Francisella tularensis in Ticks

Subjects: Microbiology

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Ticks can transmit a variety of infectious agents, including viruses, bacteria, and parasites, to humans and animals. Tularemia, caused by the Gram-negative bacterium *Francisella tularensis*, is a highly-infectious zoonotic agent that elicits flu-like symptoms in humans. In the United States, approximately half of tularemia cases are tick-associated. In a recent review by Tully and Huntley, the following points are highlighted: (1) Background information on tularemia and *F. tularensis;* (2) What is known about the four different tick vectors associated with tularemia, including their geographic ranges and other arthropod vectors historically associated with tularemia; (3) Physiological cues in ticks (compared to the mammalian host) that may prompt *F. tularensis* to modify its metabolism and protein expression to survive and persist in ticks.

Tularemia | Francisella tularensis | Tick | Arthropod | Tick-borne disease | Dermacentor | Amblyomma | Select Agent

1. Introduction

The most recent data from the United States (U.S.) Centers for Disease Control and Prevention (CDC) indicate that the reported number of tick-borne disease cases have more than doubled in the past 13 years and account for 77% of all vector-borne disease cases [1]. Although insecticides historically have been useful for controlling tick-borne diseases, resistance is becoming widespread in the U.S. [1][2]. Tick-borne diseases, including Lyme disease, spotted fever rickettsioses, babesiosis, anaplasmosis/ehrlichiosis, and tularemia, have been difficult to control because vaccines are not available. Additionally, reports of tick geographic range expansion and increases in wildlife populations that support ticks further complicate tick-borne disease control efforts [3][4][5]. Although Lyme disease accounted for 82% of all U.S. tick-borne disease cases between 2004 and 2016, tularemia cases have seen a resurgence in recent decades. Over 225 tularemia cases per year have been reported since 2015, with 314 reported cases in 2015—the most recorded since 1964

Francisella tularensis, the causative agent of tularemia, has been classified as a Tier 1 Select Agent by the CDC because of its low infectious dose, ease of aerosolization, rapid onset of severe disease, and high morbidity and mortality rates. Two *F. tularensis* subspecies cause human disease and, although they are closely related genetically, vary in their infectious dose and disease severity [6]. *F. tularensis* subsp. *tularensis* (Type A) has an extremely low infectious dose (<10 CFU) and is associated with severe, often lethal, disease. *F. tularensis* subsp. *holarctica* (Type B), has a slightly higher infectious dose (>100 CFU) and is associated with progressive disease with lower mortality rates [7]. Type A *F. tularensis* is further divided into three distinct clades, A1a, A1b and A2.

Previous studies have shown that mice infected with A1b die earlier than those infected with A1a, A2 or Type B *F. tularensis* strains, demonstrating marked differences in *F. tularensis* virulence [6]. Through repeated subculturing of *F. tularensis* subsp. *holarctica*, a live attenuated strain was created by the former Soviet Union in the 1930s and has been designated as the live vaccine strain (LVS) [8]. Despite its name, LVS is not a licensed vaccine in the U.S. due to the unresolved questions about the mechanism(s) of attenuation, adverse effects in some immunized humans, and incomplete protection against Type A aerosol infection [7][8]. However, LVS has been proven to be a useful tool to study *F. tularensis* virulence, as it causes lethal disease in mice yet can be worked with using normal biosafety precautions (i.e., biosafety level 2; BSL2) [9]. A separate *Francisella* species, *F. novicida*, rarely associated with disease in immunocompromised humans, is used as a surrogate for *F. tularensis* in some studies because of reduced biosafety requirements and ease of genetic manipulation [10].

Beyond concerns over the potential use of *F. tularensis* as a bioweapon (i.e., Select Agent designation), approximately half of tularemia cases in the U.S. are associated with tick bites [11][12]. In contrast, European tularemia cases are generally associated with ingestion of contaminated water from wells, streams, rivers, ponds, and lakes [13][14]. However, in Sweden, most tularemia cases are associated with mosquito bites [15][16][17]. A recently-published model indicated that climate change may triple the number of European tularemia cases per year, due to increases in mosquitos, higher temperatures, and increased precipitation [18]. Although ticks are generally not considered to be major drivers of European tularemia infections, *F. tularensis*-infected ticks have been reported in Spain [19], Germany [20], Denmark [21] and Poland [22][23]. Conversely, other groups have not detected *F. tularensis* in ticks collected from Poland, France, or the Netherlands [24][25][26], suggesting that more studies are needed to understand the current and future risks of tick-transmitted tularemia in Europe.

In the U.S., where tularemia was first documented, field-collected *Dermacentor andersoni* ticks (Rocky Mountain wood tick) were shown to transmit lethal *Bacterium tularense* (now known as *F. tularensis*) to guinea pigs [27]. However, the true role of *D. andersoni* ticks in U.S. tularemia cases remains unknown. Data from the CDC indicate that U.S. tularemia infections more commonly stem from *D. variabilis* (American dog tick) and *Amblyomma americanum* (Lone star tick) ticks, which are known to vary in their geographic distribution and mammalian hosts, as well as less understood factors, including tick physiology, endosymbionts, and antimicrobial defenses [4][28]. Indeed, previous studies have reported that differences in the numbers of tick phagocytic cells and prevalence/type of endosymbionts in *A. americanum*, *D. andersoni*, and *D. variabilis* ticks affected the molting success (i.e., survival between tick life stages) of these three tick species [29][30][31].

The complex life cycle of the tick (3 year progression from larvae to nymph to adult), including taking a blood meal from various hosts and molting to the next life stage after each blood meal, combined with varying lengths and severities of North American winters, indicates that upon infecting a tick, *F. tularensis* must undergo major changes over the course of >5 months to persist and replicate, before being transmitted to naive mammals. Laboratory experiments have confirmed that *F. tularensis* persists in ticks for >4 months, supporting the role of the tick as a potential environmental reservoir [32][33]. Additionally, *F. tularensis* has been shown to persist in ticks between molts (transstadial transmission) and replicate to high bacterial numbers in ticks, demonstrating that ticks serve as both a reservoir and an amplification vessel for *F. tularensis* in the environment [33][34]. Rabbits also have been implicated

as a major environmental reservoir for tularemia, as studies have demonstrated that they can survive for 3–13 days following intradermal infection (mimicking a tick infection) with Type A1a, A1b or A2 *F. tularensis* and over 14 days for Type B *F. tularensis* infection [35]. This rabbit infection data, together with the 3–7 days that both *D. variabilis* and *A. americanum* ticks (varies depending on the tick life stage) take a blood meal, suggest that infected rabbits can potentially infect large numbers of ticks [4][33][34]. Despite those studies, we still do not understand whether different tick species acquire different levels of *F. tularensis* infections from different hosts, whether different tick species promote or restrict *F. tularensis* infections, what *F. tularensis*—tick interactions occur, how *F. tularensis* is maintained through the tick molt, or how infected ticks transmit *F. tularensis* to naïve hosts.

2. Epidemiology of *F. tularensis* Transmission by Ticks in the U.S.

Tularemia has been shown to be transmitted by at least four different ticks in the U.S., including *D. variabilis*, *D. andersoni*, *D. occidentalis* (Pacific coast tick), and *A. americanum* [27][33][34][36]. Although *D. variabilis* has been implicated as the primary vector, and *A. americanum* also appears to be an important vector for tularemia, we still do not know which tick(s) poses the greatest threat to human health in the U.S. [33][37]. A 1924 study noted that *D. andersoni* ticks could transmit virulent *F. tularensis* to guinea pigs [27] and a more recent study reported that *D. andersoni* ticks could be infected by and transmit *F. novicida* (rare infection of immunocompromised humans) [38]. However, very little is known about current infection rates of *D. variabilis*, *A. americanum*, or *D. andersoni* ticks with virulent strains of *F. tularensis* or about which ticks are commonly associated with current human tularemia cases. These, and other gaps in knowledge, have resulted in a call for new research studies on tick-borne *F. tularensis* [28]. In addition, although very few studies have explored *F. tularensis*–tick interactions, such studies could provide important information that could be used to develop new strategies to reduce *F. tularensis* in the environment [12].

One area of the U.S. that has been extensively studied to understand *F. tularensis* environmental persistence and tick transmission is Martha's Vineyard. In both 1978 and 2000, two separate tularemia outbreaks occurred on Martha's Vineyard, each involving 15 patients, with 1 fatality [39][40]. Although pneumonic disease was the most common symptom in both outbreaks, *D. variabilis* tick bites remain the only proven mode of transmission in most cases [41]. Sampling studies at Martha's Vineyard have assessed *F. tularensis* infection rates in *D. variabilis* ticks, finding a median annual prevalence of 3.4% over four years, suggesting that *F. tularensis* infections are stable on the island [42]. Additionally, *D. variabilis* ticks infected with Type A *F. tularensis* have been reported to harbor over 10^8 genome equivalents/tick. Although genome equivalents may not accurately quantitate viable bacteria, those data indicate that *D. variabilis* ticks may sustain high *F. tularensis* numbers that cause significant transmission and disease in humans [37][43].

The majority of tularemia cases occur in the south-central U.S. [33][42][44]. In fact, four states accounted for 58% of tularemia cases in 2018 (most recent data from the CDC): Arkansas (24%), Oklahoma (19%), Kansas (8%) and Missouri (7%) [45]. In this region of the U.S., two major tick species exist: *D. variabilis* and *A. americanum* [46][47]. In one study from Missouri, >8500 *A. americanum* ticks were harvested over three years from various hosts, including white-tailed deer, fox, opossum and rabbits, with tick prevalence rates on these potential hosts ranging from 0.7%

to 100%. Although the presence and absence of pathogenic bacteria were not assessed in these *A. americanum* ticks, >1100 *A. americanum* nymphs were collected from a single rabbit, a natural reservoir for tularemia, highlighting the ability of one infected host to spread tularemia to thousands of other hosts, including humans [48]. In Arkansas, approx. 92% of field-collected ticks were *A. americanum* and approx. 7% were *D. variabilis*. Interestingly, none of the *D. variabilis* ticks tested positive for *F. tularensis* (>2000 ticks tested), whereas approx. 4% of *A. americanum* ticks (>5000 ticks tested) were positive for *F. tularensis* [49]. A study of over 3500 field-collected *D. variabilis* ticks from Minnesota identified Type A *F. tularensis* in 3.6% of those ticks [50]. That infection rate was similar to what has been reported at Martha's Vineyard, where the annual *F. tularensis* infection rates in *D. variabilis* ticks ranges from 2.7% to 4.3%, demonstrating that tick-borne tularemia infections are not restricted to the south-central U.S. [42]. In contrast, sampling studies in Washington state did not identify *F. tularensis* in either *D. andersoni* or *D. variabilis* ticks. However, that study examined less than 200 *Dermacentor* sp. ticks and only 25 tularemia cases were reported in Washington state between 2011 and 2016 [51]. Clearly, more studies are needed to assess *F. tularensis* infection rates in multiple tick vectors across the U.S. Further, given the geographic range expansion of various ticks throughout the U.S. [4], continuing studies will be needed to understand how this expansion will affect tick-borne disease transmission.

Although this review is focused on *F. tularensis* infections of ticks, historical data suggest that biting flies also can transmit tularemia [52][53]. However, no recent data from the CDC have linked tularemia infections with biting flies. One study used *Drosophila* as an arthropod model for *F. tularensis* infections, finding that doses as low as 200 CFU killed >90% of fruit flies injected with *F. tularensis* LVS [54], bringing into question whether flies play a significant role in tularemia transmission.

3. Factors that Affect *F. tularensis* Infections of Ticks

Tick-borne pathogens must be able to efficiently transition from mammalian to arthropod hosts following tick feeding [55][56]. *F. tularensis* has been reported to infect and cause disease in over 300 animal species, including humans, highlighting the zoonotic potential and plasticity of *F. tularensis* [57]. Although not well studied, *F. tularensis* likely undergoes substantial changes, including major changes in protein expression profiles, between the mammalian host and the tick vector. Factors such as temperature and pH have been shown to be important cues when *Borrelia burgdorferi*, the causative agent of Lyme disease, transitions between mammalian hosts and ticks. These stimuli result in modifications of bacterial surface proteins to enhance *B. burgdorferi* acquisition by ticks [58][59]. Two surface-exposed lipoproteins, OspA and OspC, are among the most well-characterized proteins that are differentially expressed in *B. burgdorferi*. OspA is highly expressed under conditions that resemble the tick environment (pH 7.5 and 23 °C) [60]. Conversely, during a tick blood meal, the pH and temperature of the tick midgut change to 6.8 and 35 °C, respectively, triggering the upregulation of OspC, and promoting the migration of *B. burgdorferi* through the tick salivary gland to the mammalian host [61][62].

In mammals, *F. tularensis* is an intracellular pathogen, infecting cell types ranging from macrophages, to neutrophils, to epithelial cells, to erythrocytes [63]. Many previous studies have identified *F. tularensis* virulence factors and have examined *F. tularensis* pathogenesis mechanisms using macrophage infection models [64]. When

comparing macrophages and ticks, *F. tularensis* encounters low pH in both the macrophage phagosome and tick midgut [62][65]. In fact, both Type A *F. tularensis* and LVS are resistant to acid stress and viable at pH 3 [66]. While it has been reported that *F. tularensis* responds to low pH by upregulating genes in the *Francisella* pathogenicity island (FPI) to escape from the phagosome [67][68], we are not aware of any study that directly examines whether *F. tularensis* uses low pH as an indicator of the transition between mammalian and arthropod hosts.

Conversely, because iron is extremely limited in the macrophage phagosome but is readily available in replete ticks (through hemolysis), it is possible that *F. tularensis* may successfully transition from mammalian to arthropod hosts by sensing changes in iron and/or altering expression of iron-regulated genes in the tick (Figure 1) [69]. Iron-regulated genes have been shown to be important for the regulation of virulence in *B. burgdorferi* [70]. One study found that *F. tularensis* LVS differentially regulated over 70 genes in iron-limiting conditions, many of which were shown to be associated with virulence or intracellular replication [71]. Although that study did not explore *F. tularensis* gene regulation under iron-replete or high-iron conditions, similar to the tick midgut after a blood meal, such data could provide important information about how *F. tularensis* initially responds and adapts to life inside a tick.

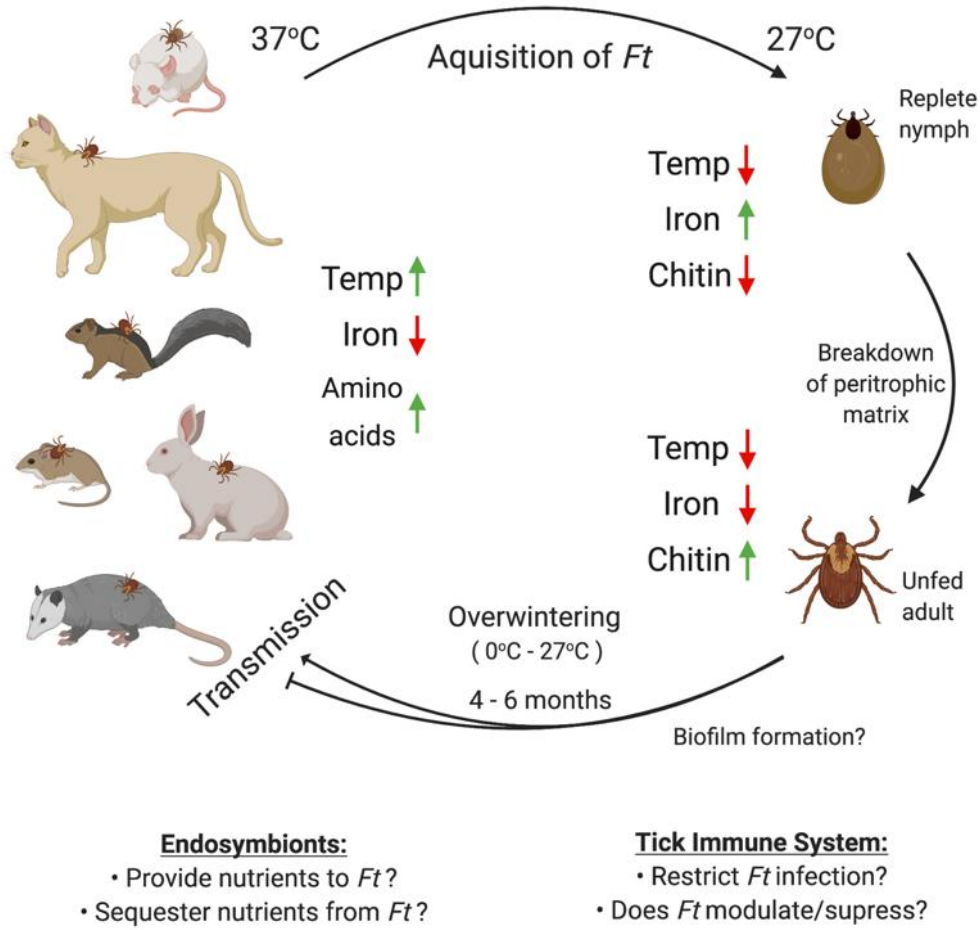

Figure 1. Possible factors affecting *F. tularensis* infection, persistence, and transmission in ticks. Ticks acquire *F. tularensis* (*Ft*) by taking a blood meal from an infected host (e.g., mouse, cat, squirrel, vole, rabbit, or opossum). Upon infecting ticks, *F. tularensis* likely regulates gene expression based on changes in temperature (37 to 27 °C)

and iron (low to high). Following processing of the blood meal, nutrients are likely limited in the tick. However, during the tick molting process, chitin fragments may be made available via chitin remodeling and/or breakdown of the peritrophic matrix. Subsequently, *F. tularensis* must overwinter in ticks, a process which may require biofilm formation, evasion/modulation of the tick immune system, and/or interactions with tick endosymbionts. However, previous studies have not examined any of these factors. Upon transmission from infected ticks to a new mammalian host, *F. tularensis* likely senses mammalian cues (e.g., increased temperature, low iron, higher concentrations of amino acids) and may alter its gene expression, including virulence genes, to promote infection. Figure created using Biorender.com.

A third environmental cue that *F. tularensis* might use to successfully transition to ticks may be temperature changes (Figure 1). One study reported that 11% of the *F. tularensis* LVS genome was differentially regulated when the bacterium was switched from ambient (26 °C) to mammalian (37 °C) temperature. Up to 40% of those identified genes were known to be important for virulence or intracellular replication, suggesting that temperature changes prime *F. tularensis* for pathogenicity in mammals [72]. Although that study may have provided information relevant to *F. tularensis* transmission from ticks to mammals, no studies have been performed to identify *F. tularensis* genes differentially regulated when transitioning from mammals to ticks. Another study found that *F. tularensis* modifies its lipopolysaccharide (LPS) structure in response to temperature changes, including altering expression of acetyltransferases, which add shorter or longer acyl chains to lipid A under ambient (18 °C) or mammalian (37 °C) temperatures, respectively [73]. Those LPS modifications were shown to promote bacterial survival and growth in cold conditions, which could be speculated to help *F. tularensis* survive and persist in ticks during the winter. However, experiments to confirm whether these LPS modifications promote *F. tularensis* persistence in ticks have not been conducted.

Likely because of the difficulties and biosafety risks of working with infected ticks, tick cell lines offer a simplified tool to understand how variables, such as temperature and tick species, can affect bacterial infection and persistence in arthropods. In one study, cell lines derived from *D. andersoni* and *Ixodes scapularis* ticks were infected with *F. novicida*, finding that at 34 °C, *F. novicida* infected and replicated 2-logs higher in *D. andersoni*-derived cells, compared to *I. scapularis*-derived cells. However, *F. novicida* infection killed up to 25% of *D. andersoni* cells, compared to *I. scapularis* cells that appeared to be unaffected by *F. novicida* up to 6 days post-infection. At 24 °C, *F. novicida* infected and replicated to similar levels in *D. andersoni*-derived cells, compared to *I. scapularis*-derived cells. However, approximately 15% less *D. andersoni* cell death was detected at the lower temperature, despite elevated bacterial numbers, indicating that low temperatures may decrease bacterial virulence, while still supporting bacterial replication in tick cells [5]. Although the above highlighted studies provided important insights into how *F. tularensis* may sense environmental cues and promote tick infections, there are still major gaps in our understanding of how virulent *F. tularensis* strains infect ticks, how *F. tularensis* adapts to life in the tick, and which ticks pose the greatest risk for tularemia transmission.

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