



The Infectious Dose of *Francisella tularensis* (Tularemia)

Rachael M. Jones, Mark Nicas, Alan Hubbard, Matthew D. Sylvester, and Arthur Reingold

University of California—Berkeley, Berkeley, California

Abstract

Quantitatively estimating an individual's risk of infection by an airborne pathogen requires knowledge of the expected dose and the pathogen's infectious dose. Based on our review of the published literature on tularemia, we conclude that the infectious dose of *Francisella tularensis* varies among individuals, but that a substantial proportion of the population can be infected by a single bacillus. We also conclude that infection can be initiated by inhaling bacilli carried on respirable particles (diameters less than 10 μm) or nonrespirable particles (diameters between 10 μm and 100 μm). Regression analyses based on two-parameter Weibull and log-normal models of human inhalation dose-infection data aggregated across three studies indicate that approximately 30% of individuals who inhale a single *F. tularensis* bacillus will develop tularemia. Further, when the organism is carried on particles with diameters on the order of 1 μm , it is estimated that the deposition of a single bacillus produces infection in 40% to 50% of individuals; thus, when *F. tularensis* is carried on respirable particles, the estimated ID_{50} via inhalation is close to one deposited bacillus. These results are consistent with separate analyses using nonparametric methods and with experimental animal models in which infection is observed after injection of a single bacillus. The risk of person-to-person transmission of tularemia is generally considered negligible, perhaps due to a low concentration of *F. tularensis* in respiratory fluids. However, viable *F. tularensis* bacilli are present in human respiratory fluids, and can be carried in inspirable particles (diameters less than 100 μm) which are emitted during coughs and sneezes.

Introduction

Francisella tularensis (formerly termed *Pasteurella tularensis* and *Bacterium tularensis*) is a bacterium that causes a spectrum of clinical illnesses termed "tularemia." *F. tularensis* is a candidate agent for bioterrorism because it can be weaponized readily and is considered to have a low airborne infectious dose (Dennis et al., 2001; Franz et al., 1999). The World Health Organization (1970) has estimated that aerosol dispersal of 50 kg of *F. tularensis* over a metropolitan area with approximately 5 million inhabitants would result in 250,000 incapacitating casualties, including 19,000 deaths. Infection by the respiratory route has been demonstrated in *Macaca mulatta* using both respirable particles (diameters less than 10 μm) and inspirable, but nonrespirable, particles (diameters between 10 μm and 100 μm) (Day & Berendt, 1972). Naturally occurring respiratory infection has been documented in Scandinavian farm workers exposed when handling hay contaminated by voles and their waste products (Dahlstrand, Ringertz & Zetterberg, 1971; Syjala, Kujala, Myllyla, & Sandstrom, 1996). Historically, laboratory personnel have become infected by bacterium-containing aerosols generated during normal laboratory procedures and accidents (Ledingham & Fraser, 1923/1924; Overholt et al., 1961; Van Metre, Jr. & Kadull, 1959). Perhaps due to the low infectious dose by inhalation, implementation of careful handling procedures for *F. tularensis* in clinical laboratories has not entirely eliminated the potential for infection (Shapiro & Schwartz, 2002).

Secondary (person-to-person) transmission of *F.*

tularensis is generally considered improbable. For example, the Centers for Disease Control and Prevention (2004) states: "Tularemia is not known to be spread from person-to-person." Similarly, the Working Group on Civilian Biodefense (Dennis et al., 2001) concludes: "Isolation is not recommended for tularemia patients given the lack of human-to-human transmission." In 1951, however, Fillmore reported the case of a nurse's aide who developed symptoms of tularemia 3 to 4 weeks after attending a patient with pleuropulmonary tularemia; the nurse's aide had no contact with domestic or wild animals.

In determining procedures to control airborne infection, it is useful to quantitatively estimate infection risk, which depends on the pathogen's infectious dose, the concentration of the pathogen in air, and the duration of exposure. Variability in host susceptibility is captured, in part, by interindividual variability in a deterministic infectious dose. Given an appreciation of the potential intensity of airborne exposure to *F. tularensis* and of the pathogen's infectious inhalation dose, one can evaluate existing biosafety protocols in laboratory and clinical settings. In this paper, we argue that the infectious dose of *F. tularensis* for a substantial portion of the population is on the order of one bacillus. We also argue that person-to-person transmission of tularemia is theoretically possible given low infectious dose values overall, and the presence of the bacillus in the sputum of some infected patients. However, the risk of secondary airborne infection may typically be low due to low pathogen concentrations in respiratory fluid and small aerosol volumes emitted in coughs commonly described as "nonproductive."

Background on Tularemia

F. tularensis is a Gram-negative coccobacillus, with diameter ranging from 0.2 to 0.7 μm (Evans, 1985). The organism is rickettsial in that it cannot replicate outside a host cell, and it is pathogenic after being phagocytized by macrophages (Sjostedt, Tarnvik, & Sandstrom, 1996). Although originally described in ground squirrels in Tulare County, California, in 1911 by McCoy, the organism was renamed for Edward Francis who described the clinical and epidemiologic features of the disease

(Francis, 1927; Francis 1983). *F. tularensis* is found globally in mammals and arthropod vectors, and two strains produce infection in humans. Type A is associated with illness in North America; Type B is less virulent and is associated with illness in Europe (Reinjes et al., 2002). Type A has been traditionally considered for use as a biological weapon (Conlan et al., 2003), and the Schu strain of Type A, isolated from a finger ulcer (Bell, Owen, & Larson, 1955), was frequently used in experimental work until the late 1960s. At that time, investigators began using the live vaccine strain (LVS) of *F. tularensis* for experimental work because it is less virulent in humans yet is virulent in mice (Conlan et al., 2003).

Note that the terms "infectivity" and "virulence" are distinct. Infectivity signifies the ability of a pathogen to penetrate into host tissue and multiply. Infectivity can be quantified by the metric of infectious dose, that is, the number of viable organisms (colony-forming units) that must penetrate into host tissue to initiate an infection, where infection is assessed by serology and clinical indicators. However, in nonhuman mammalian studies, the infectivity of *F. tularensis* is typically reported as the number of organisms that are lethal to 50% of exposed animals, termed the LD_{50} . Virulence refers to the intensity of the disease produced by pathogen infection in a given host species (Black, 2002); the term is also used to compare the intensity of disease produced by a given pathogen in different host species. For *F. tularensis*, there is evidence that virulence is influenced by the dose of organisms received. For example, among *Macaca mulatta* receiving Schu S-4 strain organisms carried on 2.1 μm diameter particles by inhalation, five inhaled bacilli infected 6/6 hosts but caused death in only 1/6 hosts, whereas higher inhaled doses caused death in a progressively greater proportion of animals (McCrumb, 1961).

Infection with *F. tularensis* can occur through ingestion, dermal contact, and inhalation of the organism, and produces an array of clinical features (Centers for Disease Control and Prevention, 2003; Dennis et al., 2001). Ingestion of *F. tularensis* typically produces oropharyngeal tularemia (pharyngitis and cervical adenitis) (Reintjes et al., 2002). Dermal contact, arthropod bites, and intracutaneous inoculation often produce an ulcerated lesion at the site

of contact and/or swelling of the regional lymph nodes, although some individuals exposed through these routes can present with fever and other signs indicative of systemic infection (Dennis et al., 2001; Evans, 1985; Saslaw et al., 1961), including pulmonary involvement (bronchopneumonia and hilar adenopathy) (Miller & Bates, 1969). Inhalation of *F. tularensis* also produces systemic disease in humans and may produce pneumonia (McCrumb, 1961; Overholt et al., 1961), oval lesions in the lungs (Overholt & Tigertt, 1960), and/or bronchial changes (Syrjala et al., 1986). Although it has been demonstrated experimentally that humans can develop tularemia through inhalation of *F. tularensis* (McCrumb, 1961; Sawyer et al., 1966), rapid disease onset and delay in examination make it difficult to determine in some cases if pulmonary involvement precedes or follows systemic infection. Cough frequently occurs in patients with and without objective pulmonary involvement (Dennis et al., 2001; Saslaw et al., 1961). Although McCrumb (1961) reports that patients exhibited a lack of sputum production and nonproductive cough, case reports indicate that some patients exhibit increased mucous and sputum production, and productive cough (Cluxton, Jr., Clifton & Worley, 1948; Syrjala et al., 1986).

If untreated, pulmonary tularemia resulting from the type A strain has a case fatality proportion of 40% to 60% (McCrumb, 1961). Mortality due to all clinical manifestations of tularemia has been approximately 5%, although in the United States, treatment with streptomycin and gentamicin has reduced the overall case fatality proportion to below 2% (Dennis et al., 2001). The type B strain found in Europe is rarely fatal (Dennis et al., 2001).

Experimental Airborne Infection

Study Descriptions

Airborne transmission of *F. tularensis* has been demonstrated using investigator-generated aerosol and respiratory aerosol emitted by infected animals. Work with investigator-generated aerosols commenced at Fort Detrick, Maryland in the mid-1940s, when infection of mice by “clouds” of *F. tularensis* was assessed (Rosebury, 1947). Animals were ex-

posed in a stainless steel chamber to aerosol generated by a Chicago atomizer from cultures suspended in water; the reported mass median diameter of the aerosol particles was less than 1 μm . The inhalation dose was estimated based on the animal's breathing rate (related to weight), duration of exposure, and the viable *F. tularensis* aerosol concentration as measured by impinger sampling of chamber air. The reported inhalation doses assumed that 100% of inhaled particles were retained in the respiratory tract. For mice, the doses ranged from 14 to 4,500 bacilli. Only one of thirty (1/30) mice receiving a dose of 14 organisms died, while all 59 mice exposed to doses of 330 or more organisms died. All animals that did not die within 16 days subsequent to exposure were autopsied and found by gross evaluation and spleen culture to be negative for *F. tularensis*. It was estimated that 70 organisms would produce mortality in 50% of exposed mice, with 95% confidence limits of 2 to 2,063 organisms. The wide confidence interval was attributed to the large variability in recovery of aerosolized organisms. Rosebury (1947) also reports the results of Henderson, which were conveyed in a personal communication. Working at Porton Down in the United Kingdom, Henderson found that the 50% lethal dose in mice exposed via inhalation was 12 organisms.

Hood (1961) exposed guinea pigs to aged *F. tularensis* (Schu D strain) aerosols generated with a Henderson apparatus and stored in a rotating stainless steel drum. Of the aerosols generated in the Henderson apparatus, approximately 98% of the dried particles had diameters less than 1 μm , and 90% of the droplets emerging from the spray apparatus had diameters less than 10 μm (Henderson, 1952). The inhaled dose was estimated based on the animal's breathing rate (related to weight), exposure duration, the viable *F. tularensis* aerosol concentration as measured by impinger sampling near the guinea pigs, and an estimated inhaled particle retention factor of 0.55. When bacterial suspensions aged 6 to 30 days were aerosolized and held in the drum for 3 seconds before the animals were exposed, the LD_{50} was 1 to 4 organisms. The investigators found that there was no significant loss of infectivity when aerosols were aged for 20 minutes in the drum, but significant infectivity was lost when aerosols were

aged for 20 hours. It is unclear, however, what portion of the decreased infectivity was due to organism die-off versus deposition on the drum walls.

A similar study was undertaken by Sawyer et al. (1966), who exposed *Macaca mulatta* and human volunteers to aerosols of the *F. tularensis* Schu-S4 strain, generated with a two-fluid nozzle and stored in a spherical static chamber. The concentration of aerosol particles was measured by a total collector, and an impinger was used to determine the number of particles with diameters of 5 μm or less; it was estimated that 65% of viable organisms were contained in particles with diameters of 5 μm or less. Caged monkeys were placed in the test chamber for 3 or 10 minutes; human subjects were exposed through a facemask for ten 1-liter breaths in 60 seconds. The inhaled dose was defined as the product of the duration of exposure, respiratory minute volume, and the concentration of viable organisms in particles with diameters 5 μm or less; the fraction of organisms retained was not considered. It was found that an inhaled dose of 80 to 180 viable organisms from an aerosol aged for 60 minutes infected three of four (3/4) humans and seven of eight (7/8) monkeys. In another series of experiments, it was found that among human subjects who inhaled 150 viable organisms, two of four (2/4) became infected when the aerosol was aged 30 minutes, and three of four (3/4) became infected when the aerosol was aged 60 minutes. Only results for aerosols aged 60 minutes or less are reported here because aerosols aged for 120 and 180 minutes showed substantially decreased infectivity for humans and *M. mulatta*.

One limitation of the Sawyer et al. study was that the dose estimate was based on particles with diameters less than 5 μm . Day and Brendt (1972) noted that all particles with diameters greater than 5 μm contained *F. tularensis* bacilli, and that while these particles may not penetrate to the pulmonary region of *M. mulatta*, infection could result from deposition in the upper respiratory tract. This observation suggests that the inhaled doses were larger than reported by Sawyer et al. On the other hand, Sawyer et al. did not adjust the inhaled dose for the fraction of organisms that deposited in the respiratory tract, which signifies that the retained respirable dose was less than the dose reported.

Low dose infectivity of *F. tularensis* aerosols in vaccinated and nonvaccinated men has been reported by two investigators (Table 1). Saslaw et al. (1961) exposed men via a facemask to an aerosol with an average particle diameter of 0.7 μm . The subjects were instructed to inhale through their nose and exhale through their mouths. Sixteen of the twenty (16/20) nonvaccinated men became infected after inhaling doses of 10 to 52 *F. tularensis* bacilli. The four men who did not develop infection had inhaled 10 to 45 organisms. The lowest doses producing infection among nonvaccinated and vaccinated men were 10 and 13 bacilli, respectively. We note that for particles with an aerodynamic diameter of 0.7 μm , the approximate deposition fraction in the human pulmonary region is 0.2 (Hinds, 1999). Therefore, 10 to 52 inhaled *F. tularensis* bacilli represent approximately 2 to 10 deposited bacilli.

McCrumb (1961) reported that among nonvaccinated individuals exposed to 20, 200, and 2,000 *F. tularensis* bacilli, all individuals (4/4, 4/4, and 2/2, in the respective dose groups) became infected; among 12 vaccinated individuals exposed to 20 organisms, four (33%) were infected. Unfortunately, McCrumb provided little information on the methodology used in these studies and did not account for the particle deposition fraction.

Statistical Analysis of Aggregate Data

To examine the infectious dose statistically, we began by pooling the inhalation dose-infection results obtained by McCrumb (1961) and Saslaw et al. (1961) for nonvaccinated individuals, and by Sawyer et al. (1966) for aerosols aged 60 minutes or less (Table 1). For this analysis, none of the inhaled doses were adjusted for the deposition fraction of the challenge particles in the respiratory tract, which is to say that the true deposited doses were less than the reported inhaled doses. We alternatively fit a discrete nonparametric maximum likelihood cumulative probability distribution and two continuous cumulative probability distributions (lognormal and Weibull) to the data to estimate the probability of remaining uninfected after being exposed to a fixed dose. These fitted probability distributions describe the survival distribution; the complement of the survival distribution is the cumulative infection distri-

Table 1

Infection dose-response data in human subjects used to determine low dose infectivity.

Estimated Bacilli Dose	Response #Infected/#Exposed	Reference
10	1/2	Saslaw, et al., 1961
12	0/1	Saslaw, et al., 1961
13	1/1	Saslaw, et al., 1961
14	1/1	Saslaw, et al., 1961
15	1/1	Saslaw, et al., 1961
16	1/1	Saslaw, et al., 1961
18	1/1	Saslaw, et al., 1961
20	5/6	Saslaw, et al., 1961; McCrumb, 1961
23	2/2	Saslaw, et al., 1961
25	1/1	Saslaw, et al., 1961
30	1/1	Saslaw, et al., 1961
45	0/1	Saslaw, et al., 1961
46	2/2	Saslaw, et al., 1961
48	1/1	Saslaw, et al., 1961
50	1/1	Saslaw, et al., 1961
52	1/1	Saslaw, et al., 1961
150	5/8	Sawyer, et al., 1966
200	4/4	McCrumb, 1961
350	2/4	Sawyer, et al., 1966
750	4/4	Sawyer, et al., 1966
2,000	2/2	McCrumb, 1961

bution (i.e., the proportion of individuals infected with increasing dose).

The survival data structure can be considered “current status.” For each dose, one knows whether the dose given was either less than a subject’s minimum infectious dose (i.e., the subject was not infected) or greater than the minimum infectious dose (i.e., the subject was infected). The nonparametric maximum likelihood estimate of the survival function for current status data has been referred to as the pooled adjacent violator (PAV); the analysis involves a nonparametric, monotonically decreasing regression on the proportion of subjects left unin-

fected at each dose (Kalbfleisch & Prentice, 2002). Inference can be derived from nonparametric bootstrapping.

Because the data are sparse and thus the nonparametric estimates are highly variable, we also explored parametric regressions by fitting both a two-parameter Weibull model and a two-parameter log-normal model to the data. The Weibull survival function has the form:

$$\text{Eq. 1} \quad S(d) = \exp(-[\lambda d]^\kappa)$$

where $S(d)$ is the probability of not being infected at dose d , and where $\lambda > 0$ and $\kappa > 0$ are scale and

shape parameters, respectively. The lognormal survival function has the form:

$$\text{Eq. 2} \quad S(d) = 1 - \Phi\left(\frac{\ln(d) - \ln(\text{GM})}{\ln(\text{GSD})}\right)$$

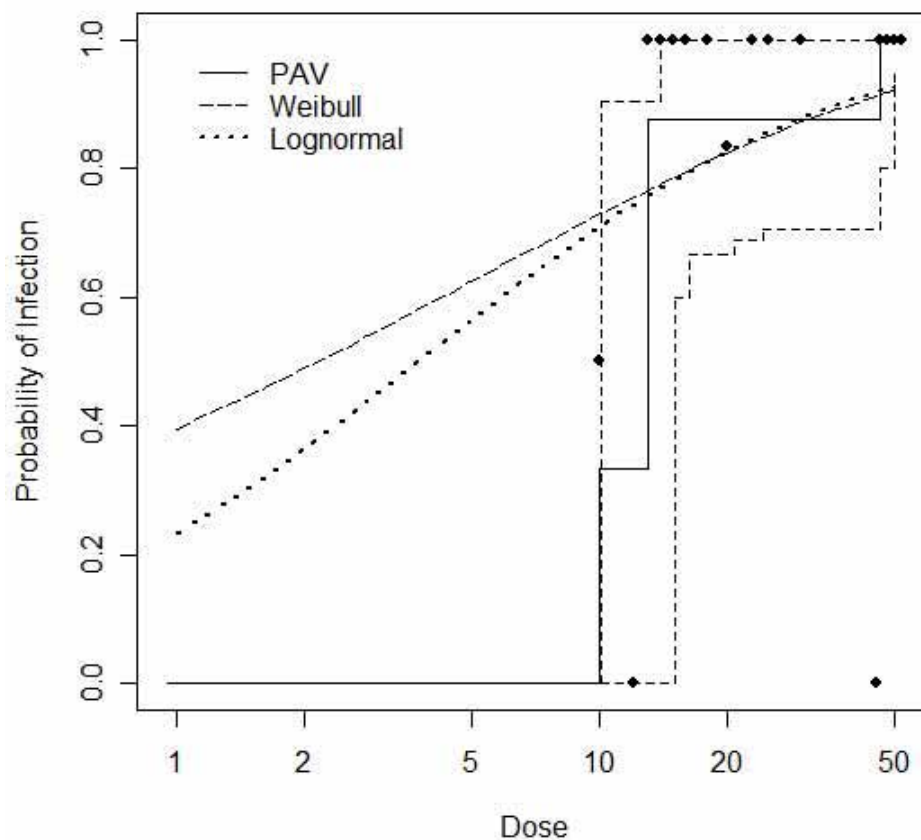
where GM and GSD denote the geometric mean (median value) and geometric standard deviation, respectively, and $\phi(z)$ denotes the cumulative standard normal distribution evaluated for the argument z . For improved readability, all fits are displayed graphically as the probability of infection, which is one minus the probability of survival.

We note that the infectious dose models explored in this analysis are “deterministic” or

“threshold” models. It is assumed that if the host receives a threshold number of organisms or more, infection is certain to occur, whereas if the host receives fewer than the threshold number of organisms, infection is certain not to occur. Variability in host susceptibility is reflected by interindividual variability in the threshold dose value, and the ID_{50} is that deposited dose which will infect 50% of the population with certainty. In contrast, a purely “probabilistic” model assumes that a single organism can successfully infect the host with probability p_{success} , such that the probability of infection is $1 - (1 - p_{\text{success}})^D$, where D is the deposited number of organisms. If the value of p_{success} is the same across individual hosts, the ID_{50} is that deposited dose which produces a 50% likelihood of infection in all

Figure 1

Estimated cumulative infection probability of humans experimentally exposed to increasing inhaled doses of *F. tularensis* based on the Table 1 data, via a nonparametric maximum likelihood analysis (black line with dashed lines representing the 95% CI), a lognormal parametric regression, and a Weibull parametric regression. The Table 1 data points are indicated by the diamonds.



hosts. A probabilistic model can also account for variability in the value of p_{success} between individual hosts.

Figure 1 displays the data listed in Table 1, the best fit curves for the lognormal and Weibull model, and the PAV fit along with the 95% pointwise confidence interval for the PAV fit. Over the range of data (inhaled doses of 10 and greater), the lognormal, Weibull, and PAV fits are consistent (the lognormal and Weibull fits are within the 95% confidence interval for the PAV fit). The lognormal and Weibull models allow extrapolation to inhaled doses below 10 bacilli. The lognormal model parameter estimates ($GM = 3.71$, $GSD = 6.01$) indicate that infection risk given inhalation of one bacillus is 23%, while the Weibull model parameter estimates ($\lambda = 0.191$, $\kappa = 0.412$) indicate that infection risk given inhalation of one bacillus is 40%. Overall, the lognormal and Weibull models suggest that 20% to 40% of the population would be infected due to inhaling a single *F. tularensis* bacillus.

The PAV fit indicates that inhaling 10 bacilli would infect approximately 30% of the population; unfortunately, this nonparametric model does not permit estimating the percent infected below the inhaled doses for which data are available. However, it does not make sense that 10 bacilli is the minimum inhalation infectious dose. If inhaling 10 organisms produces infection with 30% probability, it is reasonable that inhaling 9 organisms should produce infection with some probability less than 30% but greater than zero, unless infection is a multistep process requiring a minimum of 10 organisms. In regard to the latter idea, we note that the infectivity of just one *F. tularensis* organism is supported by animal data. In mice, guinea pigs, and rabbits, the intracutaneous and intraperitoneal injection of a single *F. tularensis* bacterium is capable of producing lethal disease (Bell, Owen, & Larson, 1955; Downs et al., 1947; Emel'yanova, 1961; Rosebury, 1947).

The distributions depicted in Figure 1 are somewhat confused by aggregating the results from aerosol exposure studies involving different or unknown particle size distributions. McCrumb (1961) did not specify the particle size distribution or describe the aerosol generating equipment, while Sawyer et al. (1966) considered the dose to be only those organ-

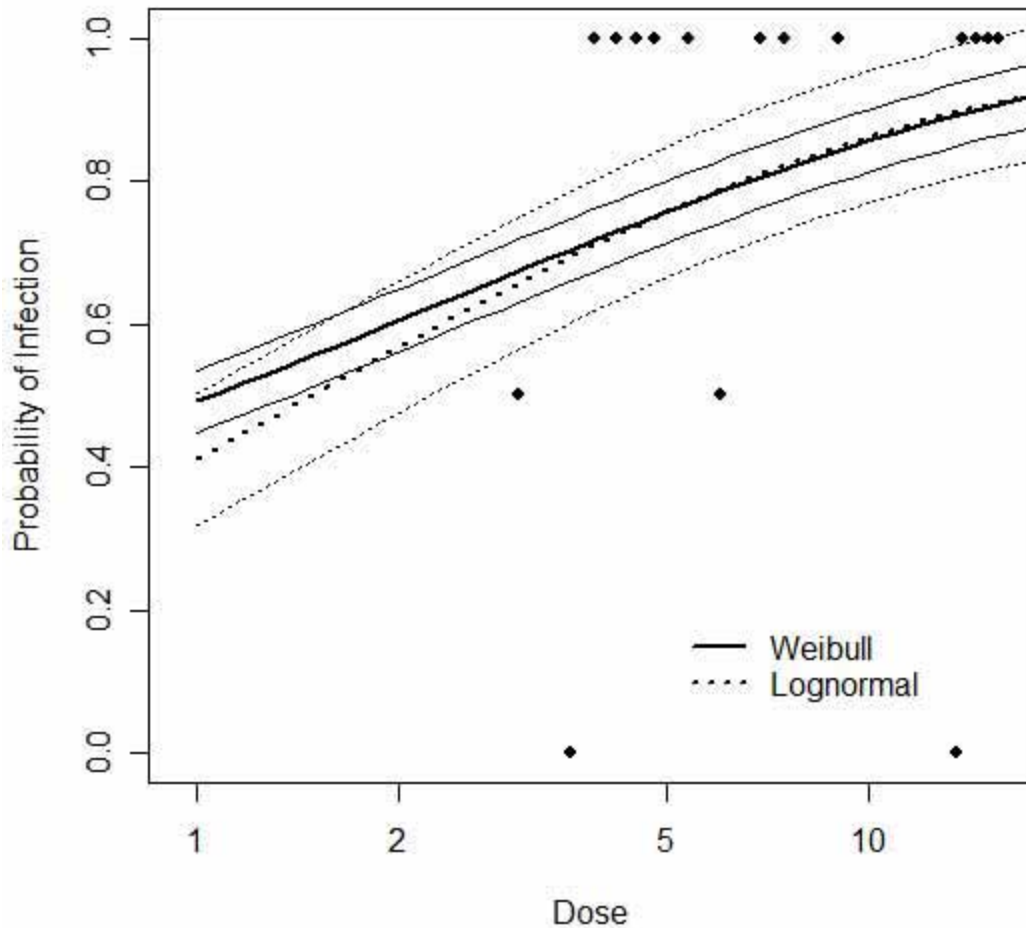
isms present in particles of diameter 5 μm or less. Because particle size is inversely related to infectivity (Day & Berendt, 1972), we applied the same statistical analyses to the data of Saslaw et al. (1961) for which the aerosol size distribution was described as homogeneous with an average particle diameter of 0.7 μm . We also decreased Saslaw's reported inhaled doses to account for a deposition fraction of 0.3 in the respiratory tract (Hinds, 1999). Figure 2 plots the Saslaw et al. (1961) data from Table 1, and the cumulative infection probability as a function of the deposited dose based on fitted lognormal and Weibull models; the 95% confidence intervals for the cumulative probability functions are also shown. The lognormal model parameter estimates are $GM = 1.48$ and $GSD = 5.75$, and the Weibull model parameter estimates are $\lambda = 0.427$ and $\kappa = 0.458$. Given the deposition of one bacillus, the lognormal model parameter estimates indicate that infection risk is 41%, while the Weibull model parameter estimates indicate that infection risk is 49%. Overall, the parametric models suggest that 40% to 50% of the population would be infected by the deposition of a single *F. tularensis* bacillus. Figures 3 and 4 show that the 95% confidence intervals for the lognormal and Weibull cumulative probability functions, respectively, fall within the 95% confidence interval of the PAV cumulative probability function. Therefore, the lognormal and Weibull probability estimates are consistent with the PAV estimates in the dose range of three bacilli or more.

Person-to-Person Transmission

Based in part on the observation that surgeons did not become ill after incising or excising suppurating lymph nodes from tularemia patients, Francis concluded in 1927 that tularemia is not transmitted from person-to-person. This conclusion has been reiterated frequently in the tularemia literature. However, it is inconsistent with the report by Fillmore (1951) of tularemia in a health care worker who had treated a tularemia patient, and with reports of droplet spray transmission resulting from experimental animals "sneezing" at persons (Aagaard, 1944; Francis, 1983; Ledingham & Fraser, 1923/1924). Further, airborne transmission between

Figure 2

Estimated cumulative infection probability of humans experimentally exposed to increasing inhaled doses of *F. tularensis*, based on the Saslaw et al. (1961) data adjusted for particle deposition, via a lognormal parametric regression and a Weibull parametric regression, including 95% confidence intervals. The Saslaw, et al. (1961) data points are indicated by the diamonds.



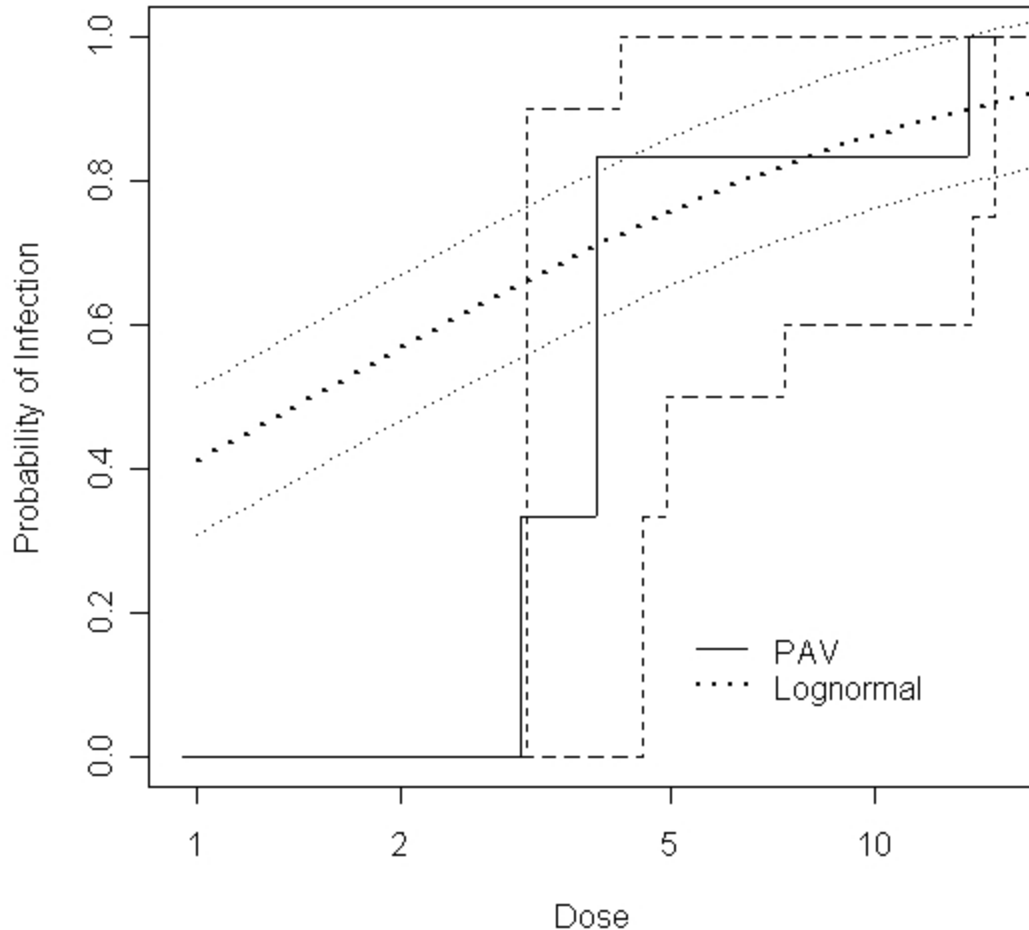
mice was demonstrated experimentally by Owen and Bucker (1956), who found that ten of twenty-eight (10/28) healthy mice strapped into tubes in near nose-to-nose contact with moribund mice succumbed to infection. For secondary airborne transmission to occur, viable organisms must be emitted from the infected animal and remain viable until reaching target tissues in the susceptible host. In this segment of the paper, we evaluate the feasibility that these events can occur and cause person-to-person transmission of tularemia.

Viable *F. tularensis* bacilli are present in the human body, including respiratory fluids. Emel'yanova (1961) compared strains of *F. tularensis* isolated from

the abscesses of two tularemia patients to strains isolated from well water which had caused a tularemia outbreak; all strains had the same biological properties and were lethal to mice, rats, guinea pigs, and rabbits in the same doses and at the same intervals. In addition, there are several reports that bacilli from throat swabs and/or sputum of tularemia patients with and without pulmonary involvement were fatal to experimental animals (Johnson, 1944; Larson, 1945). In a more extensive evaluation, gastric, pharyngeal, and sputum specimens were collected from patients with laboratory-acquired tularemia during the first 3 weeks of their illness; these specimens were inoculated into guinea pigs or cul-

Figure 3

Estimated cumulative infection probability of humans experimentally exposed to increasing inhaled doses of *F. tularensis*, based on the Saslaw et al. (1961) data adjusted for particle deposition, comparing the lognormal parametric regression to the nonparametric PAV regression, including 95% confidence intervals.



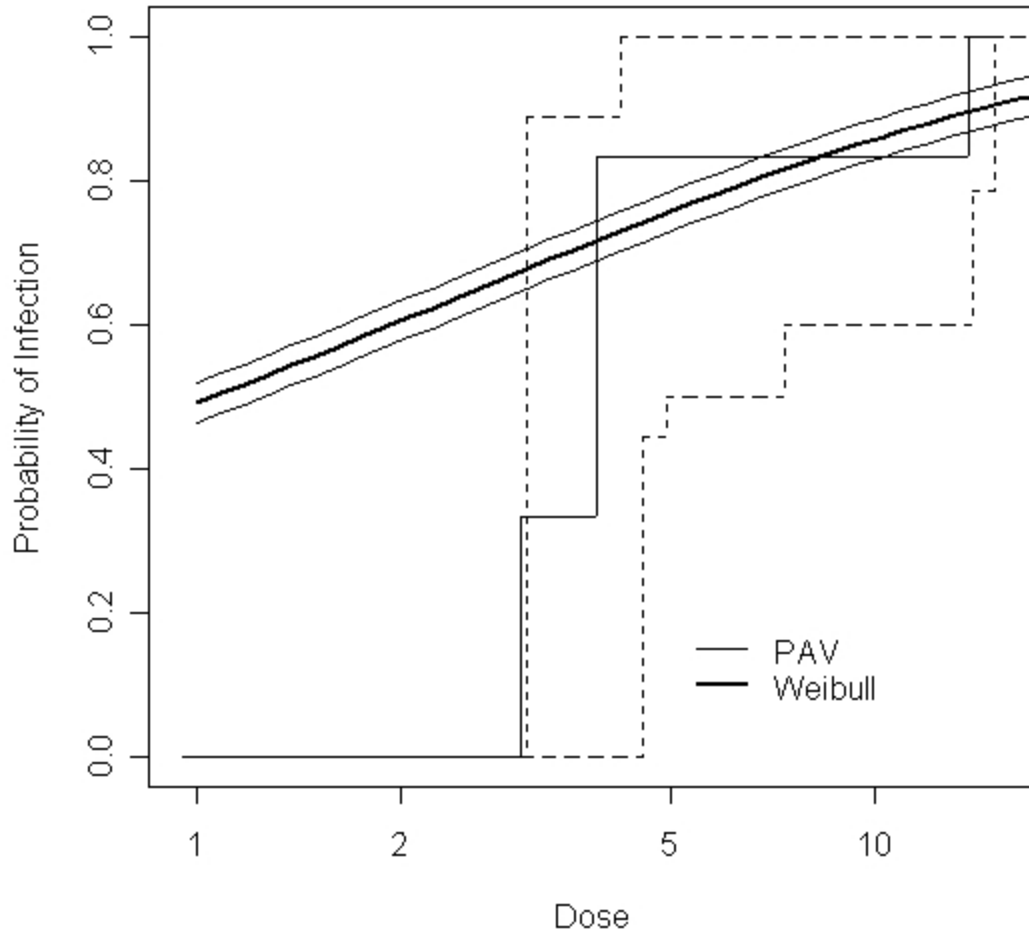
tured on glucose-cysteine blood agar plates. A total of 14/16 sputum specimens, 18/32 pharyngeal specimens, and 22/31 gastric specimens either killed the guinea pigs or produced cultures of *F. tularensis* (Overholt et al., 1961). Unfortunately, concentration data for viable *F. tularensis* bacilli in respiratory fluids have not been reported.

The presence of *F. tularensis* in respiratory fluids implies that the organism will be present in the aerosol of respiratory fluids emitted during coughs and sneezes. The numbers and size distribution of respiratory aerosol particles have been summarized by Nicas, et al. (2005). Aerosolized *F. tularensis* remain viable in air for a sufficient time to be inhaled by health care workers or family members attending a

tularemia patient. As described previously, Hood (1961) observed that *F. tularensis* aerosols retained a diminished, but still lethal, infectivity in guinea pigs when aged 20 hours. In humans, Sawyer et al. (1966) found that the inhaled dose of viable cells required to produce infection increased when the aerosol aged more than 120 minutes, but the severity of clinical illness and the incubation period did not vary with the age of the aerosol. Ambient temperature and relative humidity influence the viability of airborne *F. tularensis*, although the relationships are not linear. Peak recovery of viable airborne *F. tularensis* was observed between -7°C and 3°C (Ehrlich & Miller, 1973), and when disseminated from a wet state, survival of the organism in air was greatest at

Figure 4

Estimated cumulative infection probability of humans experimentally exposed to increasing inhaled doses of *F. tularensis*, based on the Saslaw et al. (1961) data adjusted for particle deposition, comparing the Weibull parametric regression to the nonparametric PAV regression, including 95% confidence intervals.



high relative humidity (Cox & Goldberg, 1972).

Cough is frequently associated with tularemia, and increased sputum production has been observed in some patients. Coughing emits many particles that quickly attain diameters less than 10 μm ; these particles can penetrate to and deposit in the alveolar region. Coughing also emits many particles that quickly attain diameters in the 10 μm to 100 μm range; these particles can be inspired and deposit in the upper respiratory tract (Nicas, et al., 2004). Experimental evidence indicates that *F. tularensis* remains virulent in the human host and retains viability in air for prolonged periods of time. Coupled with an infectious dose of one bacillus, these conditions indicate that airborne person-to-person trans-

mission of tularemia is possible. The overall lack of reported person-to-person transmission may be due to a low concentration of *F. tularensis* in respiratory fluids, such that the airborne concentration of the pathogen is usually low and the infection risk by inhalation is small.

Conclusion

Overall, we judge there is adequate evidence that infection can be produced by the respiratory tract deposition of a single *F. tularensis* bacillus. Human experimental work concerning inhalation infection by *F. tularensis* is limited to inhaled doses of 10 organisms or greater. However, regression parameter

estimates for the two-parameter lognormal and Weibull models indicate that the deposition of a single bacillus carried on a respirable particle will produce infection in 40% to 50% of individuals. This prediction is consistent with the observation that a single *F. tularensis* bacillus can initiate infection in experimental animals exposed via injection (Bell, Owen & Larson, 1955; Downs et al., 1947; Emel'yanova, 1961; Rosebury, 1947).

Our review of the published literature indicates that airborne tularemia transmission in the laboratory has been frequent, and that person-to-person tularemia transmission is possible. In particular, Fillmore (1951) reported the case of a nurse's aide who developed symptoms of tularemia 3 to 4 weeks after attending a case of pleuropulmonary tularemia, and virulent bacilli have been recovered from respiratory fluids of tularemia patients (Johnson, 1944; Larson, 1945; Overholt et al., 1961). Coughing, which is associated with tularemia, emits many particles of respiratory fluid that quickly attain diameters less than 100 μm ; these particles can be inspired and deposit in the alveolar region or upper respiratory tract depending on particle diameter.

The risk of person-to-person airborne infection is a multivariable function involving the pathogen concentration in respiratory fluid, the expiratory event rate, the size and volume distribution of particles emitted per respiratory event, the receptor's breathing rate and exposure duration, and the receptor's location in the room relative to the source case (Nicas, et al., 2005). Given a specified infectious dose distribution for *F. tularensis*, and the concentration of bacilli in respiratory fluids, it is possible to estimate tularemia infection risk due to bacilli in emitted respiratory aerosol. Similarly, the risk of airborne infection in the laboratory involves the pathogen concentration in the materials being handled, the size and volume distribution of the aerosolized particles, the aforementioned receptor parameters, and the nature of the pathogen's infectious dose. A quantitative estimate of infection risk, even if uncertain, serves to inform biosafety officers in their decision making about infection control procedures.

Acknowledgements

This work was supported by a collaborative agreement with the Association of Schools of Public Health, S2148-22/23. The opinions expressed are solely those of the authors and do not necessarily reflect the views of the funding agency.

References

- Aagaard, G. N. (1944). Involvement of the heart in tularemia. *Minnesota Medicine*, 27, 115-117.
- Bell, J. F., Owen, C. R., & Larson, C. L. (1955). Virulence of *Bacterium tularensis* I: A study of the virulence of *Bacterium tularensis* in mice, guinea pigs, and rabbits. *Journal of Infectious Diseases*, 97, 162-176.
- Black, J. (2002). *Microbiology: Principles and explorations*. New York: John Wiley and Sons, Inc.
- Centers for Disease Control and Prevention. (2003). Key facts about tularemia. Retrieved August 11, 2004 from www.cdc.gov/agent/tularemia/facts.asp.
- Cluxton, Jr., H. E., Clifton, E. E., & Worley, J. A. (1948). Primary tularemia of the lungs masquerading as other forms of lung pathology. *The Journal of the Arkansas Medical Society*, 44, 266-275.
- Conlan, J. W., Chen, W., Shen, H., Webb, A., & KuoLee, R. (2003). Experimental tularemia in mice challenged by aerosol or intradermally with virulent strains of *Francisella tularensis*: Bacteriologic and histopathologic studies. *Microbial Pathogenesis*, 34, 239-248.
- Cox, C. S., & Goldberg, L. J. (1972). Aerosol survival of *Pasteurella tularensis* and influence of relative humidity. *Applied Microbiology*, 23, 1-3.
- Dahlstrand, S., Ringertz, O., & Zetterberg, B. (1971). Airborne tularemia in Sweden. *Scandinavian Journal of Infectious Diseases*, 3, 7-16.

- Day, W. C., & Berendt, R. F. (1972). Experimental tularemia in *Macaca mulatta*: Relationship of aerosol particle size to infectivity of airborne *Pasteurella tularensis*. *Infection and Immunity*, 5, 77-82.
- Dennis, D. T., Inglesby, T. V., Henderson, D. A., Bartlett, J. G., Ascher, M. S., Eitzen, E., Fine, A.D., Friedlander, A. M., Hauer, J., Layton, M., Lillibridge, S. R., McDade, J. E., Osterholm, M. T., O'Toole, T. O., Parker, G., Perl, T. M., Russell, P. K., & Tonat, K. (2001). Tularemia as a biological weapon: Medical and public health management. *JAMA*, 285, 2763-2773.
- Downs, C. M., Coriell, L. L., Pinchot, G. B., Maumenee, E., Klauber, A., Chapman, S. S., & Owen, B. (1947). Studies on tularemia I. The comparative susceptibility of various laboratory animals. *Journal of Immunology*, 56, 217-228.
- Ehrlich, R., & Miller, S. (1973). Survival of airborne *Pasteurella tularensis* at different atmospheric temperatures. *Applied Microbiology*, 25, 369-372.
- Emel'yanova, O. S. (1961). The virulence of strains of *Pasteurella tularensis* isolated from man. *Journal of Microbiology, Epidemiology and Immunobiology*, 32, 853-858.
- Evans, M. E. (1985). *Francisella tularensis*. *Infection Control*, 6, 381-383.
- Fillmore, A. J. (1951). Tularemia with report of six cases. *Arizona Medicine*, 8, 27-33.
- Francis, E. (1927). Tularemia. *The Atlantic Medical Journal*, 30, 337-344.
- Francis, E. (1983). Landmark article April 25, 1925: Tularemia by Edward Francis. *JAMA*, 250, 3216-3224.
- Franz, D. R., Jahrling, P. B., Friedlander, A. M., McClain, D. J., Hoover, D. L., Byrne, W. R., Pavlin, J. A., Christopher, G. W., & Eitzen, E. M. (1999). Clinical recognition and management of patients exposed to biological warfare agents. In J. Lederberg (Ed.), *Biological weapons: Limiting the threat* (pp. 38-80). Cambridge, MA: MIT Press.
- Henderson, D. W. (1952). An apparatus for the study of airborne infection. *Journal of Hygiene*, 50, 53-68.
- Hinds, W. C. (1999). *Aerosol technology* (2nd ed.). New York: John Wiley and Sons, Inc.
- Hood, A. M. (1961). Infectivity of *Pasteurella tularensis* clouds. *Journal of Hygiene*, 54, 497-504.
- Johnson, H. N. (1944). Isolation of *Bacterium tularensis* from the sputum of an atypical case of human tularemia. *Journal of Laboratory and Clinical Medicine*, 29, 903-905.
- Kalbfleisch, J. D., & Prentice, R. L. (2002). *The statistical analysis of failure time data* (2nd ed.). New Jersey: Wiley-Interscience.
- Larson, C. L. (1945). Isolation of *Pasteurella tularensis* from sputum: A report of successful isolations from 3 cases without respiratory symptoms. *Public Health Reports*, 60, 1049-1053.
- Ledingham, J. C. G., & Fraser, F. R. (1923/1924). Tularemia in man from laboratory infection. *The Quarterly Journal of Medicine*, 17, 365-382.
- McCrum, F. R. (1961). Aerosol infection of man with *Pasteurella tularensis*. *Bacteriological Reviews*, 25, 262-267.
- Miller, R. P., & Bates, J. H. (1969). Pleuropulmonary tularemia: A review of 29 patients. *American Review of Respiratory Disease*, 99, 31-41.
- Nicas, M., Nazaroff, W. W., & Hubbard, A. (2005). Toward understanding the risk of secondary airborne infection: Emission of respirable pathogens. *Journal of Occupational and Environmental Hygiene*, 2, 143-154.

- Overholt, E. L., & Tigertt, W. D. (1960). Roentgenographic manifestations of pulmonary tularemia. *Radiology*, 74, 758-765.
- Overholt, E. L., Tigertt, W. D., Kadull, P. J., Ward, M. K., Charkes, N. D., Rene, R. M., Salzman, T. E., & Stephens, M. (1961). An analysis of forty-two cases of laboratory acquired tularemia. *American Journal of Medicine*, 30, 785-806.
- Owen, C. R., & Buker, E. O. (1956). Factors involved in the transmission of *Pasteurella tularensis* from inoculated animals to healthy cage mates. *Journal of Infectious Diseases*, 99, 227-233.
- Reintjes, R., Dedushaj, I., Gjini, A., Jorgensen, T. R., Cotter, B., Lieftucht, A., D'Ancona, F., Dennis, D. T., Kosoy, M. A., Mulliqi-Osmari, G., Grunow, R., Kalaveshi, A., Gashi, L., & Humolli, I. (2002). Tularemia outbreak investigation in Kosovo: Case control and environmental studies. *Emerging Infectious Diseases*, 8, 69-73.
- Rosebury, T. (1947). *Experimental airborne infection*. Baltimore, MD: The Williams and Wilkins Company.
- Saslaw, S., Eigelsbach, H. T., Prior, J. A., Wilson, H. E., & Carhart, S. (1961). Tularemia vaccine study II. Respiratory challenge. *Archives of Internal Medicine*, 107, 702-714.
- Sawyer, W. D., Jemski, J. V., Hogge, Jr., A. L., Eigelsbach, H. T., Wolfe, E. K., Dangerfield, H. G., Gochenour, Jr., W. S., & Crozier, D. (1966). Effect of aerosol age on the infectivity of airborne *Pasteurella tularensis* for *Macaca mulatta* and man. *Journal of Bacteriology*, 91, 2180-2184.
- Shapiro, D. S., & Schwartz, D. R. (2002). Exposure of laboratory workers to *Francisella tularensis* despite a bioterrorism procedure. *Journal of Clinical Microbiology*, 40, 2278-2281.
- Sjostedt, A., Tarnvik, A., & Sandstrom, G. (1996). *Francisella tularensis*: Host-parasite interaction. *FEMS Immunology and Medical Microbiology*, 13, 181-184.
- Syrjala, H., Kujala, P., Myllyla, V., & Salminen, A. (1985). Airborne transmission of tularemia in farmers. *Scandinavian Journal of Infectious Diseases*, 17, 371-375.
- Syrjala, H., Sutinen, S., Jokinen, K., Nieminen, P., Touuponen, T., & Salminen, A. (1986). Bronchial changes in airborne tularemia. *The Journal of Laryngology and Otology*, 100, 1169-1176.
- Van Metre, Jr., T. E., & Kadull, P. J. (1959). Laboratory-acquired tularemia in vaccinated individuals: A report of 65 cases. *Annals of Internal Medicine*, 50, 621-632.
- World Health Organization. (1970). *Health aspects of chemical and biological weapons*. Geneva: World Health Organization.

Safety Library Reference

The National Academies Press provides, free of charge, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*, 1995, in a searchable, printable format at:

<http://www.nap.edu/openbook/0309052297/html/index.html>

