Tularemia: History, Epidemiology, Pathogen Physiology, and Clinical Manifestations

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ABSTRACT: *Francisella tularensis* has been recognized as a human pathogen for almost 100 years and is the etiological agent of the zoonotic disease tularemia. Soon after its discovery, it became recognized as an important pathogen in several parts of the world, for example, in the United States and Soviet Union. The number of tularemia cases in the two countries peaked in the 1940s and has thereafter steadily declined. Despite this decline, there was still much interest in the pathogen in the 1950s and 1960s since it is highly infectious and transmissible by aerosol, rendering it a potent biothreat agent. In fact, it was one of the agents that was given the highest priority in the offensive programs of the United States and Soviet Union. After termination of the offensive programs in the 1960s, the interest in *F. tularensis* diminished significantly and little research was carried out for several decades. Outbreaks of tularemia during the last decade in Europe, for example, in Kosovo, Spain, and Scandinavia, led to a renewed public interest in the disease. This, together with a massive increase in the research funding, in particular in the United States since 2001, has resulted in a significant increase in the number of active *Francisella* researchers. This article summarizes, predominantly with a historical perspective, the epidemiology and clinical manifestations of tularemia and the physiology of *F. tularensis*.

KEYWORDS: *Francisella*; tularemia; epidemiology; pathogen physiology; clinical manifestations

A RENEWED INTEREST IN *FRANCISELLA TULARENSIS*

Francisella tularensis has been recognized as a human pathogen since the beginning of the 20th century. Reports of diseases strongly resembling tularemia preceded the first authenticated report in 1911 by McCoy.[1] For example, there were cases reported from Utah in 1908,[2] from Norway of a disease

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called lemming fever during the 1890s,[3] and of Yato-Byo (hare disease) from Japan as early as 1818.[4] There is also a description from Norway from 1653 of a tularemia-like disease of lemmings.[5] The first verified case of human tularemia occurred in Ohio in 1914.[6]

The isolation of *F. tularensis* has been reported from many countries of the Northern Hemisphere, but it is a rare pathogen in most countries. The irregular occurrences of tularemia epidemics and the high contagiousness of the pathogen have resulted in a limited experience and interest in *F. tularensis* in most clinical laboratories and also a rudimentary knowledge of its pathogenic traits. Until 2001, few researchers were actively working on the pathogen and a lack of genetic tools severely restricted research on its virulence mechanisms. Recently, however, there has been a very substantial increase in the number of *F. tularensis* researchers, most notably in the United States since 2001, as a result of the massive increase in funding of Biodefense-associated research.

For many years, *F. tularensis* has been considered as a potential biological weapon and, in the 1940s and 1950s, it was one of the agents that was given the highest priority in the offensive programs of the United States and the Soviet Union and it was stockpiled by the U.S. military even in the late 1960s.[7] A former Soviet Union scientist, Ken Alibek, who had been involved in the biological weapons program, claimed that an offensive program in the Soviet Union continued until the early 1990s and resulted in production of *F. tularensis* strains engineered to be resistant to antibiotics and vaccines.[8] There are also instances when *F. tularensis* was directly used in experiments, for example, as part of the American program on biological warfare agents, volunteers were infected with *F. tularensis* by direct aerosol delivery and by exposure in an aerosol chamber.[7] The much publicized, notorious Japanese research unit 731 in Manchuria studied the effects of biological agents on human beings before and during World War II.[9] A minor part of the work dealt with *F. tularensis*.

In 1969, the World Health Organization developed a model for predicting the outcome of bioterrorist attacks.[10] It was calculated that aerosolization of 50 kg of *F. tularensis* bacteria in a metropolitan area with a population of 5 million would result in 250,000 incapacitated individuals and some 19,000 deaths. Illness would be expected to persist for several weeks and disease relapses to occur during the ensuing weeks or months.

The model has also been used by CDC to assess the costs of a bioterrorist attack with *F. tularensis* and it was concluded that the extreme infectivity of the pathogen will lead to very high costs; $5.4 billions per 100,000 individuals affected.[11] This cost is probable higher than costs related to any other biological agent with the exception of anthrax and smallpox. The high costs and high morbidity justify the inclusion of *F. tularensis* as an agent of the highest priority together with, for example, anthrax, smallpox, and pandemic influenza. Accordingly, it is one of six agents designated as a category A Select Agent by Centers for Disease Control and Prevention (CDC), that is, agents considered

FIGURE 1. Edward Francis was one of the most prominent pioneering scientists working on *Francisella tularensis* and the bacterium was given its present name in his commemoration.

as the most likely biological threat agents. These traits are also reflected by the fact that it is highly contagious for laboratory workers.

THE TAXONOMY OF *FRANCISELLA TULARENSIS*

The original name of *F. tularensis*, first suggested by Edward Francis (FIG. 1) in 1919, was *Bacterium tularensis*,[12] named after Tulare County, California where the disease was endemic among rodents. The bacterium was during the 1920s, based on serological analysis, designated as *Pasteurella tularensis* but in 1966 it was determined by DNA hybridization that the genus

was not closely related to *Pasteurella*.[13] Later, 16S rDNA sequence analysis revealed that *Francisella* taxonomically belonged to the γ-subclass of *Proteobacteria*, but showed no close relationship to other characterized genera.[14] In 2007, *Francisella* still is the only recognized genus in *Francisellaceae*.[15] The closest related family is *Piscirickettsiaceae*, containing a number of genera, among others *Piscirickettsia*, which comprises the intracellular bacterium *Piscirickettsia salmonis*, the etiological agent of a septicemic disease in fish.[16] There are several recent reports on the identification of bacteria pathogenic for Atlantic cod that belong to the genus *Francisella.*[17,18] The distinct taxonomy of *Francisellaceae* is further supported by their unique cellular fatty acid composition of *Francisella* and the unusually high lipid content of the cell wall.[19]

The numerous reports on tularemia until the 1950s laid the basis for several classifications of the disease. The classifications depend upon the clinical picture, epidemiology, or source of infection. Based on the clinical picture, the following forms are widely recognized; ulceroglandular, or glandular, oropharyngeal, and respiratory tularemia. In 1959, Olsufiev *et al*.[20] recognized that isolates showed distinct virulence differences, for example, for hares. The organisms prevalent in North America, but not elsewhere in the world, showed very high virulence for most species assayed and caused a severe form of human illness and it was proposed to be designated *F. tularensis* biovar tularensis. The less virulent organism encountered in Europe, Asia, and North America was tentatively designated as *F. tularensis* biovar palaearctica. In 1970, the same authors proposed that the biovars should be given the status of subspecies and suggested the designation *F. tularensis* subsp. *holarctica* for the less virulent form and *F. tularensis* subsp. *tularensis* for the more virulent form.[21] Moreover, the designation *F. tularensis* subsp. *mediasiatica* for strains from the Central Asian republics of the Soviet Union was proposed. The taxonomical classification was based on the biochemical properties, the virulence, and the geographical origins of strains. Moreover, in 1983, a subdivision of the subspecies *holarctica* was proposed.[22] Strains were distinguished in view of their susceptibility to erythromycin and designated biovar I Ery^S and biovar II Ery^R. Also, Japanese strains were proposed as a third biovar, *F. tularensis* subsp. *holarctica* biovar *japonica* Rodionova. However, no or very few other phenotypic attributes have been demonstrated to support the subdivision of the subspecies *holarctica*. The designations of Type A and Type B have been proposed for *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica*, respectively.[23] Although the former designations have no formal approval, they are widely used since they are simple and unambiguous.

A bacterium closely related to *F. tularensis* was isolated from a water sample from Ogden Bay, Utah in 1951. Originally it was classified in the genus *Pasteurella*,[24] but transferred to the genus *Francisella* in 1959.[20] After originally being designated *F. novicida*, it was suggested in 1989 to constitute a

subspecies of *F. tularensis* based on DNA relatedness and biochemical characteristics.[25] *F. tularensis* subsp. *novicida* isolates have less fastidious metabolic requirements than isolates of the other three subspecies. Despite distinct growth requirements and certain metabolic reactions, the inclusion of *novicida* as a *F. tularensis* subspecies is strongly supported. Analysis of fatty acid profiles showed *F. novicida* to be similar and the DNA relatedness was more than 75% to other members of the species *F. tularensis*.[25] Moreover, virtually identical 16S rDNA sequences of *F. tularensis* subsp. *novicida* and the other three subspecies have been demonstrated.[14] Another support for the inclusion of the subspecies *novicida* in the species *F. tularensis* is the demonstration that *F. tularensis* subsp. *novicida* could be transformed with DNA from strain LVS, a derivative of a Russian *holarctica* strain.[26] The type strain (ATCC 15482) is considered nonpathogenic for humans and demonstrates lower virulence for mice than isolates of the other subspecies but *novicida*-like isolates have been identified as the etiological agents of severe diseases in man[25,27] although the affected individuals most likely were immunocompromised. Notably, the reference strain of *novicida* was isolated from water in a marsh in Utah[24] and in some cases of human disease, the suspected source has been natural water.[27]

Currently, there are four subspecies recognized, *tularensis*, *holarctica*, *mediasiatica*, and *novicida* (reviewed in Ref. 15). *F. tularensis* subsp. *tularensis* has only been isolated in North America and is the predominant form on the continent since, based on its representation in strain collections, it has been estimated to cause 70% of the human cases on the continent.[28] It is possible, however, that the relative representation in strain collections does not represent the true occurrence of the subspecies as an etiological agent of human disease since it causes a more serious form of the disease and, therefore, may be more likely to be isolated. Subspecies *tularensis* is highly virulent for humans and numerous animal hosts, including domestic rabbits. Most isolates derive from ticks and rabbits. *F. tularensis* subsp. *tularensis* is highly contagious and even in humans, doses of 10 bacteria can cause infection after subcutaneous injection and 25 organisms when given by aerosol.[29,30] The other important etiological agent of human disease is *F. tularensis* subsp. *holarctica*. There is a lack of data on its infectious dose in humans and it cannot be excluded that it is similar to that of subspecies *tularensis.* It is clear, though, that isolates of this subspecies are less virulent, especially for humans and lagomorphs since dissemination is slower and it causes a milder form of disease. *F. tularensis* subsp. *holarctica* isolates are spread throughout the Northern Hemisphere. The subspecies seems to be predominantly associated with semiaquatic rodents, for example, muskrats and beaver in North America, and ground voles in the former USSR. It is not only associated with semiaquatic rodents but has also been isolated from streams, ponds, lakes, and rivers. In Europe, tularemia is most frequently observed in hares and rodents.

F. tularensis subsp. *mediasiatica*, which has only been isolated in the Central Asian republics of former USSR, is often found in species of *Lepus* and *Gerbillinae* and in ticks.[22] Isolates of the subspecies have not been well characterized but appear to be significantly less virulent for animals such as rabbits than isolates of subspecies *tularensis*. It has been recognized that the epizootology of tularemia is highly complex and the aforementioned associations are often, but not always, true.[31] Generally, man-to-man transmission of tularemia is extremely rare or, perhaps nonexistent.

F. philomiragia was originally given the species designation *Yersinia philomiragia*[32] but subsequent analysis showed that it had no relationship to other members of the genus[33] and until 1989 was considered a species *incertae sedis*.[34] Then, it was proposed to be transferred to the genus *Francisella* on the basis of DNA relatedness and fatty acid analysis. It was designated *F. philomiragia* comb. nov.[25] and subsequently *F. philomiragia*,[15] thus being the second recognized species in the genus. The species shows a high 16S rRNA sequence similarity and the unique fatty acid profile typical of strains of *F. tularensis*, but is distinct from most *F. tularensis* strains by being capable of degrading certain carbohydrates and by a lack of requirement for cysteine for supporting growth. Like *F. tularensis* subsp. *novicida*, *F. philomiragia* is closely linked to water-borne transmission. Five isolates of *F. philomiragia*, including the type strain, were isolated from a marshy area in Utah.[32] Five out of 13 patients with a disease caused by *F. philomiragia* had a history of near drowning.[35]

The species *W. persica* and several tick endosymbionts all show the unique 16S rRNA sequence signatures of *Francisella*.[15] Thus, they should be included as members of *Francisella* in the future. At present, insufficient data exist to determine their relationship at the species level. Also *Caedibacter taeniospiralis*, a protozoan endosymbiont, shows high 16S rRNA similarity to members of *Francisellaceae.*[36] In addition, several isolates identified in soil samples in the Houston area of the United States possibly are members of new *Francisella* species.[37] Some of them demonstrate high 16S rRNA similarity to *F. tularensis*.

FRANCISELLA TULARENSIS—A FACULTATIVE INTRACELLULAR BACTERIUM

The bacterium was suggested to be an intracellular parasite already in the 1920s and Francis demonstrated that it had the ability to proliferate inside cells.[38] Much later, in the 1960s, results suggested that the bacterium not only survived, but also multiplied intracellularly in mononuclear phagocytes, although the findings were not conclusive (reviewed in Ref. 39). Moreover, the critical finding was made that resistance to tularemia in mammals was conferred by living lymphoid cells, but not by antiserum.[40] The latter results

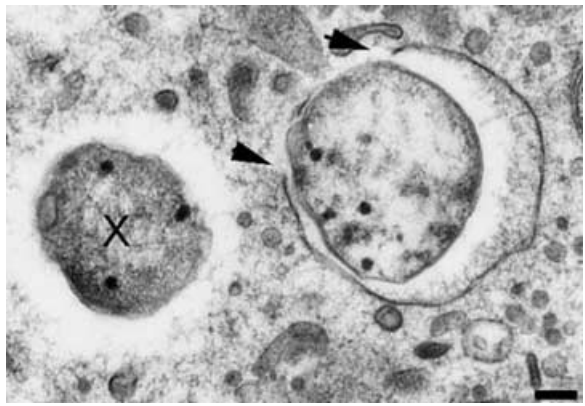

FIGURE 2. An *Francisella tularensis* LVS infected mouse peritoneal exudate cell. One bacterium is located in the cytoplasm without any phagosomal membrane surrounding it (marked with an X) and the other is enclosed by a membrane with an obvious defect (the defects are marked with arrowheads). Bar: 0.24 μm.

are similar to those obtained on other intracellular organisms. Most studies *in vitro* have employed various forms of macrophages as the target cell. It should be remarked that *F. tularensis* is capable of infecting a large number of cell types and it is possible that other cell types than macrophages are important as reservoirs for the replication *in vivo*. In fact, in the experimental mouse model, it has been demonstrated that hepatocytes are heavily infected throughout the course of infection and after aerosol administration, bacteria are initially localized in dendritic cells and subsequently in alveolar Type II cells.[41–43] Moreover, old studies investigated a wide range of cells and observed that *F. tularensis* was capable of multiplying in endothelium of guinea pigs and *Dermacentor andersoni* as well as in HeLa cells and in mouse fibroblasts (reviewed in Ref. 39). In a primate model, *F. tularensis* was localized in macrophages and extracellularly in respiratory bronchioles after aerosol administration.[44]

A number of studies during the last few years have elucidated the outcome of the *F. tularensis* infection of macrophages. The first findings that contradicted the long held dogma that *F. tularensis* survived within the phagosome of phagocytic cells were presented by Golovliov *et al*., who demonstrated a cytoplasmic localization of *F. tularensis* LVS in mouse and human monocytic cells (FIG. 2).[45] Subsequently, this finding was corroborated by a number of studies using also *F. tularensis* subsp. *novicida* and virulent isolates of subspecies *holarctica* and *tularensis*.[46–48]

Several recent studies have elucidated the early host–parasite interaction during the intramacrophage *F. tularensis* infection. Irrespective of whether

F. tularensis LVS, *F. tularensis* subsp. *novicida*, or a clinical isolate of *F. tularensis* subsp. *tularensis* was studied, bacteria initially colocalized with the late endosomal/lysosomal markers LAMPs, but not with cathepsin D.[46,47] Moreover, the phagosomes containing *F. tularensis* bacteria were not significantly acidified.[46] In both macrophages of human and mouse origin, bacteria started to egress from the phagosome within at most one hour and soon thereafter, a large majority of bacteria appeared to be free and replicating in the cytoplasm.[45–47] Subsequently, a majority of bacteria were enclosed within large, juxtanuclear, LAMP-1-positive vacuoles.[48] Thus, the findings indicate that *F. tularensis* bacteria are capable of altering the maturation of the phagosome, as evidenced by the exclusion of cathepsin D, lysosomal tracers, and the lack of acidification and, then, by an unknown mechanism; of disrupting the phagosomal membrane but later via an autophagic mechanism; and of reentering the endocytic pathway.

A study on *F. tularensis* LVS demonstrated that activation of peritoneal exudate cells by addition of IFN-γ rendered the bacteria less capable, but did not prevent the escape from the phagosome,[49] whereas in IFN-γ-activated human-derived macrophages, the escape of *F. tularensis* subsp. *novicida* was completely inhibited.[47] No study has addressed how the intracellular localization of virulent strains of *F. tularensis* is affected when macrophages are activated.

In monocytic cells, *F. tularensis* LVS and human virulent *F. tularensis* strains grow until the viability of the host cells is compromised. The mechanisms of this cytopathogenic effect have been elucidated in a series of studies and it was observed that the *F. tularensis* LVS-infected cells of the mouse macrophage cell line J774 are killed by apoptosis within 24–48 h.[50,51] The apoptosis is mediated via the intrinsic pathway with critical involvement of the mitochondria, as evidenced by a change of the mitochondrial potential and release of cytochrome *c*, followed by activation of caspase-9, formation of the apoptosome, and subsequent activation of caspase-3.[51] By contrast, the *iglC* mutant of *F. tularensis* LVS induces no apoptosis.[52] It remains to be determined if the host cell death resulting from infection with virulent strains also is mediated via apoptosis. The scarce data that exist indicate that infection with the SCHU S4 strain of subsp. *tularensis* induces more rapid cytopathogenicity than does LVS.[53] A study, employing *F. tularensis* LVS, demonstrated that not only apoptosis occurred in J774 cells but the infected cells subsequently developed signs of necrosis.[54] Moreover, the study identified that concomitant with the development of apoptosis, phosphorylation of p42/p44 MAPK (Erk1/2) occurred and it was demonstrated that levels of apoptosis were markedly diminished if cells were treated with an inhibitor of MEK1/2. In agreement with the study by Telepnev *et al*.,[55] it was also noted that a significant inhibition of p38 MAPK activity occurred in infected cells and it was proposed that the inhibition is an important component in the pro-apoptotic activity of *F. tularensis*.[54]

THE EPIDEMIOLOGY OF TULAREMIA

"The bubo is usually about the size of a pea and the gland structure is generally replaced by a firm caseous mass." This first description of tularemia was published in the paper "A Plague-like Disease of Rodents" in 1911 written by George McCoy, a pathologist working in California.[1] The first human cases were reported by Wherry and Lamb in 1914.[6] As suggested by Edward Francis, the etiological agent became known as *Bacterium tularense*, named after Tulare County where the disease was endemic among rodents. A similar disease was reported under different names from various regions of the United States during the next decade. For example, Francis published a report in 1919 entitled "Deer-fly fever—a disease of man of hitherto unknown etiology."[12] The same author wrote a comprehensive review in 1921, in which he suggested that the disease was transmitted to man from rodents by bites of blood-sucking insects.[56] Moreover, he proposed the disease be named tularemia. In the commemoration of the pioneering work of Francis, the bacterium eventually received its present day name, *Francisella tularensis*. After these pioneering publications, reports on tularemia quickly accumulated; by 1929 more than 800 cases had been reported in United States, and by 1945 more than 14,000.[57] A peak incidence was recorded 1939 with 2,291 cases. By this time, it was obvious that *F. tularensis* and tularemia were confined not just to North America, since Japanese and Russian researchers reported on a similar disease in the 1920s.

Extensive epidemiological information was gathered about tularemia during the first decades after its discovery. Despite all this knowledge, there were no uniform descriptions of the life cycle of *F. tularensis*. It was clear that understanding the epidemiology of tularemia was far more difficult than other diseases resembling tularemia, for example, plague. There are a number of reasons for this. First, the occurrence of *F. tularensis* is much localized, some endemic areas encompass no more than a few hundred square kilometers and neighboring areas may have a very low incidence of the disease. Second, many epidemiological studies are based on the assumption that there is a correlation between the occurrence of a pathogen and the resulting disease. However, it appears likely that *F. tularensis* may persist in the environment without concomitant outbreaks of tularemia in the same geographical regions. There is evidence that *F. tularensis* persists in endemic areas but that an active infectious cycle requires a number of other prerequisites such as certain climatic conditions.[58] Therefore, an understanding of its life cycle requires simultaneous analysis of the occurrence of the bacterium in the environment and in vectors and as well as recording of tularemia cases among animals and humans. Thus, a correct assessment requires extensive trapping of the primary mammalian reservoirs of *F. tularensis*, such as rodents and lagomorphs, and of vectors, ticks, flies, and mosquitoes. In most countries, such epidemiological

investigations are not made currently since they are very time-consuming and expensive.

The irregular distribution of *F. tularensis* and cases of tularemia make it almost impossible to accurately illustrate its distribution worldwide. At the same time, it is obvious that the bacterium does not have a pan-global distribution since only isolates of subspecies *novicida* have been found in the Southern Hemisphere.[59] Thus, the currently known distribution of tularemia coincides with the boundaries of the holarctic animal region.

After the first descriptions of the disease in the 1910s and 1920s, detailed epidemiological investigations were made for many decades in the former Soviet Union, some countries of Eastern Europe and in some parts of the United States. Besides the epidemiological information, it became clear that there are fundamental properties that distinguish *F. tularensis* strains and they had to be taken into account when describing the epidemiology of tularemia. Crucial to this was the finding by Olsufiev *et al*. in 1959 of the marked differences in virulence of the subspecies *holarctica* and *tularensis*.[20] The latter has a distinctly higher virulence for certain mammals such as lagomorphs. The underlying differences between the two biovars were further emphasized by Jellison who introduced the concept of two distinct types of tularemia, Type A and Type B, distinguished not only with regard to virulence but also with regard to biochemical and epidemiological properties.[23] It was suggested that transmission of subspecies *tularensis* strains are associated with rabbits, ticks and sheep, whereas strains of subspecies *holarctica* are often isolated in association with streams, ponds, lakes, and rivers and water-associated species such as water voles.

THE DISTRIBUTION OF HUMAN TULAREMIA CASES

For a long time, endemic foci of tularemia have existed in Russia, Kazakhstan, and Turkmenistan[60] as well as in Finland and Sweden.[61] In the former Soviet Union, the total numbers of cases have decreased significantly and, since 1990, have not exceeded a few hundred cases annually.[61] Cases are reported every year from many countries in Eastern Europe but it is a rare disease in western Europe although extensive outbreaks have occurred during the last decade in Spain.[62,63] Other regions with significant outbreaks have been in Sweden and Kosovo, each comprising many hundreds of cases.[64,65] A new focus of tularemia has emerged during the last decade in an area surrounding the city of Örebro in central Sweden.[64] Cases are also reported annually from Japan and the northern regions of China. In the Western Hemisphere, there are each year cases reported from United States and Canada and historically a few cases have been reported from Mexico. On average, some 120 cases have been reported annually in the United States from 1990 to 2000.[66] More than half of these cases have occurred in Arkansas, Missouri, South Dakota, and Oklahoma. Small outbreaks have been reported during the last decades

from the island Martha's Vineyard in Massachusetts.[67,68] No cases of human tularemia have been reported from the Southern Hemisphere.

TULAREMIA IN THE FORMER SOVIET UNION AND IN RUSSIA

In the Former Soviet Union extensive investigations of the occurrence of *F. tularensis* were made from the 1930s until the 1970s. Tularemia was an endemic disease in many regions and a considerable research effort was undertaken to define the natural foci, to understand the reasons for its persistence and to implement preventive measures. Numerous classification schemes were proposed; Olsufiev and co-workers suggested one of the most elaborate (reviewed in Ref. 58). They proposed that the natural foci could be classified as six types; swamp-floodland, grassland-meadowland, woodland, steppe land, cisalpine-stream type, and desert-flood land. The former two foci are the most common and well-defined foci, usually characterized by agricultural outbreaks and in the wintertime water-borne outbreaks due to common voles in the grassland-meadowland areas and to vector-borne disease originating from the water vole in the swamp-floodland areas. The epidemiological data was based on screening tests performed by skin-testing of the human populations potentially exposed to *F. tularensis* such as hunters and herdsmen as well as serological analysis of cattle and sheep. The presence of *F. tularensis* was verified by cultivation of the pathogen from *Ixodidae*, for example, *Dermacentor* species, collected in large quantities from domestic animals, such as cattle and sheep, and from tularemia-susceptible rodents. Prophylactic measures in these endemic areas included vaccination of humans. Gaisky and Elbert developed the first live *F. tularensis* vaccine in 1942.[69] Other prophylactic measures included poisoning of water rats and common voles by toxic baits. The latter were also eradicated by fumigation of the entrances of vole burrows. In agricultural areas, stubbles were removed and thorough plowing was performed. Organized hunting was performed to limit the populations of hares, water rats, and other game rodents and ticks were actively controlled by treatment of, for example, cattle and/or the vegetations by pesticides. Also implementation of sanitary rules and regulation of water sources were enforced. Besides such directed research efforts to eradicate the disease in the Former Soviet Union, there was also extensive research performed to characterize the ecology and epidemiology of tularemia. Numerous naturally infected arthropod vectors, animal hosts, and modes of contracting human disease were identified. From this work it is evident that the ecology of the disease is highly complex in Eurasia. In Soviet Union, the involvement of certain rodents (*Arvicola terrestris*, *Microtus* spp., *Mus musculus*, and *Clethrionomys* spp.), the arctic hare (*Lepus timidus*), and several hard tick species (*Ixodidae* spp.) as well as mosquitoes (*Aedes*, *Culex*, and *Anopheles* spp.) and horse flies (*Tabanidae*) have been proven (reviewed in Ref. 58).

In 1936, O1sufiev and Golov demonstrated experimental transmission of tularemia by the bites of various tabanid species from sick and dead guinea pigs and from dead water rats to healthy sheep, rabbits, rats, and guinea pigs.[70] Preservation of *F. tularensis* for 56 h in the fly gut was demonstrated. The authors had observed that tularemia cases were not directly related to exposure to rodents that existed most frequently during the tabanid season and the patients displayed lesions on exposed body surfaces with subsequent localized lymphadenopathy. These observations in conjunction with their experimental results led them to conclude that horse flies are important vectors for the dissemination of tularemia. Later, Olsufiev was successful in isolating *F. tularensis* bacteria from naturally infected tabanids of the same species as those used as experimental vectors.[58] Other vectors were also demonstrated to mechanically transmit *F. tularensis*, for example, *Aedes, Mansonia*, and *Anopheles* species.[71]

Despite these convincing data on the potentially important role of tabanids and mosquitoes for transmission, there is an uncertainty regarding the natural source of fly/mosquito infection. It should be noted that *F. tularensis* has never been demonstrated to reside in the salivary glands of any arthropod species. It cannot be ruled out that the spread is mechanical, by mouthparts that become infected when a fly or a mosquito bites an infected host or that *F. tularensis* is rubbed into the skin when the arthropod is swatted. It has been suggested that water contaminated with *F. tularensis* might serve as a reservoir of infection of flies or mosquitoes, possibly at their larval stage.[58]

Altogether, the aforementioned Russian data, together with American data,[57] indicate that tabanids, in particular *Chrysops* species (deer flies), are primary means whereby tularemia is transmitted in endemic regions of Russia and western United States. However, the role of tabanids in the natural transmission of *F. tularensis* in areas other than the aforementioned endemic regions is not known but the flies are prevalent in almost the whole world and females of all species are blood-sucking. Also ticks (*Ixodes* spp) have been implicated in the transmission of tularemia. This is particularly true in the United States, but Russian authors have also published evidence supporting this hypothesis. The natural occurrence of *F. tularensis* in ticks in the former Soviet Union appears to be low, however; a prevalence of only 0.01% infected ticks were detected among 120,000 adult *I. ricinus* collected between 1960 and 1964.[72] *F. tularensis* has been detected in at least 20 flea species of eight genera but their role in the spread of the bacterium is unclear.[72]

Not only human tularemia cases have decreased considerably in the Soviet Union/Russia during the last 50 years, but also the widespread epizootics that were commonly reported during the 1930s and 1940s. Already long ago, it was demonstrated that the epizootics have followed after an increase in the rodent population size. Moreover, a recent publication demonstrated that the number of human cases of tularemia in the Novosibirsk region from 1956 to 2000 was correlated to the density of the water rat population.[73]

TULAREMIA IN NORTH AMERICA

After the first description of human tularemia in 1914 by Wherry and Lamb, the number of reported cases quickly accumulated and by 1945 more than 14,000 cases had been reported.[23] Very high numbers of cases were reported from Illinois and neighboring states. For example, until 1945, 2,267 cases had been reported from Illinois.[23] Concomitantly health authorities started to investigate the routes of infection and it soon became apparent that hunting and sale of cottontail rabbits (*Sylvilagus* sp.) were the predominant route in vast areas of the eastern states. In 1942, Francis estimated that two thirds of all American tularemia cases could be linked to contacts to *Sylvilagus*.[74] Tularemia had also spread from the Midwest/Eastern focus to New England, which originally had been free from the disease, since rabbits from the Midwest were imported as a source of game.[67] The importance of *Sylvilagus* as a source of infection for man lies in its very high degree of susceptibility to an infection with *F. tularensis*. In particular, the populations appear to be very susceptible during epizootic periods and the death rates are very high.[75] Historically, *Lepus* species (*L. articus, americanus, townsendii,* and *californicus*) have not represented an important source of tularemia in North America, possibly because they are more resistant than *Sylvilagus* species. Moreover, they have not been as widely hunted as the latter species.

Another important source of human infection was tick bites. Although the original source of these infections is difficult to trace, it was suspected that ticks acquired the infection from cottontail rabbits or rodents. Of 700 cases identified between 1938 and 1948 in Arkansas, 56% percent were identified as the result of tick bites and 3l% by contact with infected rabbits.[76] These numbers agree well with more recent experiences; a summary of more than 1,000 cases during the 1980s in Arkansas, Kansas, Louisiana, Missouri, Oklahoma, and Texas demonstrated that 63% reported an attached tick and 23% exposure to rabbits.[77] These cases represented 60% of all reported tularemia cases in the United States during the period. Today, Arkansas has the highest number of reported tularemia cases with an incidence of more than 30 per 1 million population. After the peak number of cases were recorded in the late 1930s and early 1940s, the number of cases steadily declined and in contrast to the more eastern occurrence of the United States tularemia cases during the first half of the 20th century, most human cases currently occur in the south, central, and western States, especially Arkansas, Missouri, Oklahoma, South Dakota, and Montana.

In the 1942 review by Francis, it was apparent that an endemic focus also existed in the western parts of the United States, predominantly in Montana and Nevada, in which other *F. tularensis*-susceptible species than *Sylvilagus* resided.[74] Already in 1921, Francis and Wayne proved that *F. tularensis* could be transmitted to man by the bite of *Chrysops discalis*, which subsequently was substantiated by epidemiological data.[23] Jellison reviewed tularemia cases

from 1915 to 1949 with the simultaneous occurrence of *C. discalis* and concluded that the fly must represent the most likely mode of transmitting *F. tularensis* in many areas of North America. One reason for its importance is the predilection to feed on rodents and rabbits. Historically, this appeared to be a common infectious cycle in the western parts of the United States.

During the first decades after the discovery of *F. tularensis*, an infectious cycle of great economic impact in North America was described. In 1923, Parker proved that sheep were susceptible to *F. tularensis*. Subsequently, severe epizootics were reported that involved the loss of large numbers of sheep.[78] It was hypothesized that the outbreaks followed after epizootics among rodents or lagomorphs hadexisted. The infection often occurred in spring and was transmitted by *Dermacentor andersoni.*[78]

Besides the aforementioned terrestrial cycles, there has also existed a common aquatic-associated life cycle of *F. tularensis* in North America. The bacterium was isolated in river water in Russia already in 1934 and this alerted North American authorities to the possibility of such a life cycle. A review by Parker summarized more than 100 human cases of aquatic-associated tularemia in the United States.[79] A serological study on reactivity among 3,000 Indians in a border region between Ontario and Manitoba where rodent epizootics had occurred, revealed that 12% were sero-positive.[80] Jellison suggested that the presence of *F. tularensis* in watercourses and surrounding marshland is a precondition for the outbreak of epizootics among muskrats and beavers and that the bacteria are secreted into the environment by the excrements of the infected animals.[57] For example, experimentally infected voles acquired chronic infection with bacteriuria, thereby amplifying water contamination.[81] Parker *et al*. demonstrated that *F. tularensis* might survive for at least three months in mud and water and this suggested that the aquatic persistence might not require any mammalian link.[79] There were numerous instances of water-associated epizootics, for example, in beavers and muskrats in Montana, Idaho, Wyoming, Oregon, and Washington in 1942 and human cases resulting from contacts with beavers or muskrats occurred up to 1950 in many states.[79] Similar epizootics were described in Alaska and in a National Park in Alberta in the winter of 1952–53.[23] Today, the water-living muskrat (*Ondatra zibethicus*) is considered the main source of tularemia caused by subspecies *holarctica* in Canada.[3] Also the Canadian beaver (*Castor canadensis*), in contrast to the European beaver, appears to be susceptible to *F. tularensis* subsp. *holarctica* and may serve to sustain the aquatic cycle.[3] Overall, however, the numbers of water-associated tularemia epizootics in North America are far fewer than those reported in Soviet Union. Presumably this is due to the fact that most or all of these occurrences are related to bacteria belonging to subspecies *holarctica* and that the subspecies is not as prevalent in North America.

More recently, other types of epizootics have been described in North America, for example, among mink[82] and prairie dogs.[83] The latter outbreak occurred in a Texan exotic pet facility. In July 2002, more than 250 prairie dogs

died and *F. tularensis* subsp. *holarctica* was isolated from many of the dead prairie dogs. Some of the infected animals had been wild-captured and it was suspected that they introduced the bacteria into the facility. The disease spread by cannibalism and all infected prairie dogs displayed signs of oropharyngeal tularemia. It was suggested that prairie dogs might act as chronic carriers of *F. tularensis* since *F. tularensis* was cultivated from seropositive prairie dogs.

In a recent American study, it was found that human infection cases by subspecies *tularensis* and subspecies *holarctica* differed with respect to affected populations, anatomic site of isolation, and geographic distribution.[28] Moreover, molecular typing with pulsed-field gel electrophoresis defined two subpopulations of Type A (A-east and A-west) that differ with respect to geographic distribution, disease outcome, and transmission. The data suggested that Type A-west infections are less severe than either Type B or Type A-east infections. The same subpopulations were also described in a study of the genetic relatedness of *F. tularensis* strains.[84] They were then designed AI (A-east in the former publication) or AII (A-west).

TULAREMIA IN SCANDINAVIA, CONTINENTAL EUROPE, AND JAPAN

A publication by Francis refers to cases of human tularemia in Scandinavia in 1929 in Norway and in 1931 in Sweden.[85] The former cases were also described in a publication by Thjötta.[86] In central Europe, the first cases of tularemia were reported in the Moravin Basin in 1936.[87] In the central European foci, hares appeared to represent the main carrier and the source of tularemia in man whereas ticks harbored by hares were the perennial reservoir of *F. tularensis*. Outbreaks of tularemia among hares in Europe have persisted to date.[63,88] In the late 1990s, several outbreaks of tularemia were reported from Spain, a country with no previously reported cases of human tularemia. A total of 559 cases of tularemia were reported in 1997 with 519 cases from the community of Castille-Leon in northwestern Spain. A great majority had had previous contact with hares.[63] Ulceroglandular tularemia was the most common form and isolates of subspecies *holarctica* were isolated from patients and also from hares in the region. A suspected source of the emergence of tularemia in Spain is the widespread import of hares for hunting from Central Europe.[63] However, the role of rabbits and hares in the spread of *F. tularensis* subsp. *holarctica* to other mammals and humans is relatively rare in most parts of the world and this infectious cycle may be predominantly related to hunters[87]. In the environment, *F. tularensis* subsp. *holarctica* appears to be spread mainly by various terrestrial and aquatic mammals such as ground squirrels, rabbits, hares, beavers, muskrats, and, in particular, rodents such as meadow voles and water voles.

Widespread epizootics of tularemia often have been associated with a preceding increase in the density of the rodent population size. For example,

the number of human cases of tularemia in the Novosibirsk region of the Russian Federation from 1956 to 2000 correlated to the density of the water rat population.[73] Similarly, a strong correlation between peaks in vole and hare populations and outbreaks of tularemia in humans in Sweden were reported during the 1960s and 1970s,[89] but this correlation has not occurred during the recent decades. In countries of continental Europe, there have been occasional associations between the size of rodent populations and tularemia outbreaks. For example, storage for industrial sugar production attracts large rodent populations and this resulted in tularemia outbreaks among sugar-factory workers in Southern Moravia (Czech Republic) in 1961–62 and Ukraine in 1948–49.[90] In Sweden, the largest number of cases, 700, was reported during the winter of 1966–1967, mostly from the province of Jämtland, and was related to large increases of rodent and lemming populations, many of which died of tularemia.[91] The carcasses contaminated hay, which led to aerosol dissemination of *F. tularensis* to humans.

Tularemia had never been reported in Kosovo before year 2000 when a number of patients were identified with fever, pharyngitis, and pronounced cervical lymphadenopathy.[65] Serological testing confirmed 327 cases of tularemia in various regions of Kosovo, a majority of which presented as the oropharyngeal form. A follow-up case-control study indicated that the outbreak was food and water related.[65] It could not be ruled out that the disease had existed in Kosovo previously but not been recognized in the absence of a large number of human cases. Human and animal tularemia cases had occurred in neighboring regions.

In Scandinavia, the predominant form is ulceroglandular tularemia. It normally results from arthropod bites, most often by mosquitoes, or sometimes from direct contact with infected animals. In Sweden, naturally infected *Aedes cinereus* were reported as early as 1942[92] and, in fact, a Russian conference proceeding[71] mentioned that naturally infected *A. cinereus* were already found in Sweden in 1938. During the last decades, epidemiological data indicated that mosquitoes were the predominant vectors responsible for transmission during outbreaks of tularemia.[64] The other regularly reported form of tularemia in Scandinavia is the respiratory variant that results from aerosol dissemination of bacteria, often related to farming activities, such as piling of hay.[64]

Human tularemia cases in Sweden appear to have a strong aquatic association since, by far, the highest incidence of disease occurs in areas with close proximity to rivers or lakes. This association is mostly supported by epidemiological data whereas experimental evidence demonstrating the presence of *F. tularensis* in water or in vectors is scarce and how the acquisition of *F. tularensis* by mosquitoes occurs has not been clarified. It has been suggested that it occurs already at their larval stage while feeding in water. Alternatively, the mosquitoes may become infected when feeding on an infected animal and the infection is spread via the infected mouth area. Lemmings and beavers in Scandinavia might play a role in maintaining the water association

of the bacterium.[3,89,93] There is also evidence that the bacterium can persist for months in watercourses, possibly in association with protozoa.[94] An association of *F. tularensis* with semiaquatic animals is supported by data from a Swedish survey,[93] in which agglutinating antibodies against *F. tularensis* were assayed in cattle, moose, beaver, and mountain hare. Some 20% of the beavers demonstrated significant titers whereas no titers were found in any of the other animals. However, it is unknown whether the occurrence of the organism in natural water is only the result of contamination from mammals or if water may represent a mammal-independent reservoir of *F. tularensis*.

In Spain, a unique, water-associated tularemia outbreak occurred in the central province of Cuenca in 1997. Cases presented as ulceroglandular tularemia after contact with crayfish.[62] This was the first time tularemia had been associated with fishing. Transient contamination of the river water was implicated as the cause of the outbreak and it was suspected that most individuals acquired infection through skin cuts or abrasions while fishing. By PCR, samples identified the presence of *F. tularensis* in samples from the river, crayfish, and human lymph node aspirates.

Oropharyngeal tularemia results from intake of contaminated food or water. Despite the strong aquatic association of *F. tularensis* in Scandinavia, this appears to be an exceedingly rare form of disease in the region. Luotonen *et al*. has summarized 127 cases that occurred in Finland from 1967 through 1983.[95] The source was hare meat in five cases and strawberries in another five, whereas the sources in the other cases were unknown. Only one known outbreak of water-borne tularemia has occurred in Sweden.[96] Nine individuals became ill in 1973 and the likely origin was a well that served as a source of drinking water.

Although the oldest published description of tularemia in Japan may be a report from 1818, the epidemiology of tularemia in Japan has not been well characterized. Almost all cases occur in the northeastern part of the main island.[97] The annual incidence has decreased and since the middle of the 1960s, there have been less than 10 cases per year. A great majority of cases have been associated with contact with infected wild rabbits. There appears to be an ecological system based on the spread from ticks to rabbits. The changes in the occurrence of tularemia are presumably related to the change of life style caused by the rapid growth of the Japanese economy after World War II.

FRANCISELLA TULARENSIS: A WAR-RELATED PATHOGEN

One of the strongest correlations to an increased incidence of the tularemia is the occurrence of war. For example, during World War II, there were massive increases in the populations of field and house mice in Soviet Union since large areas of arable land was left uncultivated, harvests were delayed, and grain store

houses destroyed. This together with poor sanitary conditions and destruction of buildings resulted in unsurpassed exposure of humans to rodents. Normally, *A. terrestris* was the main source of tularemia in the forest belt from the Volga to the Caucasus and all the way to Poland, but during the war, field and house mice became the most prevalent species. This rapid increase in rodent populations subsequently resulted in major tularemia outbreaks. In Russia, during the winter of 1941–1942, 67,000 cases were reported from the region surrounding Rostov-on-Don and the lowlands of Caucasus.[87] Significant numbers of cases of tularemia have also been recorded during other wars, for example, during the Continuation War between Finland and the Soviet Union in Karelia between 1941 and 1944[98] and more recent experiences from the civil wars in Bosnia and Kosovo in the 1990s and early 2000s, where tularemia had not been described previously.[65] Altogether, these experiences suggest that in Europe, tularemia is a disease that might have a considerable impact during war and after natural disasters that lead to severe disruptions of the sanitary conditions.

A scientist from the former Soviet Union, Ken Alibek, has suggested that tularemia outbreaks that affected 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.[8] However, there is no direct proof to substantiate this claim and, in fact, the claim has been analyzed in great detail and its accuracy challenged.[98] The reasons for the significant number of cases during the war illustrate a number of important prerequisites for tularemia epidemics. In an analysis, Rogozin, head of the Anti-Epidemic Department of the People's Health Commissariat, observed that there had been a massive multiplication of infected rodents and concluded that "a decisive source of the infection [with *F. tularensis*] revealed the inhalation of dust when contaminated straw was used as mattresses."[99] One very important reason for the rodent explosion was that the crops were not harvested due to the intense fighting in the areas surrounding Rostov-on-Don and Stalingrad. Another reason was that the systems established to prevent plague and tularemia had totally collapsed as a consequence of the war. *F. tularensis* were spread from dead rodents or from feces of infected rodents and were disseminated by aerosols from the fields or via food stuff.[100] Occasionally bacteria were transmitted by insects (especially mosquitoes and ticks), by contaminated water, or by biting mice.

The massive dissemination of *F. tularensis* during World War II led to ectoparasites being infected, which resulted in the persistence of the bacterium even after the normalization of the mouse populations. During the 1940s, there were about 100,000 annual tularemia cases in the Soviet Union.[87] These cases did not only occur as a result of the exposure to live rodents, but also persisted during winter months since dead animals and their excrements had contaminated grain supplies and led to respiratory tularemia of individuals working with the grain. Also, widespread water-borne epidemics occurred due to contamination of water supplies by dead rodents. Subsequently, the number of cases decreased and only a few hundreds of cases were reported in the

mid-1950s, presumably due to greatly improved sanitary conditions and massive vaccination campaigns.[87] By comparison, between 100 and 400 annual cases were recorded in Russia between 1987 and 1997.[61]

TULAREMIA—AN EMERGING OR DISAPPEARING DISEASE?

For the areas of the world where systematic investigations have been made, the reported occurrence of tularemia during the last 90 years probably quite accurately reflects the actual occurrence. However, it can be assumed that the disease existed well before the first reports were published since there were no dramatic changes in the ecology of the relevant areas and, therefore, the identification of tularemia cases in several countries most likely was partly coincidental, partly due to an improved epidemiological surveillance and better diagnostic techniques. Nevertheless, it is unlikely that tularemia was a common disease worldwide before the 20th century since the disease displays a number of clinical characteristics, such as a typhoid form, a localized respiratory form, and a conjunctival form, and, therefore, it appears unlikely that thorough medical investigations made in the 19th century would have overlooked tularemia if it had been frequently occurring. Assuming that tularemia in fact was an emerging disease during the early 20th century, there are no clear-cut answers as to why.

The number of tularemia cases has dramatically decreased in certain areas of the world, in particular, in Russia and the United States. The annual number of human cases today in these countries, which do not exceed a few hundreds, represents only a small proportion compared to the peak incidence of the disease in the 1940s. Although the extreme numbers of cases in Soviet Union was to a large extent dependent on the war, in both countries, areas that once were considered endemic have experienced dramatic decreases of tularemia; for example areas along Rocky Mountains, such as in Nevada and Montana, and in Siberia. One obvious explanation is the general change in the societies with far fewer individuals having rural occupations such as hunting or farming, thus decreasing human exposure to *F. tularensis*. Since the number of epizootics appear to have diminished as well, it is possible that subtle ecological changes have occurred that have diminished the persistence and spread of *F. tularensis*.

At the same time, the disease is clearly emerging or increasing in other areas of the world, as evidenced by the aforementioned outbreaks of tularemia in Kosovo and Spain and increasing number of tularemia cases and newly emerging areas in Sweden.[63–65] Some of this may be related to human activities, such as the war-like conditions in Kosovo in the late 1990s that predisposed for the disease but also natural disasters may predispose to tularemia outbreaks as exemplified by epidemics that have occurred in Turkey in the aftermath of earthquakes.[101] Some of the outbreaks in Spain among hares are suspected to be due to the trade of hares and other wild animals in Europe.[63] The spread of

tularemia by commercial sale of hares also occurred historically in the United States when the disease was introduced in New England.[67] Most likely, the endemic focus at Martha's Vineyard is a result of such import from the Midwest. Another example of human activities leading to the spread of tularemia is the aforementioned outbreak in prairie dogs that occurred not only in Texas but, after shipping of the prairie dogs from the United States, also in the Czech Republic and Japan.[83] Human activities alone appear not to explain the emergence and spread of tularemia in Europe, however, and it appears reasonable to postulate that subtle ecological changes have contributed to the emergence and increase of tularemia in certain parts of Europe. At the same time, other, equally subtle ecological might have resulted in the disappearance of the disease in certain parts of the United States and Russia. As a result of the changing climatic conditions in many parts of the world, it is likely that tularemia in the future will disappear in some locations but appear in new locations and populations at any time. In fact, this possibility was already suggested in 1934 by Parker when he stated: "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."[102]

CLINICAL PRESENTATIONS OF TULAREMIA

Tularemia normally presents after an incubation period of 3–5 days. Initially, symptoms are quite nonspecific with fever, malaise, chills, and headache. Depending on the route of entry and the infecting *F. tularensis* subspecies, other specific symptoms may present. If the infection is transmitted through the skin or mucous membranes, ulceroglandular tularemia will result. It is often the result of vector-borne transmission or, sometimes, direct contact with infected animals. A primary ulcer develops at the site of infection, often a solitary papule that subsequently develops into an inflamed pustule. Many patients do not recognize the initial ulcer and it often heals within a week. The lymph nodes draining the ulcer subsequently enlarge and become palpable and tender. Without administration of antibiotics within 7–10 days, the enlargement of the lymph nodes continues and suppuration may occur. This occurs in 30–40% of the cases and is one of the most serious complications of an infection caused by *F. tularensis* subsp. *holarctica*. The ulceroglandular form comprises more than 90% of the European tularemia cases. It is the predominant form of tularemia caused by *F. tularensis* subsp. *holarctica* but infection by all subspecies may present as ulceroglandular tularemia.

Respiratory tularemia results from inhalation of *F. tularensis*. Farmers appear to be at risk to contract this form from work with contaminated hay[64] but also other activities such as lawn-mowing may occasionally give rise to the disease.[68,103] Outbreaks of this form rarely occur but can affect a considerable

number of individuals. The contaminated aerosols most likely result from animal carcasses or excretions from infected animals. Normally the disease is systemic presenting with fever but may lack respiratory symptoms and also diagnostic X-ray findings.[104] The outcome of respiratory tularemia depends on the etiological agent; infection with *F. tularensis* subsp. *holarctica* results in a nonfatal respiratory infection, whereas inhalation of bacteria of subspecies *tularensis* gives an acute, serious infection characterized by high fever, malaise, chills, and cough and sometimes delirium and pulse–temperature dissociation. The latter form is potentially life-threatening and, historically, had a case-fatality rate of >50%, most of which occurred before effective treatment was available.[105] Today, modern antibiotic regimens have reduced the fatality rate in the United States to less than 2%.[7]

Other, uncommon forms of tularemia include direct inoculation of the eye resulting in oculoglandular tularemia. The patient usually presents with unilateral conjunctivitis, prominent swelling of the eyelids, photophobia, and a purulent secretion. Another uncommon form, oropharyngeal tularemia, is the result of ingestion of contaminated water or food. Typhoidal tularemia is used to describe tularemia patients with severe systemic symptoms but without any obvious signs of the port of entry.

FRANCISELLA TULARENSIS VACCINES

During the early years of *Francisella* research, the relative incidence of tularemia in particularly ín the United States and the Soviet Union led to an intensive research for an effective vaccine. The hopes were based on the observation that previous tularemia infection results in efficient protection against secondary infection. This protection is, however, not absolute and, as a matter of curiosity, Francis contracted the disease at least three times but he exposed himself deliberately to *F. tularensis* by performing autopsies of animals without wearing gloves.[23]

During the 1930s and 1940s, there was an intensive hunt for a vaccine and this resulted in the development of live, attenuated strains in the Soviet Union that appeared to confer efficient protection (reviewed in Ref. 106). The first experiments on humans were carried out in 1942 using a strain that had been attenuated by subjecting it to immune serum and by drying at thermostat temperature.[69] This strain was found to be completely harmless. Six months after inoculation, the individuals were subjected to an experimental infection with a virulent strain. The test fully confirmed its high protective effectiveness. After use on several thousand individuals this strain was lost. Subsequently, the efficacy of several strains, one of which was designated strain 15, were evaluated and methods to prepare them in an egg yolk suspension in saline and administer them by the intradermal route were developed. When the vaccine was given in to a large number if individuals in endemic areas, almost

all individuals given the vaccine experienced local reactions, 94% of 950 people in one study, but systemic reactions did not occur and there was no time lost from work.[69] After vaccination, it was noted that tularemia occurred in 0.36% of vaccinated individuals whereas 4.3% of nonvaccinated individuals contracted the disease. Quite sophisticated methods for preserving the vaccine were developed. For example, in one publication it is described that the vaccine was prefrozen, then dried for 14 to 18 h without added heat, and sealed under vacuum.[107] The authors believed that the sugar in the preparation "fixes the amount of water" and "safeguards the microbes against excessive dehydration." Such a vaccine was immunogenic for man after storage at 2–4°C for 1.5 years.

As many as 60 million individuals were immunized with live tularemia vaccines between 1946 and 1960 in the Soviet Union.[108] In 1956, one of the live vaccine strains of *F. tularensis* was brought to the United States. A passaged isolate of this strain was denoted *F. tularensis* LVS (live vaccine strain) by Eigelsbach *et al*.[30] It has been extensively used in the United States and elsewhere since then. The incidence of laboratory-acquired infections with *F. tularensis* before and after the introduction of the live vaccine strain has been reviewed by Burke.[109] He showed that the incidence of respiratory tularemia dramatically decreased, whereas the incidence of ulceroglandular tularemia remained unchanged. *F. tularensis* LVS has also found a widespread use in experimental models, as it remains virulent for mice and causes a disease similar to human tularemia (reviewed in Ref. 110).

The duration of the period of immunity following vaccination was examined by numerous Russian investigators. In 1949, annual revaccination was recommended but subsequently the period was extended and an editorial footnote to an article published in May 1958[111] states that "at the present time the period of revaccination for children and adults living in natural foci of infection is officially set at five years." The reason for this advice seems to have been that a few cases of tularemia occurred in vaccinated individuals but often more than five years after vaccination.

The successful adaptation of *F. tularensis* to the intracellular habitat is the probable explanation for the need for cell-mediated immunity for long-term protection (reviewed in Ref. 39). This requirement may explain previous work on the efficacy of *F. tularensis* vaccines. In experimental systems, protection against tularemia was afforded either by natural infection or by vaccination with live attenuated strains, whereas killed vaccines induced only a low level or no protection against virulent *F. tularensis*. Recent findings have provided a slightly modified view since it has been shown that a vaccine based on part of the LPS conjugated to bovine serum albumin in an experimental mouse model protected well against intradermal challenge with a strain of subspecies *holarctica* but afforded only marginal protection against an aerosol challenge with the same strain and did not protect against any form of challenge with a strain of subspecies *tularensis*.[112] Thus, this LPS-induced, presumably

antibody-dependent, protective mechanism may be relatively efficacious against the most common form of tularemia, ulceroglandular tularemia caused by subspecies *holarctica*, whereas priming of T-cell-mediated immune responses appears to be a prerequisite for protection against highly virulent strains belonging to subspecies *tularensis*. Regardless of the form of tularemia, however, the evidence indicates that vaccines priming cell-mediated immune responses will be more efficacious than those priming antibody-responses only.[39] Moreover, this evidence indicates that specific immune sera will be of no or very limited use for prevention or treatment of tularemia.

The live vaccine strain, *F. tularensis* LVS, and other attenuated strains of *F. tularensis* have been used to immunize risk groups such as laboratory staff. These live vaccines appear to provide good albeit not full protection against tularemia, but licensing of the LVS strain as a tularemia vaccine has been difficult since its history is uncertain and the basis of its attenuation is unknown.[110] Due to these problems, currently there is no licensed vaccine available in Western countries.

The need for effective cell-mediated immunity to protect against *F. tularensis* necessitates that testing for correlates of immunity in volunteers is based on measurement of such immune responses. Investigations on the longevity of cell-mediated immunity after vaccination with *F. tularensis* LVS demonstrated that it persists for at least 25 years[113] and since infections in vaccinated laboratory staff are extremely rare, the presence of a cell-mediated immune response may correlate with a relative protection against laboratory-acquired infection.

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