may exhibit suppuration or require surgical intervention, are symptoms of oropharyngeal tularemia. This clinical presentation is rather unusual for a pathogen acquired by ingestion of contaminated water and differs from disease symptoms normally associated with waterborne pathogens. Gastrointestinal symptoms can occur with accompanying diarrhea and intestinal lesions, but a relatively high threshold dose is required for infection and would likely only be associated with ingestion of highly contaminated food or water. The 50% infective dose (the dose required for 50% infectivity) required for gastrointestinal infection in humans has been reported to be $10^8$ colony-forming units (cfu) (Hornick et al. 1966). The organism exhibits a degree of innate acid resistance (Humrighouse et al. 2011), but laboratory studies have found that the bacterium is susceptible to conditions that would normally be encountered in the human stomach (Adcock et al. 2014). Gastrointestinal symptoms can sometimes also result from tularemia acquired by means other than ingestion, such as arthropod bites—e.g., ticks (Zaidi & Singer 2002).


The severity of the illness can be ameliorated by prompt administration of antibiotics. Infected individuals normally respond well to a 10-day course of treatment of an aminoglycoside such as streptomycin or gentamicin. Ciprofloxacin and doxycycline have also been used as chemotherapeutic agents. A seven-day regimen of these latter two antibiotics has been prescribed for post-exposure prophylaxis (Adalja et al. 2015). Currently there is no vaccine readily available for prevention of this disease in the United States.

**DRINKING WATER OUTBREAKS**

Drinking water outbreaks of tularemia have been reported from Bulgaria, Georgia, Germany, Italy, Kosovo, Norway, Russia, Sweden, Turkey, and the United States. A list of these reports published in the English literature is provided in Table 1. The largest number of outbreaks have occurred in Turkey, and two review articles (Akalin et al. 2009, Helvaci et al. 2000) summarize many of these outbreaks, including those cited only in the Turkish literature. The role of drinking water in outbreaks in Kosovo is discussed in review articles by Grunow and Finke (2002) and Reintjes et al. (2002). In an earlier comprehensive review by Pollitzer (1967), numerous outbreaks attributed to contaminated drinking water in the former Soviet Union were reported from White Russia, Eastern Siberia, Armenia, Kazakhstan, and the Altai Mountain region.

Since the majority of outbreaks have occurred in Eurasia, it is assumed that most have been caused by *F. tularensis* subsp. *bolarctica* because subsp. *tularensis* has only rarely been reported from this geographic region. In several of these outbreaks, the causative agent has been subtyped to the subspecies level and in all instances was found to be *F. tularensis* subsp. *bolarctica* (Gürçan et al. 2006, Kantardjiev et al. 2006, Christova et al. 2004, Gürçan et al. 2004, Hoel et al. 1991).

Various water sources have been implicated in these outbreaks; among these are community water systems, reservoirs, rivers,
springs, streams, and wells (Table 1). Invariably these were sources that were untreated or had only intermittent or inadequate disinfection. In some instances, the drinking water was reported to be of unsanitary quality on the basis of the presence of coliform bacteria (Meric et al. 2010; Willke et al. 2009; Gürcan et al. 2006, 2004; Nordahl et al. 1993). Seasonally, the majority of these incidences have occurred in late fall, winter, or early spring (Gürcan et al. 2004).

DETECTION IN WATER

The detection of the causative agent of a waterborne outbreak in the implicated water source can be a challenging task. Though a fastidious organism in its growth requirements, F. tularensis has been identified in water in several outbreak reports and ecological studies. A variety of detection methodologies, occasionally preceded by sample concentration, have been used, including animal inoculation, bacterial culture, molecular assays, and immunoassays.

Concentration and preliminary treatment of samples have been employed in some investigations. Meric et al. (2010) used membrane filtration and found the procedure to be superior to centrifugation as a means of concentration prior to analysis. Similar procedures were used by Şimşek et al. (2012) in an occurrence study in environmental waters. Using an ultrafiltration procedure, Francy et al. (2009) were able to concentrate the attenuated strain (F. tularensis subsp. holarctica live vaccine strain [LVS]) spiked at approximately $10^5$ cfu into 100 L of municipal drinking water and well water. Recoveries varied between 0.2 and 40.4% on the basis of culture results. In a study designed to evaluate the use of ultrafiltration prior to culturing for a variety of microorganisms, it was reported that F. tularensis was one of the most difficult organisms to recover. The addition of 1% weight per volume ammonium chloride to the ultrafiltration concentrates was reported to increase recovery (USEPA 2011).

Animal inoculation followed by bacteriological culture of infected tissues has been used for initial isolation of F. tularensis from water. In this approach, a sample is injected into an animal. If the animal succumbs to the infection, the animal is dissected, and the tissue is examined by bacteriological culture to isolate

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Implicated Water Source—Comments</th>
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<tbody>
<tr>
<td>Bulgaria</td>
<td>Kantardjiev et al. 2006</td>
<td>Well—food also implicated</td>
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<td></td>
<td>Christova et al. 2004</td>
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<td>Georgia</td>
<td>Chitadze et al. 2009</td>
<td>Community water supply</td>
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<td>Germany</td>
<td>Dlugaczyk et al. 2010</td>
<td>Surface water</td>
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<td>Italy</td>
<td>Mignani et al. 1988</td>
<td>Community water supply</td>
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<td></td>
<td>Greco et al. 1987</td>
<td>Community water supply—spring source</td>
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<tr>
<td>Kosovo</td>
<td>Grunow &amp; Finke 2002</td>
<td>Unspecified water source—food also implicated</td>
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<tr>
<td></td>
<td>Reinjes et al. 2002</td>
<td>Unspecified water source—food also implicated</td>
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<tr>
<td>Norway</td>
<td>Larsen et al. 2011</td>
<td>Well</td>
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<td></td>
<td>Brantsæter et al. 2007</td>
<td>Well</td>
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<td></td>
<td>Berdal et al. 2000</td>
<td>Well</td>
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<td></td>
<td>Nordahl et al. 1993</td>
<td>Well—contaminated ice covering implicated</td>
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<td></td>
<td>Hoel et al. 1991</td>
<td>Well</td>
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<td></td>
<td>Mair et al. 1973</td>
<td>Well—perhaps other water sources</td>
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<tr>
<td>Russia</td>
<td>Pollitzer 1967</td>
<td>Review of outbreaks in former USSR</td>
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<td></td>
<td>Karpoff &amp; Antonoff 1936</td>
<td>Stream—first recorded outbreak</td>
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<td>Sweden</td>
<td>Tärnvik et al. 1997</td>
<td>Well</td>
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<td>Turkey</td>
<td>Küpeli &amp; Tekin 2011</td>
<td>River</td>
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<td></td>
<td>Meric et al. 2010</td>
<td>Reservoir</td>
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<td></td>
<td>Akalin et al. 2009</td>
<td>Review of Turkish outbreaks</td>
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<td></td>
<td>Şencan et al. 2009</td>
<td>Springs and well</td>
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<td>Willke et al. 2009</td>
<td>Spring</td>
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<td></td>
<td>Celebi et al. 2008</td>
<td>Spring</td>
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<td></td>
<td>Leblebioglu et al. 2008</td>
<td>Spring and community water supply—nonfunctioning chlorinator</td>
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<td>Kandemir et al. 2007</td>
<td>Unspecified water source</td>
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<td>Ozdemir et al. 2007</td>
<td>Community water supply</td>
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<td>Sahin et al. 2007</td>
<td>Reservoir—inadequate chlorination</td>
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<td></td>
<td>Celebi et al. 2006</td>
<td>Unspecified water source</td>
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<td></td>
<td>Gürcan et al. 2006, 2004</td>
<td>Spring and reservoir—intermittent chlorination</td>
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<td></td>
<td>Karadenizli et al. 2005</td>
<td>Stream and community water supply—unspecified treatment</td>
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<td></td>
<td>Arkan et al. 2003</td>
<td>Well</td>
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<td></td>
<td>Helvacı et al. 2000</td>
<td>Review of Turkish outbreaks</td>
</tr>
<tr>
<td>United States</td>
<td>Jellison et al. 1950</td>
<td>Stream and pond</td>
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</table>

All water sources were untreated unless specified otherwise.
the organism. This was the approach used by Karpoff and Antonoff (1936) in the first documented waterborne disease outbreak and also in the first isolations from water reported in the United States from outbreak (Jellison et al. 1950) and non-outbreak situations (Parker et al. 1951, Foote et al. 1943, Jellison et al. 1942). In these early reports, guinea pigs were used as the animal model, and this model continues to be used for this purpose (Kantardjiev et al. 2006, Young et al. 1969). Direct isolation from water has also been accomplished using the white mouse as an animal model (Chitadze et al. 2009, Young et al. 1969).

*F. tularensis* has been isolated directly from water samples by culture-based techniques using selective bacteriological media. Cysteine heart agar with blood has often been used as a basal medium. Various antibiotic supplements have been added to assist in the isolation of the organism (Humrighouse et al. 2011, Petersen et al. 2009). Using these techniques, Christova et al. (2004) isolated *F. tularensis* subsp. *bolaricita* in one of 10 wells examined as part of an outbreak investigation. Şimşek et al. (2012) were able to isolate the organism using a membrane filtration procedure and antibiotic-containing medium. Detection of target colonies on agar media can be difficult because of the presence of high numbers of background organisms. *F. tularensis* is a fastidious, slow-growing organism, and more rapidly growing indigenous aquatic bacteria can often interfere with detection. To counter this problem, the use of an acid pre-treatment step (similar to the procedure used for the analysis of *Legionella* spp. in water), coupled with the use of a selective antibiotic-containing agar medium, has been proposed for direct isolation from water samples (Humrighouse et al. 2011).

Molecular genomic methods have been used for detecting the organism (Forsman et al. 1995). The complete genome sequence has been published (Larsson et al. 2005). For analysis of water samples, a number of reports cite the use of the polymerase chain reaction (PCR) procedure (Berrada & Telford 2011, Larssen et al. 2011, Leblebicioglu et al. 2008, Brantsæter et al. 2007, Ozdemir et al. 2007; Gürcan et al. 2006, Forsman et al. 1995), as well as the quantitative PCR procedure (Broman et al. 2011, Lundström et al. 2011, Meric et al. 2010, Francy et al. 2009). A non-PCR microarray approach has also been proposed as an alternative method (Brinkman et al. 2013). The choice of primers and probes is an important consideration for genomic methods, and nucleic acid sequencing is often used to verify positive responses. The genes that have been most commonly targeted in these assays are *tul4* and *fopA*, which encode for outer-membrane proteins; *ISFtu2*, which encodes for an insertion element-like sequence; and the 23 kDa gene, which encodes for a protein associated with macrophage infection (Versage et al. 2003). Because of the similar genetic makeup of *Francisella* species, some primers may exhibit a low specificity for the subspecies of most interest from a public health perspective. This point was recently made in an outbreak investigation in which a PCR assay incorrectly identified *F. novicida* as *F. tularensis* (Brett et al. 2014). These findings highlight the need to continually evaluate primer sequences on the basis of the most current genomic databases. Molecular procedures do not provide information on viability, a factor of particular importance for evaluating the efficacy of various treatment strategies.

Several immunological methods have been used for detection of the organism in water. Berdal et al. (2000) and Grunow et al. (2008) used enzyme-linked immunoabsorbent assays and rapid immunochromatographic tests designed to detect lipopolysaccharide antigen. An immunoaffinity chromatographic procedure (Grunow et al. 2008) and a piezoelectric immunosensor detection system (Pohanka & Skládal 2007) have also been proposed. Currently the majority of these methods are not able to detect low levels of the organism in an aqueous matrix.

**PERSISTENCE IN WATER**

*F. tularensis* may persist in water for extended periods. The survival of the organism in sterilized tap water has been reported in two separate studies. Using culture-based procedures for determining viability, Gilbert and Rose (2012) reported that *F. tularensis* subsp. *bolaricita* (NY98) persisted for 28 days, and *F. tularensis* subsp. *bolaricita* LVS persisted for 21 days at 8°C in sterile tap water. Under similar conditions, Forsman et al. (2000) reported that it took 70 days for this strain to decrease to an undetectable level. These observed differences may be due in part to the assay procedures used for growing the organisms or to the varying chemical/physical compositions of the two tap waters. *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *bolaricita* LVS have been reported to be able to survive in filter-sterilized brook water held at 4°C for seven to 10 days (Berrada & Telford 2011). These studies were conducted in sterilized water and, while informative, would not take into account biotic factors such as predation; therefore, they may not be totally indicative of survival in natural water samples.

Using flow cytometric and PCR methods, Forsman et al. (2000) found that nonculturable cells were present, but they did not manifest virulence on the basis of animal infectivity. Attempts to restore viability using temperature gradients and different culture media were unsuccessful. Similar results regarding loss of virulence with extended storage in lake water have been reported by Thelaus et al. (2009). Loss of pathogenicity for nonculturable cells was also seen for the fish pathogen *F. noatunensis* (Duodu & Colquhoun 2010). In a study on survival of *F. tularensis* subsp. *tularensis* in brackish water, Berrada and Telford (2011) questioned the relative risk of infection from organisms detected by culture-independent methods. It was suggested that bacterial growth in culture may more closely approximate the minimum threshold of viability that would be required for animal or human infection.

In earlier studies using animal infectivity, Jellison et al. (1942) reported persistence of the bacterium in a beaver pond for a period of up to 31 days but not after 41 days. Using naturally contaminated water stored in the laboratory at 7°C, Parker et al. (1951) reported that the pathogen survived for at least 23 days but not more than 35 days. Naturally contaminated mud samples stored under these same conditions gave varying results, with persistence lasting throughout a four- to 10-week period. These researchers also isolated the organism from ice formed from naturally contaminated water. Freezing of contaminated water in the laboratory showed that the organism survived for a period of not less than 12 days.
Experimental studies have been conducted with *F. tularensis* and free-living protozoa to evaluate the potential effect this interaction may have on persistence in the aquatic environment. Studies with *F. novicida* and *F. philomiragia* in water indicated that interaction with amoebae may support persistence (Verhoeven et al. 2010). Using the vaccine strain *F. tularensis* subsp. *holarctica* LVS, Abd et al. (2003) reported that in co-culture with the free-living amoeba *Acanthamoeba castellanii*, the bacterium was found in both trophozoites and cysts. The organism multiplied within intracellular vacuoles and eventually killed many of the host protozoan cells. Using the same protozoan model, El-Etr et al. (2009) found that in culture media, most virulent strains responded differently from the vaccine strain. The amoeba host rapidly encysted in response to *F. tularensis* infection and did not replicate in large numbers, but survived for a period of three weeks. In a lake-water microcosm study using a fluorescently labeled strain of *F. tularensis* subsp. *holarctica*, Thelaus et al. (2009) found that high nutrient conditions and predation by free-living protozoa affected survival of the pathogen.

It has been hypothesized that *F. tularensis* would likely form biofilms, which would enhance persistence in the aquatic environment (van Hoek 2013). Biofilm formation would be consistent with the lifestyle of many other bacteria. In laboratory studies, *F. tularensis* subsp. *holarctica* LVS, *F. novicida*, and *F. philomiragia* have been induced to form biofilms in wells of plastic plates (Mahajan et al. 2011, Margolis et al. 2010). The significance of the formation of biofilms and interaction with free-living protozoa in relation to the persistence of *F. tularensis* in the aquatic environment awaits further elucidation.

**MAMMALS AS SOURCES OF CONTAMINATION**

Carcasses and excreta of infected animals have been implicated as primary sources of water contamination. This finding includes aquatic mammals such as beaver (Parker et al. 1951) and muskrats (Young et al. 1969, Parker et al. 1951). In studies of epizootic tularemia in the northwestern United States, water contamination was common in any stream where there were infected muskrats or beaver (Parker et al. 1943). Carcasses of guinea pigs that died from laboratory infections were able to contaminate water for a period of at least 10 days (Parker et al. 1951). Rossw et al. (2014) reported experimental infection in voles, resulting in high levels of bacteria in excreta and carcasses, and suggested that these carcasses could serve as sources for environmental contamination. Laboratory studies demonstrating prolonged shedding of the organism in the urine of infected voles led Bell and Stewart (1975) to hypothesize that cannibalism of infected carcasses could potentially bestow a partial immunity, with a resulting chronic bacteriuria that could be responsible for protracted contamination of watersheds. Population-cycling dynamics of animal species also appears to be an important factor in the ecology of *F. tularensis* subsp. *holarctica*. The two- to three-year cycling nature of the vole population has been linked to enhanced disease transmission in eastern Hungary (Gyuranecz et al. 2012). However, because tularemia readily kills most infected rodent species, it is unlikely that they would serve as true reservoir hosts during nonepizootic periods (Gyuranecz et al. 2011).

Various mammalian species have been implicated as sources of contamination in numerous drinking water outbreaks. Animals cited include mice (Merc et al. 2010, Jellison et al. 1950), lemmings (Berdal et al. 2000, Mair et al. 1973), rats (Sencan et al. 2009, Gürcan et al. 2004, Arikan et al. 2003, Politzer 1967); and voles (Nordahl et al. 1993, Hoel et al. 1991, Dahlstrand et al. 1971). In the former Soviet Union, drinking water contaminated by infected rodent carcasses was reported to account for more than 20% of all tularemia cases (Pollitzer 1967). In Bulgaria, an increase in the rodent population was thought to favor environmental contamination in a food/waterborne tularemia outbreak investigation in which *F. tularensis* subsp. *holarctica* was isolated from an open well (Christova et al. 2004). Karadenizli et al. (2005), reporting on a waterborne outbreak in Golcuk, Turkey, suggested that following the 1999 earthquake, severe damage to water infrastructure increased the probability of pollution of natural springs by infected rodents. Contamination by infected rodents and hares has been cited as a major influence in the recent re-emergence of waterborne tularemia in Turkey (Akalin et al. 2009).

**TREATMENT STUDIES**

The first published report on the inactivation of *Francisella tularensis* by chlorination appeared in this journal in 1943 (Foote et al. 1943). The study was conducted by personnel from the Montana State Board of Health (Division of Water and Sewage) in conjunction with federal investigators from the National Institutes of Health at the Rocky Mountain Laboratory in Hamilton, Mont. Since contaminated water had been shown to be a means of transmission of tularemia (Karpoff & Antonoff 1936), the efficacy of chlorination was examined after detecting the organism in local natural waters (Parker et al. 1943). Laboratory experiments were conducted by adding an inoculum (four drops) of a turbid culture into water samples that were subsequently chlorinated. Survival factors (time in minutes multiplied by chlorine concentration, mg/L) were used for comparing inactivation at different exposure times and chlorine concentrations. In pond water at temperatures between 15.5 and 18.5°C, pH 7.3, no viable organisms were detected after exposure to 0.30 mg/L in 45 min, and at 0.45 mg/L in 30 min. Viability was determined by intraperitoneal injection using the guinea pig model.

Rather amazingly, these investigators also reported finding sufficient indigenous levels of the organism in a Montana stream to allow them to conduct a field investigation. The streamflow was diverted into a chlorine contact chamber composed of a wooden trough with “over” and “under” baffles to prevent short-circuiting (Figure 1). The exposure time was determined by controlling the volume flowing through the trough (approximately 1 gpm). Guinea pigs were injected in the field, using four animals per exposure time. After a preliminary set of experiments, a second study (labeled experiment 19) was conducted. In the unchlorinated control, all of the animals (four out of four) succumbed to the disease. Under these natural stream conditions, a free chlorine residual of 1.0 mg/L with an exposure time of 15 min (a simple *C × T* value of 15 mg-min/L) was required to prevent the death of any of the animals. Assuming a neutral pH, a low chlorine demand, and a cold water temperature (the field
experiments were conducted in February), this \( C \times T \) value is remarkably close to the findings of a recent laboratory study by O’Connell et al. (2011) in which a mean \( C \times T \) value of 16.7 mg-min/L was required to inactivate virulent strains of \( F.\) \textit{tularensis} by three orders of magnitude (3 log\(_{10}\)) at 5°C, pH 7.0, in an oxidant demand–free buffer.

Shortly after the publication of Foote et al. (1943), Russian researchers (Gotovskaia & Magaram 1945) reported on the bactericidal effect of chlorine as a drinking water disinfectant. In this study, animal (mouse) inoculation was used for determining viability. Pure cultures of \( F.\) \textit{tularensis} were inoculated into effluents from slow and rapid sand filters collected at a municipal drinking water treatment plant. When these waters were chlorinated at an initial level of 1.25 mg/L of available chlorine for 1 h, none of the animals exposed to the slow sand filter effluent (0/28) or rapid sand filter effluent (0/64) became infected. However, when \( 10^3 \) to \( 10^4 \) organisms/mL were inoculated directly into tap water with a residual of 0.3–0.4 mg/L available chlorine, after 1 h of exposure, all of the animals (20/20) became infected. The authors concluded on the basis of these findings that it was unlikely that the typical residual chlorine levels present in tap water would protect users if water was contaminated with “tularemia microbes” in the distribution system; they recommended that chlorine levels be maintained at 1.5–2.0 mg/L to prevent a waterborne tularemia outbreak.

Although there has not been a reported drinking water outbreak of tularemia in the United States in more than 65 years, there has been renewed interest in \( F.\) \textit{tularensis} as a potential biothreat organism. This concern prompted research on the innate resistance of the bacterium to commonly used drinking water disinfectants, using oxidant demand–free buffer as the suspending medium. In an initial investigation of chlorine disinfection for several bacterial biothreat agents, \( F.\) \textit{tularensis} exhibited the highest level of resistance to free available chlorine among the various vegetative bacteria examined, including \textit{Burkholderia} spp., \textit{Brucella melitensis}, and \textit{Yersinia pestis} (Rose et al. 2005). Further research was conducted by O’Connell et al. (2011) with free available chlorine using several isolates of both virulent subspecies—\textit{F. tularensis} subsp. \textit{tularensis} and \textit{F. tularensis} subsp. \textit{bolaarctica}—and the attenuated \textit{F. tularensis} subsp. \textit{bolaarctica} LVS. These were quantitative studies with results based on plate-count data derived from culturing on agar media. Because temperature and pH are important parameters, \( C \times T \) values were developed for 2–4 \( \log_{10} \) levels of inactivation at two temperatures and two pH levels (Table 2). The \( C \times T \) values for the two subspecies were not significantly different from each other. Under the most favorable conditions for inactivation (25°C, pH 7.0), the mean \( C \times T \) value for a 4 \( \log_{10} \) level of inactivation was 1.4 mg-min/L. At the least favorable condition of 5°C and pH 8.0, a mean \( C \times T \) of 76.1 mg-min/L was required to achieve a 4 \( \log_{10} \) level of inactivation. \textit{F. tularensis} was more resistant to inactivation by free chlorine than other vegetative bacterial biothreat agents and other waterborne bacterial pathogens (Rose & Rice 2014).

More limited studies with monochloramine and chlorine dioxide using only one virulent strain of \textit{F. tularensis} subsp. \textit{bolaarctica} indicated the organism responds in a manner similar to other vegetative bacterial cells (Table 3). Using preformed monochloramine at pH 8.0 in oxidant demand–free buffer, a 3 \( \log_{10} \) inactivation was achieved with a \( C \times T \) of 116 mg-min/L at 5°C (Rose et al. 2007). Under the same conditions, a \( C \times T \) value of only 1.1 mg-min/L was required to achieve the same level of inactivation with chlorine dioxide (Shams et al. 2011). These results indicate that \textit{F. tularensis} is inactivated by these chemical disinfectants in a manner similar to other vegetative bacterial waterborne pathogens.

One important finding in the studies of Rose et al. (2005) and O’Connell et al. (2011) was the lower \( C \times T \) values required for inactivation of the attenuated \textit{F. tularensis} subsp. \textit{bolaarctica} LVS compared with virulent strains, suggesting that this strain would not be a good surrogate organism for determining effectiveness of chlorination for virulent strains of \textit{F. tularensis}. In all instances for inactivation by free available chlorine, the \( C \times T \) values for the attenuated vaccine strain were always less than those of the virulent strains under the same conditions. The reason for this
observation is unclear but may be linked to differences in the capsular composition and the cell wall lipopolysaccharide layer of virulent strains. This difference was also observed with monochloramine (Rose et al. 2007) and chlorine dioxide (Shams et al. 2011); however, it was not as pronounced and suggests that the mode of inactivation by free chlorine may be a factor in these findings.

Using a collimated beam apparatus and a low-pressure ultraviolet lamp (254 nm), Rose and O’Connell (2009) reported on the agent’s resistance to ultraviolet irradiation. A fluence of 6.3 and 8.7 mJ/cm² was required to inactivate the virulent strain \( F.\) tularensis subsp. holarctica (NY98) by three and four orders of magnitude (log₁₀), respectively. \( F.\) tularensis subsp. holarctica LVS was less resistant, requiring 4.8 and 6.6 mJ/cm² to achieve the same level of inactivation. All of the vegetative biothreat bacterial agents examined required a fluence of <12 mJ/cm² to achieve a 4 log₁₀ reduction in viability.

### EFFECTIVENESS OF TREATMENT FOR CONTROLLING OUTBREAKS

Several case studies of tularemia outbreaks in drinking water have demonstrated disinfection to be an effective means for controlling the spread of the disease. The lack of adequate water treatment has been cited as a major factor associated with drinking water outbreaks (Şencan et al. 2009, Leblebicioglu et al. 2008). The issuance of an initial boil-water advisory followed by subsequent chlorination of the system was successful in controlling an outbreak in the Republic of Georgia (Chitadze et al. 2009). The importance of chlorination was emphasized by Greco et al. (1987) in an epidemiological study of an outbreak that occurred in Sansepolcro, Italy. The town was served by two separate water systems, one of which was chlorinated while the other was not, and a clear association was seen between tularemia cases and consumption of the unchlorinated water. Intermittent and inadequate chlorination have been cited as compounding factors for several outbreaks in Turkey (Table 1). Several Turkish tularemia outbreaks have been reported to subside with the institution of chlorinating the water supply and other remediation efforts such as reservoir cleaning (Meric et al. 2010, Celebi et al. 2008, Sahin et al. 2007). Pollitzer (1967) noted that in a Russian drinking water outbreak of tularemia, there were no new cases once the system was “thoroughly chlorinated” and a residual chlorine concentration of 1.0 mg/L was maintained in the system.

### CONCLUSIONS AND RECOMMENDATIONS

Contaminated drinking water can serve as a vehicle for the spread of tularemia. Disease occurrence has been most commonly associated with untreated community and domestic water supplies. \( F.\) tularensis is of interest both from the standpoint of naturally occurring waterborne outbreaks and from its potential use as a biothreat organism. In some instances, especially in war-torn areas where the disease is endemic, distinguishing between these two possible scenarios has been a matter of concern (Grunow & Finke et al. 2002). The organism can persist in the
aquatic environment, and occurrence has often been associated with contamination from various mammal species. Methods exist for detection in water, but further research is needed in the development of procedures that combine specific identification and viability determinations. While capable of being inactivated by commonly used drinking water disinfectants, *F. tularensis* does exhibit an increased resistance to chlorination in comparison with other waterborne vegetative bacterial pathogens. However, current US guidelines regarding required levels of treatment for encysted protozoa and enteric viruses would be sufficient for controlling this organism (USEPA 1991).

When cases of oropharyngeal tularemia are encountered, medical and public health authorities should consider ingestion of contaminated water as a potential source of infection. Public health education efforts, especially in areas of known waterborne outbreaks, should emphasize the avoidance of nonpotable water (such as stream water) as a drinking water source. In a natural outbreak situation or in the case of an intentional contamination event, the public would need to be notified and provided with alternate sources of drinking water.

Guidelines for emergency water treatment using chemical disinfectants provided by governmental agencies and international health organizations (WHO 2006) should be instituted in outbreak situations. McCoy and Chapin (1912) reported a thermal death point for the bacterium of 56°C. A more recent thermal resistance study indicated that normal pasteurization parameters used for the destruction of foodborne bacterial pathogens were sufficient for inactivating *F. tularensis* (Day et al. 2008). This finding would justify the issuance of boil-water advisories in which water is brought to a rolling boil for 1 min, or for 3 min at altitudes above 5,000 ft (1,524 m), as a ready means for providing safe drinking water during an outbreak of waterborne tularemia.

Because of the role of rodents in the waterborne transmission of this disease, efforts should be directed at minimizing this source of contamination whenever possible. Since many reported tularemia outbreaks have been from contaminated wells (Table 1), measures to prevent rodent entry in wells and water storage facilities are recommended. Institution of chlorination has been found to be effective in controlling and preventing waterborne tularemia. Many outbreaks have occurred in small community water systems that lacked any form of water treatment. System managers wishing to initiate disinfection of surface water sources as a control measure would need to evaluate whether disinfection alone would be sufficient or if other unit processes would be required. Physical characteristics that would assist in making this determination would include turbidity of less than or equal to 1 ntu, color of 15 acu, and a chlorine demand of no more than 2 mg/L. (Geldreich et al. 1990).

**DISCLAIMER**

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