Francisella tularensis: an Overview

Ongoing research progresses toward understanding pathogenesis and virulence mechanisms, and may help lead to an improved vaccine

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Francisella tularensis was first isolated in 1912 from rodents suffering from a plague-like disease in Tulare County, Calif. Subsequently, investigators learned that this small, gram-negative bacterium infects many animal species and is associated with a wide range of diseases. In humans, the bacterium causes a serious, sometimes fatal disease, called tularemia, which is also known as rabbit fever, hare fever, dearfly fever, or lemming fever. Tularemia in humans occurs in many countries in the Northern but not the Southern Hemisphere. The reason for this distribution is not known.

Terrestrial and aquatic mammals such as ground squirrels, rabbits, hares, voles, muskrats, water rats, and other rodents are thought to serve as reservoirs for this disease. In particular, increases in rodent tularemia are closely linked to epidemic outbreaks of human tularemia. Additionally, F. tularensis survives in amoebae, a finding that appears to explain the association of this bacterium with waterways.

Although our understanding of F. tularensis is limited, ongoing research is elucidating its pathogenesis and virulence mechanisms. Researchers who are sequencing the genomes of the Schu S4 and LVS strains expect to complete these projects soon, and information from them is already providing valuable insights. In parallel, large-scale microarray and proteomic analyses are being used to identify differences among these and other strains. Such information will surely prove valuable for developing new diagnostic, prophylactic, and therapeutic agents for F. tularensis.

F. tularensis Has Few Close Relatives

Currently, experts recognize three subspecies of F. tularensis (Table 1). However, an older system of nomenclature often refers to F. tularensis types A and B, and these designations correspond to F. tularensis subspecies tularensis and F. tularensis subspecies holarctica, respectively. In addition, Francisella novicida is now usually classified as a subspecies of F. tularensis. Generally, the different subspecies are associated with different regions of the world. For example, subspecies tularensis is found predominantly in North America, while subspecies holarctica is predominant in Europe.

In humans or in rabbits, strains belonging to subspecies tularensis cause the most severe form of disease, but isolates belonging to all subspecies are highly virulent in mice (Table 1). In addition to differences in virulence for humans and rabbits, the different subspecies can be distinguished on the basis of the activity of citrulline ureidase, an enzyme that converts L-citrulline to ornithine, and acid production from glycerol or glucose. More recently, when investigators began using genetic typing methods, they found that there is a significant degree of genetic identity among isolates belonging to different subspecies.

On the basis of both 16S rDNA sequence comparisons and DNA-DNA hybridizations, F. tularensis has no close relatives, although the family Francisellaceae is relatively closely related to the Piscirickettsiaceae, a family that includes certain fish pathogens. Several endosymbiotic bacteria of various ticks and a ciliate also appear to be closely related to members of
Francisellaceae. The most closely related, albeit distant, human pathogens are *Coxiella burnetti* and *Legionella* species, both of which share aspects of their lifestyles with *F. tularensis*.

**Virulence Mechanisms Remain Hazy**

Although *F. tularensis* can be cultured in the laboratory, it requires enriched growth media. In contrast, the bacterium can readily infect and replicate in a range of cultured cells, and it is generally accepted that *F. tularensis* is a facultative intracellular pathogen in vivo (Fig 1). Macrophages are thought to be the major host cell, but the mechanisms that allow survival and growth in this cell type are poorly understood. The capsule of *F. tularensis* is considered a virulence factor, although its biochemical makeup and precise role in virulence are not known.

In part, this paucity of information reflects the difficulties of working with this pathogen—to date, only low-virulence strains have proved amenable to genetic manipulation. Even with these strains, mutagenesis is not simple. Improved genetic tools that could be used to identify virulence mechanisms of *F. tularensis* are urgently needed.

Notwithstanding these difficulties, researchers are characterizing *F. tularensis* and learning more about how it causes disease. For instance, the bacterium appears to enter macrophages via a cytochalasin B-insensitive pathway and without triggering a respiratory burst. Macrophages treated with chemicals to inhibit endosome acidification show a much-reduced ability to support the growth of *F. tularensis*, possibly reflecting the reduced ability of the bacterium to acquire iron, which would otherwise be released from transferrin under acidic conditions. These findings suggest that, after uptake, the bacterium replicates within acidified endosomes.

**Table 1. Subspecies of *F. tularensis***

<table>
<thead>
<tr>
<th>Biotype</th>
<th>Previous names</th>
<th>Geographical location</th>
<th>LD₅₀ dose in rabbits*</th>
<th>LD₅₀ dose in mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Francisella tularensis</em> subsp. tularensis</td>
<td><em>Francisella tularensis</em> type A</td>
<td>Primarily North America</td>
<td>1–10 CFU</td>
<td>&lt;10 CFU</td>
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<td></td>
<td><em>Francisella tularensis</em> subsp.</td>
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<td>nearctica</td>
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<tr>
<td><em>Francisella tularensis</em> subsp. holarctica</td>
<td><em>Francisella tularensis</em> type B</td>
<td>Primarily Europe, former</td>
<td>&gt;10⁶ CFU</td>
<td>&lt;10 CFU</td>
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<tr>
<td></td>
<td></td>
<td>Soviet Union, Japan, and</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>North America</td>
<td></td>
<td></td>
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<tr>
<td><em>Francisella tularensis</em> subsp.</td>
<td><em>Francisella tularensis</em></td>
<td>Kazakhstan, Uzbekistan</td>
<td>&gt;10⁶ CFU</td>
<td>&lt;10 CFU</td>
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<tr>
<td>mediasiatica</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Francisella tularensis</em> subsp. novicida</td>
<td><em>Francisella novicida</em></td>
<td>Primarily North America</td>
<td>Resistant</td>
<td>10¹ - 10² CFU</td>
</tr>
</tbody>
</table>

*Subcutaneous challenge route.

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**How Hazardous is *F. tularensis***?

“I know of no other infection of animals communicable to man that can be acquired from sources so numerous and so diverse,” remarked R. R. Parker from the U.S. Public Health Laboratory in Montana 70 years ago. “In short, one can but feel that the status of tularemia, both as a disease in nature and of man, is one of potentiality.”

Exposure to airborne bacteria poses a particular hazard because the infectious dose is so low. Studies from the 1950s showed that inhaling 10–50 CFU of *F. tularensis* reliably causes disease among human volunteers. The disease, whose symptoms include a fever of 103–104°F, headache, sore throat, myalgia, and nausea, develops within 3–5 days of exposure to bacteria. Fortunately, antibiotic treatment typically permits full recovery.

Prior to the availability of live vaccines, accidental infection with *F. tularensis* in laboratory workers was a frequent occurrence. Nowadays such cases of tularemia are rare because most lab workers are vaccinated, and labs studying this pathogen are required to use appropriate containment facilities to minimize the risk of accidental exposure. However, occasional cases of respiratory tularemia in the general population serve to remind us of the hazard posed by this pathogen.
Frustrated by the shortage of information on the biochemical and genetic makeup of *F. tularensis*, researchers coordinated an international program to sequence the approximately 2-Mbp genome of several strains belonging to the different subspecies. This project, which is expected to be completed in November 2003, involves researchers at the Walter Reed Army Institute for Research, the Lawrence Livermore National Laboratory, the Department of Energy (DOE) Joint Genome Institute, and the Centers for Disease Control and Prevention (CDC) facility in Fort Collins, Colo., in the United States; the Swedish Defence Research Agency, Umeå University, and the University of Uppsala in Sweden; and the Defence Science and Technology Laboratory and the London School of Hygiene and Tropical Medicine in the United Kingdom.

The genome-sequencing project has already yielded a preliminary annotation of the sequence of a strain of *F. tularensis* subspecies *tularensis*, identifying 1,804 candidate open reading frames (ORFs). Many of these ORFs encode proteins with no ready database match, suggesting that *F. tularensis* contains a high proportion of unique genes. The full analysis of this genome sequence and a comparison with genome sequences of other strains could identify putative virulence determinants.

### Clinical Presentation of Tularemia Is Related to Route of Infection

The infectious dose of highly virulent strains of *F. tularensis* in humans is a remarkably low 10–50 colony forming units (CFU). Most cases of tularemia in humans result from arthropod bites from individual insects that previously fed on infected mammals. Mosquitoes and ticks are especially implicated in the spread of disease. In cases of ulceroglandular tularemia, an ulcer forms at the site of the bite and typically there is involvement of the regional lymph nodes. These lymph nodes can swell to resemble the characteristic bubo seen in cases of bubonic plague (Fig. 2). The patient often complains of flu-like symptoms with fever, chills, headache, and muscle aches. Glandular tularemia presents with similar symptoms but with an unknown route of entry.

Glandular and ulceroglandular tularemia, which are by far the most commonly encountered forms of the disease, are rarely fatal. In fact, many cases of arthropod-borne tularemia involving low-virulence strains are probably not diagnosed as such. The glandular and ulceroglandular forms of the disease are especially associated with hunters, trappers, and others who come into contact with infected animals or with infected arthropod vectors.

The respiratory disease that results from inhaling *F. tularensis* is a much more serious form of tularemia. Moreover, when the infecting strain belongs to subspecies *tularensis*, the mortality rate for this form of disease is higher than 30%. Prominent symptoms include hilar

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**FIGURE 1**

*F. tularensis* grows to high numbers intracellularly. The picture shows infected hepatocytes with numerous *F. tularensis* LVS bacteria in the cytoplasm. (Photo obtained through the courtesy of Wayne Conlan, National Research Council, Ottawa, Canada.)
lymph node enlargement, dry cough, and retrosternal pain. Although some reports refer to typhoidal and intestinal tularemia, these terms are not clearly defined. The infection may spread to the lungs in all clinical forms of tularemia.

**Incidence and Risk Factors for Tularemia**

During the first half of the 20th century, tularemia was a considerable public health problem in the Soviet Union and in the United States. A decline in tularemia cases in these countries since the 1950s may be due to less-frequent exposure of humans to rodents, rabbits, and hares—in turn, reflecting fewer hunters and trappers and a more general reduction in rural populations.

During the winter of 1941–42, 67,000 cases were reported from the region surrounding Rostov-on-Don, in the former Soviet Union. Later, large-scale vaccination campaigns contributed to control of the disease. A more recent experience during the civil wars in Bosnia and Kosovo suggests that tularemia and other zoonotic diseases can increase significantly during and after warlike conditions—or natural disasters—that disrupt the normal hygiene and sanitary conditions of a society.

In the United States, health officials recorded 2,291 cases of tularemia in 1939, whereas during the past 10 years, the average yearly incidence was 125 cases. Most of these cases are the consequence of arthropod bites, particularly tick bites, or contact with infected mammals, particularly rabbits. The peak number of cases occurs in late spring and in the summer months when arthropod bites are most common. A similar pattern occurs in Scandinavia, where the peak incidence of disease is usually during late summer.

When outbreaks are reported, they often are associated with inhalation of *F. tularensis*. For example, a recent Swedish study identified an association between farming and the pulmonary form of the disease, tracing transmission to traditional farming procedures in Scandinavia such as piling hay, which is contaminated with *F. tularensis* from rodent urine or feces, onto drying racks. In the United States, an outbreak of pulmonary tularemia on Martha’s Vineyard, Mass., in 2000 was similarly attributed to exposures from inhaling this pathogen when individuals were mowing lawns or cutting brush (see box, above). Although the exact routes by which respiratory tularemia is transmitted are not known, in endemic areas such outdoor activities undoubtedly confer an elevated risk of acquiring the disease.

Another concern is that *F. tularensis* might be used illegitimately as a bioterrorist or biowarfare agent. Despite recently heightened anxiety about the possible deliberate spread of this pathogen, this concept is not new. From 1932–1945, for example, special research units in Ja-
“Lawnmower” Tularemia

The term “lawnmower tularemia” was first used about a decade ago to describe two cases of pneumonic tularemia in adolescent males who ran over a dead rabbit while mowing a grassy area. Neither youth touched the remains of the animal, suggesting an aerosol of bacteria was sufficient to cause infection. Both individuals were hospitalized, treated with streptomycin, and recovered.

A more recent outbreak of tularemia on Martha’s Vineyard, Mass., involved 15 patients, 11 of whom presented with a primary pneumonic tularemia. One infected adult male died, and F. tularensis type A bacteria were isolated from his blood and lung tissues. While the precise origin of these cases is not certain, the evidence again points towards the patients inhaling aerosols of the bacteria that were generated during lawnmowing or brush-cutting activities. Hence, in endemic areas, use of protective clothing and face masks may be warranted.

Renewed Interest in Developing an Improved Tularemia Vaccine

With such concerns in mind, there are obvious needs for a more thorough understanding of the pathogenesis of this disease and for improved means of protecting against it, such as developing a better vaccine for at-risk personnel. Since the 1930s, several nonliving vaccines to protect against tularemia were developed and evaluated. Some early studies showed that immunizing mice or nonhuman primates with killed bacterial cells provides a low degree of protection against fully virulent F. tularensis.

Moreover, although immunizing humans with killed, whole-cell vaccines reduces both the incidence and severity of disease, those encouraging results were not consistently reproduced, a finding that led researchers to focus on developing live-attenuated vaccine strains. Such vaccines were used extensively in the former Soviet Union, with as many as 60 million individuals immunized until 1960.

Some of these vaccine strains were transferred to the United States in the 1950s, and one of them was subsequently developed as the live vaccine strain (LVS) of F. tularensis. During the early 1960s, U.S. researchers conducted small-scale clinical trials to evaluate the efficacy of the LVS vaccine. In one such study, 20 volunteers, only some of whom received LVS, inhaled either 10, 100, or 1,000 infectious doses of F. tularensis Schu S4, a highly virulent strain. All those who were not vaccinated developed pneumonic disease. Individuals who received LVS were fully or partly protected, especially against lower challenge doses. For instance, of the group challenged with 10 infectious doses, five of six remained asymptomatic, while the sixth volunteer developed mild disease. In the group challenged with 1,000 infectious doses, one individual developed pneumonic disease, while two others developed mild disease.

A U.S. Army Medical Research Institute of Infectious Disease epidemiologic study also indicates LVS efficacy. After LVS vaccinations became routine at this facility, the incidence of tularemia among laboratory workers fell sevenfold. However, during a 10-year period, 11 individuals who received LVS nonetheless developed tularemia. Thus, this vaccine, which is administered intradermally, appears to provide at least partial protection against tularemia. However, because the basis of attenuation and other facts concerning the history of LVS are not known, this vaccine remains unlicensed.

As with other potential bioterrorism agents, development of a vaccine against tularemia recently received renewed priority. Since experience with LVS proves in principle that a live-attenuated product can be safe and effective, one option is to create another attenuated mutant, using an approach similar to that pioneered with live-attenuated Salmonella vaccines. Indeed, several specific metabolic pathways, including the purine and aromatic amino acid biosynthetic pathways that were disrupted in the case of Salmonella to yield rationally attenuated vaccines, also appear to be present in F. tularensis. Alternatively, researchers may choose to develop a subunit vaccine for protecting against tularemia, although F. tularensis contains no obvious immunodominant antigens to test.
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