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Environmental Adaptation of *Francisella Tularensis*

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Abstract

Concerns over weaponizable bacteria have recently prompted considerable interest in *Francisella tularensis* (*Ft*). In addition to its potential illicit use, *Ft* occurs naturally in diverse ecological niches including mammals, arthropods, and fresh water protozoans. Here we review the current knowledge of *Ft* adaptation which has ramifications for both basic and applied research.

1. Introduction

The genus Francisella contains fastidious, aerophilic gram-negative bacteria found in diverse environments and associated with disease in both vertebrates and invertebrates. Within the species *Ft*, three subspecies (ssp.), *tularensis*, *holarctica*, and *novicida* can cause a potentially serious disease (tularemia) in humans or in animal models of tularemia. Consequently, studies of these *Ft* ssp. or their derivates comprise the bulk of our current knowledge of adaptation of *Ft* to different environments.

The environmental reservoir(s) of *Ft* are not as well defined as those of many other zoonotic pathogens. *Ft* has been found in an array of warm- and cold-blooded hosts (mammals, insects, arthropods, fresh water protozoans) [1,2] indicating remarkable bacterial adaptability (Figure 1). The *tularensis* subspecies (found in North America) is thought to be maintained in lagomorphs and rodents with transmission via ticks and tabanid (deer, horse, etc) flies with little evidence for an aquatic reservoir. In contrast, the *holartica* ssp. which is distributed throughout the northern hemisphere has a stronger association with water-borne disease and, perhaps consequently, with transmission by mosquitoes (along with ticks and biting flies). In addition to the broad range of hosts, it has recently become appreciated that within both mammalian and protozoan infection models, the bacterium has both intracellular (replicative) and extracellular (transmissive) phases [3-7] suggesting additional environments to which *Ft* must adapt. Furthermore, a growing body of evidence indicates that as *Ft* progresses through the intracellular cycle (phagocytosis and phagosomal escape, cytoplasmic replication, induction of autophagic vacuoles and cellular escape) the bacterium adapts to distinct micro-environments by up-and down-modulating expression of many genes [8-10].

To date, no study has cataloged a complete collection of adaptive changes that *Ft* undergoes as it transits from one native environment to another. However, studies of environmental influences on *Ft* have made meaningful inroads. Herein we abstract these studies which

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establish a foundation for the continued investigation of this bacterium's remarkable adaptability.

2. Defined environmental cues

2.1 Temperature

Ft experiences a temperature change during transmission from an environmental source (an arthropod or protozoan host) to a warm-blooded animal. For many vector-borne bacteria such as *Borrelia burgdorferi* (the causative agent of Lyme disease - transmitted by ticks) and *Yersinia pestis* (the plague bacterium - transmitted by fleas), temperature is a cue which alters expression of host-specific, bacterial genes [11-13]. Based on the premise that temperature might similarly alter gene expression of the tularemia bacterium, Horzempa *et al.* [14] performed a genome-wide microarray analysis of *Ft* LVS (the live vaccine strain – a *holartica* derivative) grown in defined broth media at 26°C and 37°C - temperatures frequently used to mimic environmental and mammalian environments. In total, ~11% of the bacterium's genes were differentially-regulated; the mammalian temperature induced 95 genes and repressed 125 others. Approximately half of the induced genes were of unknown function; this ratio increased to ~2/3s for the repressed genes. Among the genes with a known or presumed function, those induced by mammalian temperature included a significant portion of those encoding chaperones/heat-shock proteins (consistent with prior reports of a heat-shock response in *Ft* [15]) and those involved in amino acid metabolism and a select group of tRNAs. These latter observations may suggest that a shift from ambient to mammalian temperature cues *Ft* to prepare for a different nutritional environment. Consistent with the notion that the induced, protein-encoding genes are likely mammal-specific, a significant portion (40%) of these genes had previously been shown to be important for growth and/or virulence in mammalian systems. Just as importantly, the authors analysis did not detect induction of genes in the *Francisella* pathogenicity island (FPI) known to be important for growth in both mammalian and insect/protozoan systems such as PdpA and B or the intracellular growth locus (Igl) proteins A, B, C, D [16,17]. In contrast, one FPI gene (PdpC) that is required for infection of mammalian cells [18] but not for mosquito cells [17], was induced by a shift to mammalian temperature. The authors also mutated a highly-induced, hypothetical gene of unknown function (FTL1581/FTT482); the resulting strain had a diminished capacity for growth in two models of mammalian infection. Cumulatively, these findings suggest that an ambient-to-mammalian temperature shift prompts *Ft* to induce mammal-specific genes and repress genes whose function is related primarily to survival in either arthropods or protozoans. The nature of the regulatory mechanism controlling these temperature-dependent changes has not been reported but could involve the alternative sigma factor, σ^{32}, thought to control expression of several genes encoding heat-shock proteins [19].

Many gram-negative bacteria with both environmental and mammalian phases will synthesize modified forms of lipopolysacchride (LPS) in response to cues such as temperature or [Mg/Ca]. Modifications to the lipid A portion of LPS can alter the bacterium's susceptibility to antimicrobial peptides, diminish the molecules pro-inflammatory properties, and would be predicted to influence the fluidity of the outer membrane. For example, the *Y. pestis* lipid A produced during growth at environmental temperatures is mainly hexa-acylated and elicits a robust inflammatory response from mammalian macrophages whereas the molecule produced during growth at mammalian temperatures is tetra-acylated and immunologically silent [20]. To determine if *Ft* undergoes temperature-dependent LPS modification, Shaffer *et al* used tandem mass spectroscopy to analyze the lipid A moieties of the *novicida* ssp. grown at 25°C and 37°C [21]. Although remodeling of the fatty acid constituents was noted, the *Ft* lipid A remained tetra-acylated at both growth temperatures. Tetra-acylation coupled with a lack of free phosphate moieties likely contribute to the well-recognized “immunological inertness” of *Ft* LPS [22].

2.2 Iron

The struggle between host and microbe for iron is a central paradigm in bacterial pathogenesis [23]. In many bacteria, the low levels of free iron within a mammalian host (~10^{-9} μM) are sensed by an Fe-binding, transcriptional repressor protein (Fur/DtxR) which leads to de-repression of genes involved in Fe-acquisition and frequently, mammal-specific bacterial virulence genes [23]. Not surprisingly, the impact of Fe-limitation on *Ft* has been studied by many groups, most extensively by those of Ramakrishnan and Hansen [24-29]. Collectively, these two groups have characterized an iron-acquisition operon (alternately termed *fsl* - *Ft* siderophore locus, or *fig* - *Ft* iron genes) which lies downstream of the *fur* gene and a canonical Fur-binding site [24,26,28,29]. The *fsl*/*fig* ABCD operon is strongly-induced by Fe-limitation in a Fur-dependent manner and appears to be involved in the synthesis and secretion of a rhizoferrin-like siderophore which chelates extracellular, ferric iron. A down-stream gene (*fsl*/*fig*-E) appears to encode an outer membrane protein receptor for the Fe-siderophore complex [28]. To identify additional Fe-responsive genes, Deng *et al* used genome-wide microarray to analyze the transcriptomes of *Ft* LVS grown in a Fe-replete or deficient defined media [24]. In addition to the siderophore genes (whose expression was increased by ~20 fold), 55 other genes showed increased expression and roughly another 20 (several heat-shock and ribosomal genes) were down-regulated. The 55 up-regulated genes were induced ~2-3 fold and contained a variety of metabolic genes and, strikingly, nearly all 17 members of the FPI (whose functions are unknown but required for infection of mammalian, arthropod, and protozoan cells). Similar findings for several FPI proteins were noted in a proteomics study by Lenco *et al* [27]. It should be noted that Fe-mediated changes in FPI expression are relatively modest compared to those of the siderophore genes or when compared to FPI induction by other environmental cues such as amino acid limitation [25,30]. For at least one FPI gene, *iglC*, the mechanism of Fe-dependent change is likely not attributable directly to Fur [30], but perhaps to a recently characterized FPI activating protein FevR [31] that is induced under low-Fe growth [24]. Cumulatively, it appears that upon encountering conditions of limiting Fe, *Ft* robustly engages an iron-scavenging system and secondarily up-regulates genes required in a range of hosts. Given the scarcity of free Fe within mammalian hosts, the bacterial Fur–regulon is likely engaged during infection of animals; whether this also is true within arthropod or protozoan hosts is currently unknown.

2.3 Stress

Most bacteria exhibit relatively characteristic responses to defined laboratory insults such as exposure to elevated temperature and/or oxidants; these responses are thought to reflect reactions to stress encountered in the bacterium's natural setting. As suggested above, *Ft* appears to have a canonical heat-shock response which has been characterized in the context of both wildtype (WT) and mutant *Ft* lacking specific heat-shock proteins [15,19]. Ericsson *et al.* observed that *in vitro* exposure of *Ft* LVS to 42°C resulted in elevated levels of ~ 15 proteins, three of which were identified as the chaperones DnaK, GroEL, and GroES [15]. Given the transmission cycle of *Ft* and the above-noted findings of Horzempa *et al.* [14] it is likely that the bacterium's heat-shock response is engaged upon entry into a mammalian host. Consistent with this notion, *Ft* lacking ClpB, a heat-inducible enzyme which helps disaggregate and refold denatured proteins, are less fit than their WT counterparts in models of mammalian tularemia [19]; to our knowledge, a similar analysis in a arthropod or protozaon system has not been conducted.

Ericsson and colleagues also noted that exposure of *Ft* LVS to 5 mM H_2O_2 induced the above chaperones [15] and a fourth peroxide-inducible protein, IglC [32]. Others, however, have found the degree of peroxide-mediated induction of IglC to be modest [33,34]. In addition to DnaK, GroEL, and GroES, Lenco *et al.* [33] detected peroxide-induction of other LVS chaperones (ClpB, HtpG), a protease (HslUV), and the antioxidant enzyme, AhpC – a

peroxidase subunit which receives reducing equivalents from AhpF, thiroredoxin reductase or NADH oxidase to detoxify both hydro- and alkyl- peroxides [35-37]. Interestingly, in the single study conducted to date, the peroxide response of the *tularensis* ssp. appears to be rather distinct from that of LVS with the former characterized by a strong induction of Fe-dependent superoxide dismutase (SodB) and AhpC, with minimal induction of chaperones [34]. Whether this is a true difference among *Ft* ssp or a reflection of lab-to-lab variation remains to be determined. As antioxidant-deficient *holarctica* strains (ΔSodB [38] and Δcatalase-KatG [39]) are less fit in models of mammalian tularemia, the bacteria are presumably exposed to superoxide and hydrogen peroxide within a mammalian host. Interestingly, in contrast to LVS strains, many *tularensis* antioxidant mutants do not show significant growth defects *in vivo* [39,40] suggesting either that these ssp. have significant redundancy in their antioxidant armamentarium or that these ssp. experience less oxidative stress *in vivo*. In either case, the location of oxidant exposure remains enigmatic as *Ft* appears to inhibit formation of the host's superoxide-generating, phagosomal NADPH oxidase [41]. The degree to which *Ft* is exposed to oxidants within arthropod or protozaon hosts is currently unknown as is the ability of antioxidant mutants of *Ft* to survive in these hosts.

3. Distinct Growth Environments

In addition to examining the responses of *Ft* to specific stimuli, several investigations have taken a more holistic approach and compared the phenotypes of *Ft* grown in pure culture to those of bacteria exposed to various aspects of the mammalian host environment. To our knowledge, a similar analysis of bacterial responses to arthropod or protozoan environments (other than low temperature) has not been reported. Below, we offer a synopsis of these studies which are grouped based upon their major findings. In large measure, this grouping also follows a temporal progression and lays out our evolving conception of *Ft*-host cell interactions and the distinct micro-environments to which *Ft* must adapt.

3.1 *In vitro* vs. *in vivo*

By comparing *Ft* proteins synthesized during growth in a chemically-defined media to those produced during co-culture with murine macrophages, Golovliov *et al.* identified IglC as a cytoplasmic bacterial protein that is prominently-induced in the presence of macrophages [32]. Subsequently, Twine *et al.* performed a detailed proteomics analysis of a *tularensis* strain either grown in Muellar Hinton broth (MHB) or harvested from murine spleens four days post-infection (PI) [34]. In bacterial lysates from each growth condition Twine *et al.* were able to discern greater than 400 protein spots. Among these, 78 (~20%) proteins were differentially represented, empirically revealing the vast differences between MHB-grown *Ft* and those derived from a mammalian source. Notably, there was minimal congruence between the proteins differentially-regulated *in vivo* and those affected by H_2O_2 *in vitro* [34]; SodB (up-regulated by H_2O_2) was down regulated *in vivo*; KatG (not detected as H_2O_2 responsive) was increased *in vivo*. Among the 78 proteins, the annotations and/or predicted functions ran the gamut from co-factor synthesis and utilization, to energy and amino acid metabolism; 12 of the 78 proteins (including IglC-upregulated ~ 4 fold *in vivo*) had unknown functions.

3.2 Intracellular vs. extracellular

Following the studies of Golovliov and Twine, it became increasingly apparent that *Ft* has a significant extracellular phase [4,5,7]; results from these more recent reports suggest that at the time that *Ft* was harvested in the Twine study (4d PI) that the majority of hematogenous *Ft* were extracellular. A subsequent study revealed that intracellular and extracellular *Ft* are phenotypically distinct and that many of the differences noted by Twine (elevated KatG, IglC, FsaP and decreased SodB, MglB) were characteristic of *Ft* that had emerged from macrophages to become extracellular [25]. Although the observation was underappreciated, previously

Golovliv *et al.* had adroitly noted elevated IglC expression among extracellular *Ft* [32]. This more recent report also indicated that the characteristics of extracellular *Ft* could be recapitulated during *in vitro* growth through the use of a bacterial media that replicated the mammalian environment, and that expression of several of the FPI genes was influenced by levels of free amino acids [25] which are known to vary between the extra- and intracellular milieus (~ 2 mM and ~50 mM respectively [42]).

3.3 Phases of intracellular infection/cellular micro-environments

Several recent investigations have examined more closely the timing of bacterial gene expression as *in vitro*-grown *Ft* transits through the intracellular growth cycle [8-10]. De Bruin *et al*. observed that expression of the *novicida* FPI protein IglA began at 8 hrs PI, peaked at 10 hrs, and diminished starting at 12 hr [9]. In a similar study using the *tularensis* ssp., Chong *et al.* noted that levels of the FPI proteins IglC and PdpC (both expressed at low levels *in vitro*) peaked at 2 hrs PI and were diminishing at 4 hr PI, the last time point examined [8]. The observations of Chong were interpreted as evidence that the environment of the early phagosome cues induction of IglC and PdpC (phagosome acidification as a possible cue was eliminated) [8]. Others, however, have reported that when *in vitro*-grown *Ft* is placed in culture with macrophages, IglC is induced in both intracellular, and to a greater extent, extracellular *Ft* [25,32]. These later observations suggest that an early burst of IglC expression by *in vitro*-grown *Ft* placed in co-culture with macrophages is not specifically a response to the early phagosome, but more simply, a response of these *in vitro*-grown *Ft* to a more mammalian-like environment which is not replicated by most *Ft* cultivation media. An experiment modeled on that of Chong *et al.* but initiated with *Ft* harboring abundant pre-formed IglC (such as macrophage-derived, extracellular bacteria) would more closely replicate the natural course of disease and would address this issue of “phagosome vs. *in vivo*”-mediated, early induction of IglC.

Wehrly *et al.* recently performed an elegantly-comprehensive microarray analysis of *Ft* ssp. *tularensis* gene expression over 24 hrs of growth within macrophages [10]. Prior to initiating the mRNA analysis, the authors very carefully tracked the bacterium's intracellular location over the course of cellular infection. Consequently, the authors were able to correlate changes in bacterial gene expression with residence in particular intracellular compartments. Both the earliest phase [phagosomal uptake and escape, ~ 1 hr PI] and the latest phase of cellular infection [residence in a late autophagic vesicle immediately prior to cellular egress (~20-24 hr PI)] were characterized by elevated transcripts for chaperones (DnaK, GroEL, ClpB, HtpG), proteases (ClpXP, Lon, HslUV), and select antioxidant enzymes (AhpC, SodC). Between hrs ~4 to 16, the bacteria exhibited rapid cytoplasmic replication and the transcriptional profile was characterized, particularly in hrs 12 and 16, by induction of di-/tripeptide transporters and amino acid metabolism genes. This induction is consistent with depletion of the host's cytoplasmic pool of free amino acids, the observed cessation of bacterial replication (16 hrs) and subsequent induction of host-cell autophagy [10,43] a process known to be induced by nutrient depletion [44].

Consistent with a role for amino acid levels in regulation of the FPI [25], this is also the time (12-16 hr) in which the most significant induction of the FPI genes occurred [10]. While five FPI genes (*vgrG*, *iglF*, *iglG*, *iglJ*, and *pdpE*) showed minimal intracellular transcription, the remaining genes displayed a modest blip of early (~1 hr) expression, several hours of minimal transcription followed by a spike of induction at either 8-12 hr (*pdpA*, *pdpB*, *iglE*, and *iglI*) or 12-16 hrs (*pdpD*, *iglA*, *iglB*, *iglC*, and *iglD*). In addition to the above transcriptional analysis of macrophage-grown bacteria, the authors also probed the same samples for several FPI proteins by western blot. IglC protein levels went from undetectable at time zero to an early zenith at 4 hrs; stabilized at a lower level from hrs 8-12; became undetectable by 16 hrs; and

showed maximal expression at 24 hrs [10] prior to cellular egress. In light of the author's transcriptional data, the extensive re-modeling of IglC protein levels suggests that post-transcriptional processes may play an important role in Igl regulation.

4. Mediators of *Ft's* adaptive responses

While a detailed discussion of the transacting regulatory elements of *Ft* is beyond the scope of this reveiw, a brief synopsis of the known actors which mediate adaptive responses is warranted. Seven regulatory proteins (MglA, SspA, FevR, PmrA, Hfq, MigR, Fur) have been empirically characterized. To date, this analysis has focused primarily upon using WT and mutant *Ft* to define the transcriptomes of each of these proteins. With the exception of Fur (see above), little is known about the environmental signals sensed by these regulators or the mechanisms by which *Ft* integrates signals from multiple stimuli to fine-tune its adaptive responses.

MglA (macrophage growth locus A, FTT1275, 23.6 kDa) and SspA (FTT458, 24 kDa) are homologs of the *E. coli* stringent starvation protein A [45] and, as determined thus far, act as heterodimers which interact with the RNA polymerase complex [46]. The *E. coli* SspA is a transcription factor which responds to nutrient limitation and is involved in the regulation of genes contributing to the stringent starvation response. Immediately downstream of the enteric *sspA* is a co-transcribed gene that encodes an accessory factor (SspB) which modulates substrate utilization by the ClpXP protease [47]. Similarly, downstream of MglA is a SspB homolog (MglB) which is annotated as a ClpXP specificity factor [45]. Although known to be required for virulence in mammalian systems [48,49], the role of MglB and ClpXP in governing the expression of *Ft* genes remains largely unexplored. Interestingly, transcriptional analysis indicates that MglA participates in the transcriptional regulation of ~ 100 *Ft* genes [50] while proteomics analysis indicates that the abundance of ~350 proteins are altered in the absence of MglA [51]. These observations suggest that *Ft* makes extensive use of MglA-dependent, post-transcriptional regulatory mechanisms to “sculpt” its proteome.

FevR (Francisella effector of virulence regulation, FTT383) is a small (13 kDa) protein whose expression is significantly dependent on MglA, SspA, and MigR [30,46] and, to a lesser extent, influenced by PmrA and Fur [24,52]. Microarray analysis of parental, *mglA*, *sspA*, and *fevR* mutant strains grown *in vitro*, reportedly indicates that these three regulators have virtually-identical transcriptomes [31,46]. Brotcke *et al.* have proposed a feed-forward mechanism in which MglA and SspA induce FevR which then acts in parallel with MglA/SspA; such a scenario involving FevR could help explain why *mglA* and *sspA* transcript levels are highest at a time when the *igl* transcripts are minimal and vice versa [31,46]. A related notion is that maximal expression of the target genes (such as the *igl*s) requires a certain ratio of the activator proteins i.e. high levels of FevR or PmrA coupled with modest levels of MglA.

MigR (macrophage intracellular growth regulator, FTT694) is a large protein (79 kDa) with a putative AMP-binding domain and homology with acyl-transferase/ligase proteins. MigR has been described as an activator of *igl* transcription; this activation may be indirect as MigR is a potent activator of FevR, and to a lesser extent, SspA [30]. Microarray analysis will be required to determine the relationship between the MigR and FevR/MglA/SspA transcriptomes. Interestingly, in contrast to Δ*fevR Ft* which had replication lesions in both macrophages and epithelial cells, Δ*migR Ft* were only replication defective in macrophages [30]. This observation suggests that MigR and FevR either respond to distinct stimuli and/or direct different transcriptional responses. Consistent with distinct functions of these proteins, the *fevR* and *migR* mutants traffic to different intracellular compartments within macrophages [30].

PmrA (FTT1557, 25.5 kDa) is one of *Ft*'s two response-regulator proteins and serves to activate 52 genes and repress 13 others [52]. Typically, response regulators are cytoplasmic and become competent to bind DNA and affect transcription upon phosphorylation by a membrane-spanning sensor kinase. While identification of the sensor kinase(s) that activates PmrA has yet to be formally established, the impact of PmrA on gene expression is known to have both commonalities and distinctions from that of MglA/SspA/FevR. For example, in contrast to the MglA/SspA/FevR regulon, which most strongly induces *iglAB* but includes all the *igl*s, PmrA appears to preferentially activate transcription of *iglC* and *IglD* [52]. Thus, maximal expression of the Igls appears to require both the MglA/SspA/FevR regulon and that of PmrA. *Ft* encodes two sensor kinases, KdpD (FTT1736) and QseC (FTT94), which could potentially activate PmrA. However, chemical inhibition of *Ft* QseC, which in other bacteria senses host epinephrine or the bacterial molecule auto-inducer 3, led to a transcriptional profile that is distinct from that of *pmrA* mutant *Ft* [53]. Despite the commonly-noted absence of *pmrA* from the MglA/SspA/FevR transcriptome, Guina *et al.* have reported [51] that PmrA protein levels are lower in *mglA Ft*. These observations indicate that PmrA protein levels are not solely the result of transcriptional alterations of *pmrA*.

Hfq (host factor for bacteriophage Q β replication, FTT630) is a small (12.5 kDa) RNA-binding protein that facilitates base-pairing between mRNAs and complementary small, non-coding RNAs. This RNA-RNA interaction impacts mRNA stability and/or translational efficiency. In *Ft*, Hfq negatively regulates transcript levels for 88 genes and positively regulates mRNAs of 16 others [54]. Similar to the MglA/SspA/FevR and PmrA transcriptomes, the Hfq regulon contains a pleotropic set of genes which partially overlap with the other regulons [54]. While deletion of *hfq* clearly has a significant impact of the bacterium's ability to survive many standard laboratory insults (heat, osmotic stress, detergents) [54] the Hfq regulon has a comparatively small complement of recognizable stress-response proteins likely indicating that the natural function of Hfq extends beyond tolerating stress.

5. Summary and perspective

With its capacity to survive/replicate in a diverse array of protists, arthopods, and mammals, *Ft* is a fascinating organism to study and is perhaps one of the more adaptable bacteria known. In recent years, our understanding of the environmental complexity to which this bacterium is exposed (even within a single host) has blossomed. The field has gone from recognizing that bacteria grown *in vitro* and *in vivo* are distinct, to recognizing that "*in vivo*" actually encompasses extracellular and intracellular (and the multiple intracellular micro-environments to which *Ft* is exposed). Furthermore, all of the hosts for *Ft* have multiple tissue and/or cell types - each of which may present *Ft* with unique challenges. When we consider the breadth of potential hosts, the evolutionary success of *Ft* would suggest that we have just begun to understand this bacterium's remarkable adaptability.

Clearly, as researchers continue to uncover new regulatory/adaptive mechanisms employed by *Ft*, the next series of challenges includes defining the signals to which these regulators respond and identifying the environments in which such regulation is engaged. Once the co-activators, co-repressors, and other cues are identified, we then will be poised to begin understanding the hierarchy of signal integration that ultimately underlies the bacterium's environmental versatility. While unraveling such a complex web will undoubtedly be intellectually challenging and ultimately gratifying, such efforts are not mere lofty, academic pursuits. Understanding a pathogen's pattern of differential gene expression can provide significant benefit to the development of effective prophylactic and/or therapeutic countermeasures. History tells us that while it is tempting to pursue vaccine development before a comprehensive understanding of a bacterium's adaptability has emerged; such an approach has potential pitfalls. The now defunct Lymerix vaccine and its target (OspA) of *Borrelia burgdorferi*, the

Lyme disease spirochete, provides a salient example of what can happen when vaccinology outpaces the basic understanding of a microbe's biology/adaptability. Based largely upon work with *in vitro* grown spirochetes and syringe-infected mice, OspA was characterized as an abundant, surface-exposed outer membrane protein which could serve as a protective antigen. After many years of development and commitment of financial resources on the vaccinology front, it was discovered (on the basic research front) that spirochetes dramatically down-regulate OspA expression as the tick vector takes a bloodmeal and transmits the bacterium to the mammalian host. Consequently, the host-adapted (OspA negative) spirochetes were “immune” to the host's Lymerix-induced immunity [55], an observation which correlated very well with the vaccine's sub-optimal efficacy. Despite a decade of effort and many millions of dollars invested in its development, production of Lymerix was discontinued in 2002. As students of *Ft*, a highly adaptable vector-borne bacterium, we have the opportunity to learn from history rather than repeat it.

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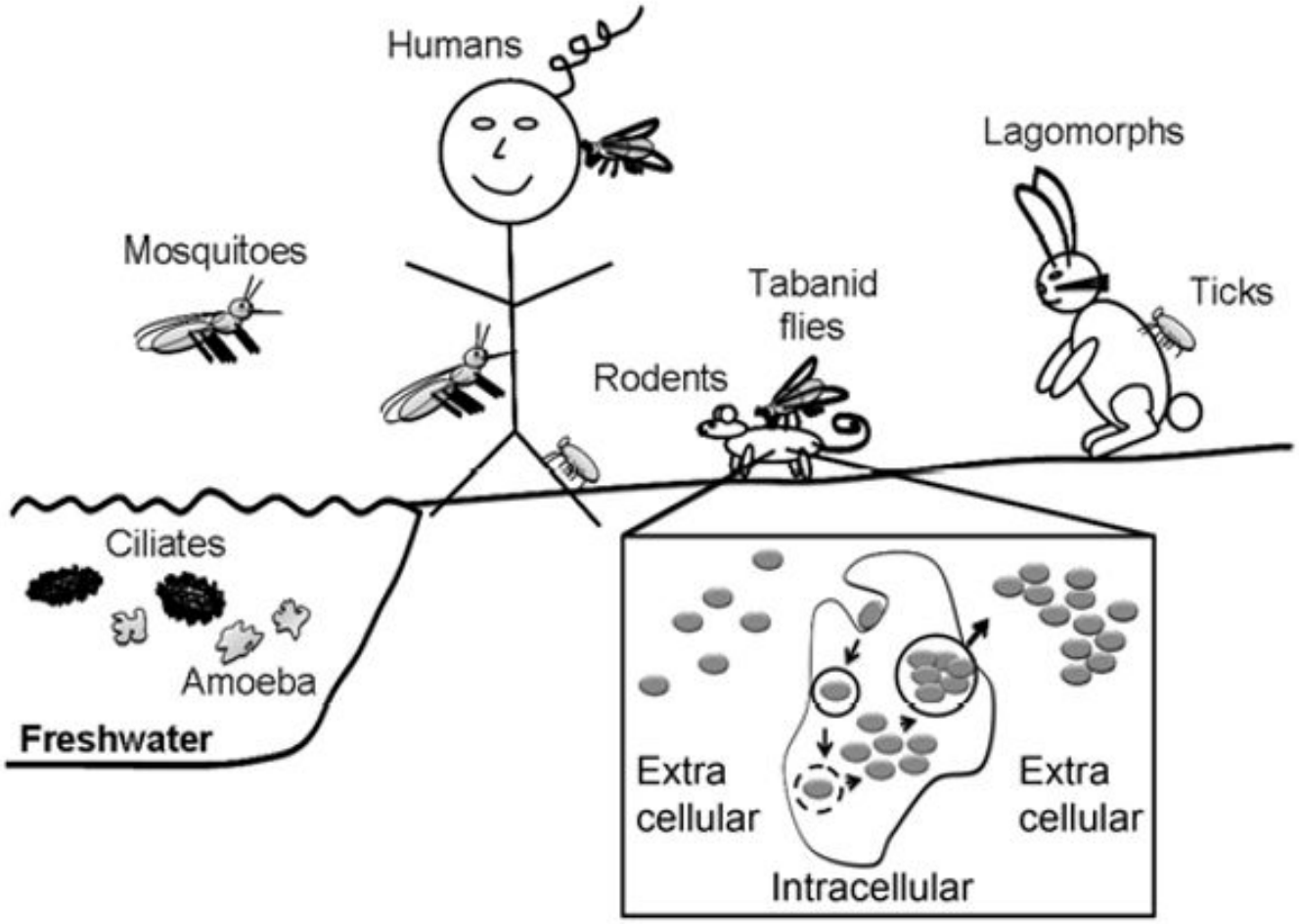

Fig 1.

The many and varied environments of *Francisella tularensis*. The *tularensis* ssp. is restricted to terrestrial organisms whereas the *holartica* ssp. is found in both aquatic and terrestrial environments [1]. All *Ft* ssp. analyzed to date exhibit extracellular and intracellular phases [3-7].