Tularemia vaccine: past, present and future

Petra C.F. Oyston* and Janine E. Quarry
Defence Science and Technology Laboratories, Porton Down, Salisbury, Wiltshire, SP4 0JQ; *Author for correspondence (e-mail: pcoyston@dstl.gov.uk; phone: 01-980-613641)

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Abstract

Francisella tularensis is a Gram negative intracellular pathogen that causes the highly debilitating or fatal disease tularemia. F. tularensis can infect a wide range of animals and can be transmitted to humans in a variety of ways, the most common being by the bite of an infected insect or arthropod vector. The attenuated F. tularensis live vaccine strain (LVS) has been used previously under investigational new drug status to vaccinate at-risk individuals. However the history of the strain and lack of knowledge regarding the basis of attenuation has so far prevented its licensing. Therefore the focus of current research is on producing a new vaccine against tularemia that would be suitable for licensing.

Francisella tularensis

Francisella tularensis is a small Gram negative bacterium that is able to infect a wide range of animal species. It was originally isolated in 1911 from ground squirrels in Tulare County, California (McCoy and Chapin 1912), but it is now known to be endemic across the Northern hemisphere. There are two species in the Francisella genus based on 16S rDNA sequencing and fatty acid composition; F. tularensis and Francisella philomiragia (Hollis et al. 1989). There are currently four recognised subspecies of F. tularensis (Table 1). Microarray analysis revealed limited genetic variation within F. tularensis, but it was possible to differentiate subspecies tularensis and holarctica (Broekhuijsen et al. 2003), the two most commonly isolated subspecies. F. tularensis subspecies tularensis is the most virulent of the four subspecies and is found primarily in North America, whereas F. tularensis subspecies holarctica is less virulent and is found mainly in Europe and Asia. F. tularensis subspecies mediasiatica is isolated in central Asia and F. tularensis subspecies novicida primarily in North America. However, recently a novicida-like organism was isolated in Australia indicating a wider range for Francisella than had been previously thought. (Whipp 2003). Subspecies mediasiatica and novicida rarely cause disease in humans. The highly virulent F. tularensis subspecies tularensis strains have infectious doses for humans of less than 10 cfu (Table 1), making this one of the most highly infectious bacterial pathogens known.

Tularemia has been isolated from several parts of the Northern Hemisphere but has rarely been found in the Southern Hemisphere. The disease has been regularly reported in the US, Sweden, Finland, Czech Republic, Slovakia, Russia, Kazakhstan, Uzbekistan and Japan. Typical average rates of infection in the US and Sweden are around 125 and 90 cases per year respectively. F. tularensis is probably maintained in the environment by mammals such as rabbits, hares and rodents,
although its environmental niche is not known. Hunters, walkers, farmers and veterinarians in endemic zoonotic areas are at the greatest risk of contracting tularemia due to contact with infected wild animals. Transmission to humans usually results from the bite of an insect or arthropod vector, such as biting flies, ticks and mosquitoes, that has recently fed on an infected animal. This results in ulceroglandular tularemia, which has a mortality rate of less than 3% without treatment (Evans et al. 1985). Infection can also occur through inhalation of infectious aerosols and following ingestion of contaminated food and water. The symptoms and severity of this disease vary depending on the route of transmission, the infecting dose and the Francisella subspecies involved. Pneumonic tularemia and typhoidal tularemia are the most severe, with mortality rates of 30–60% (Gill and Cunha 1997). However, even in non-fatal cases of tularemia, the infection is severely debilitating for extensive periods.

**The live vaccine strain**

Live attenuated strains were developed prior to the Second World War in the former Soviet Union. This was achieved by either repeatedly sub-culturing a virulent strain of *F. tularensis* subspecies *holarctica* on media containing antiserum or by drying the organisms (Khatenever 1943). Several strains were identified as attenuated including strains 15 and 155, which were transferred from the Institute of Epidemiology and Microbiology (Gamaleia Institute), Russia, to the US Army Medical Research Institute of Infectious Diseases (Tigertt 1962). From these a suitably attenuated strain was isolated, tested for safety and efficacy and subsequently designated *F. tularensis* Live Vaccine Strain (LVS) (Eigelsbach and Downs 1961).

Although various routes of delivery have been evaluated, including oral (Hornick et al. 1966) and aerogenic (Hornick and Eigelsbach 1966) immunization, the LVS vaccine is routinely delivered by scarification. Retrospective studies on the efficacy of the LVS vaccine based on laboratory acquired infections have shown that it affords good, but not complete, protection against typhoidal tularemia, leading to a dramatic decrease in cases. However the incidence of ulceroglandular tularemia is not reduced in vaccinated individuals, although there appears to be a reduction in the severity of the clinical symptoms (Sandstrom 1994). Previous publications have reviewed the human immune response following infection with virulent *F. tularensis* or vaccination with LVS (Tarnvik 1989; Sandstrom 1994), and the response to LVS infection in mice (Elkins et al. 2003). Intra-dermal inoculation with $10^5$ cfu LVS was able to protect mice against subsequent intra-dermal challenge with over 50 LD$_{50}$ of a fully virulent subspecies *tularensis* strain (Shen et al. 2004). However, the immunised mice remained susceptible to challenge by the aerosol route.

The LVS vaccine was assigned Investigational New Drug status by the US Food and Drug Administration (FDA) in the early 1960s. Thus, LVS has only been used to vaccinate at-risk personnel. However, the LVS vaccine remains unlicensed due to several significant problems with the vaccine. The first drawback with the strain is that the basis of attenuation and protection is not known. Secondly, the LVS strain retains virulence for mice. The median lethal dose varies from $10^7$ cfu when delivered subcutaneously to less than

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<table>
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<tr>
<th>Subspecies</th>
<th>Primary geographic distribution</th>
<th>Human LD$_{50}$ (cfu)$^a$</th>
<th>Mice LD$_{50}$ (cfu)$^a$</th>
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<td><em>mediasiatica</em></td>
<td>Central Asia</td>
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$^a$ All doses were given subcutaneously.
NR – Not reported.

Table 1. Geographical distribution and virulence of *F. tularensis* subspecies.