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Survival of *Francisella tularensis subsp. novicida* after exposure to different high temperatures

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ABSTRACT:
Francisella tularensis is a gram-negative facultative intracellular bacterium that can cause a fatal disease, tularaemia, in human and animals. This organism has been isolated from over 250 wildlife species, including fish, birds, amphibians, rabbits, squirrels, hares, voles, ticks, and flies. It resists harsh environments, and has been shown to survive in water and mud for more than a year. There are four closely related subspecies of *F. tularensis*: *tularensis* (type A), *holartica* (type B), *mediasiatica* and *novicida*. The aim of the study was to follow survival of *F. tularensis* subsp. *novicida* after exposure to different high temperatures: 42 °C, 55 °C, 60 °C and 65 °C. Our results showed that *F. tularensis* subsp. *novicida* survives in all exposed temperatures during 60 minutes of incubation.

KEY WORDS:
F. tularensis subsp. *novicida*, High temperatures, Survival

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INTRODUCTION

Francisella tularensis is a Gram-negative facultative intracellular bacterium that can cause the fatal disease tularemia in humans and animals [1,2,3,4]. It was first isolated in 1911 from rodents suffering from a plague-like disease in Tulare County, CA (USA), and designated *Bacterium tularense*. Edward Francis showed that different diseases in humans (Ohara's disease, tick fever, rabbit fever, deer fly fever and lemming fever) are all manifestations of the same disease, known today as tularemia [4]. The genus has been designated *Francisella* in recognition of Francis' work.

F. tularensis can be acquired by contact with, or ingestion of, contaminated material, including food and water, and by inhalation of infectious particles.

The organism has been isolated from more then 250 species, including fish, birds, amphibians, rabbits, squirrels, hares [4,5]. A wide range of arthropod vectors have been implicated in the transmission of the pathogen causing tularaemia between mammalian hosts, including mosquitoes, ticks and deer flies [6]. These vectors can also transmit the pathogen to man. In addition, *F. tularensis* can be acquired by contact with, or ingestion of, contaminated material, including food and water, and by inhalation of infectious particles. Rural populations, and especially those individuals who spend periods of time in endemic areas, such as farmers, hunters, walkers and forest workers, are most at risk of contracting tularaemia [7]. Outbreaks associated with contaminated water supplies can involve large numbers of cases, but usually the incidence of the disease is low [8]. These water-associated outbreaks are mainly caused by subspecies *holarctica*; subspecies *tularensis* has never been linked to water-borne infections [9].

Invasion and intracellular replication of *F. tularensis* within ameba might explain the association of this organism with waterways [10,11], similar to the interaction of *Legionella pneumophila* with amebae [12]. Because of the ease of its dissemination, its multiple routes of infection and its high infectivity, morbidity and rates of mortality, *F. tularensis* has been classified as a category A select agent.

There are four closely related subspecies of *F. tularensis*: *tularensis*, *holarctica*, *mediasiatica* and *novicida* [13,14]. The attenuated live vaccine strain (LVS) of *F. tularensis* is derived from *F. tularensis* subsp. *holarctica*. Analyses of unidirectional genomic deletions and single nucleotide variations have shown that the four *F. tularensis* subspecies have evolved by vertical descent and that *F. tularensis* subsp. *novicida* is the oldest in evolutionary terms [15,16]. In humans, *F. tularensis* subsp. *tularensis* is the most virulent of the subspecies, whereas *F. tularensis* subp*. holarctica* and *mediasiatica* are less virulent and *F. tularensis* subsp. *novicida* is attenuated. It is unclear why *F. tularensis* subsp. *novicida* is attenuated, given its robust replication within human-derived macrophages and its virulence in the mouse models of tularemia: as few as ten organisms can cause tularemia in mice. There is no evidence of reistance of *F. tularensis* subsp. *novicida* to changes in the temperature. In our studies we examined surivival of *F. tularensis* subsp. *novicida* after exposure to differnt high tepmerature up to 65 °C over different time points.

MATERIALS AND METHODS

Bacteria

In this experiment the WT of *F. tularensis* subsp. *novicida* strain U112 was used. Bacteria were grown on a blood agar supplemented with 0.1 % cysteine for 2 days in CO_2 incubator at 37 °C.

Exposure of bacterium to different high temperatures

For growth kinetics, 10^9 cfu/mL of bacterium was inoculated into phosphate buffered salt (PBS) in triplicate, and exposed to different temperatures during 60 minutes. At 5, 15, 30, 40 and 60 minutes after incubation at temperatures of 42 °C, 55 °C, 60 °C and 65 °C the number of *F. tularensis* subsp. *novicida* was determined by plating serial dilutions on blood agar plate.

RESULTS AND DISCUSSION

Tularaemia is a zoonosis of veterinary and public health concern. The general ecological factors, which might influence both the reservoir hosts and the vectors participating in the circulation of *Francisella tularensis* as well as the survival of the infectious agent in the environment, have been examined by J. Pikula et al in Czech Republic [17].

They showed that the natural foci of tularaemia occur mostly in the warmest lowland areas of the Czech Republic that support the highest population densities of the European Hare, small rodent reservoirs and blood sucking ectoparasites [17]. It is also known that *Francisella tularensis* is resistant to exterior factors and survives in environment for several months up to several years [11].

Tularemia is a zoonosis of veterinary and public concern. *F. tularensis* is resistant to exterior factors and survives in environment for several months up to several years.

Our results showed that *F. tularensis* subsp. *novicida* survived high temperatures of 42 °C, 55 °C, and 60 °C up to 65 °C (Fig. 1, 2, 3 and 4). In addition, we examined the time of survival with consideration on exposure to different temperature in distance of 5 min, 15 min, 30 min, 40 min and 60 min. Most of Gram negative bacteria are destroyed after exposure on temperature higher then 60 °C. Although optimal temperature for growth and replication of *Francisella tularensis* is 37 °C, our results show that the large number of bacteria survives on temperature of 65 °C up to 60 min after incubation (Fig. 4). On all temperatures examined, 42 °C, 55 °C, 60 °C and 65 °C the number of inoculated bacteria of 10^9 cfu/mL decreased proportionally with the increase of the temperature. Meanwhile, at 42 °C we determined around 10^8 cfu/mL of bacterium 60 min after incubation. On highest temperatures 55 °C, 60 °C and 65 °C the number of bacteria decreased after 5 min of incubation to 10^4 cfu/mL and remained constant trough the all time of incubation.

Our results clearly show that *F. tularensis* subsp. *novicida* can resist high temperatures which might have an importance in the epidemiology and ecology of tularaemia.

Figure 1. ***F. tularensis* subsp. *novicida* survives temperature of 42 °C.** At the indicated time points, bacteria were plated, and then CFUs were enumerated. The error bars represent the standard deviation of triplicate samples.

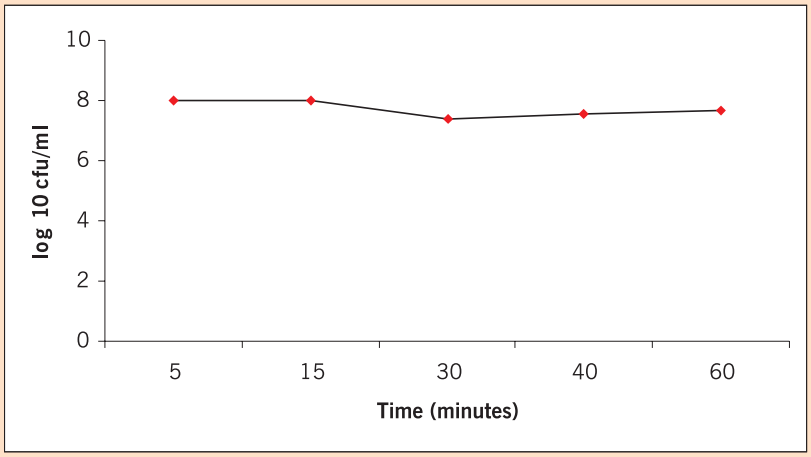


Figure 2. ***F. tularensis* subsp. *novicida* survives temperature of 55 °C.** At the indicated time points, bacteria were plated, and then CFUs were enumerated. The error bars represent the standard deviation of triplicate samples.

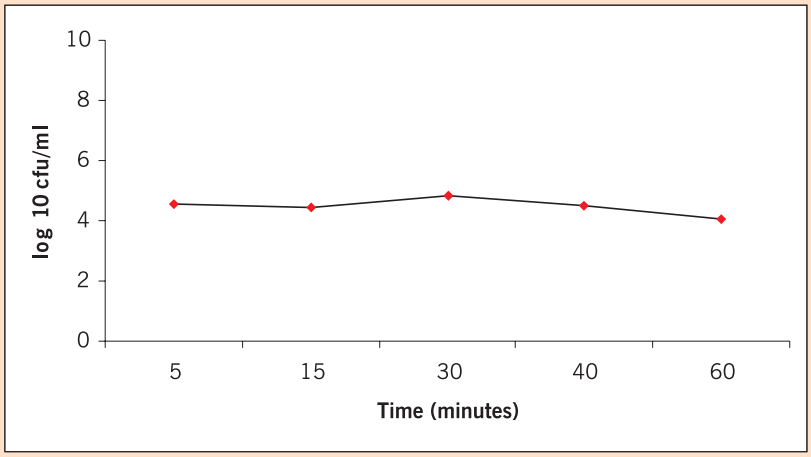


Figure 3. ***F. tularensis* subsp. *novicida* survives temperature of 60 °C.** At the indicated time points, bacteria were plated, and then CFUs were enumerated. The error bars represent the standard deviation of triplicate samples.

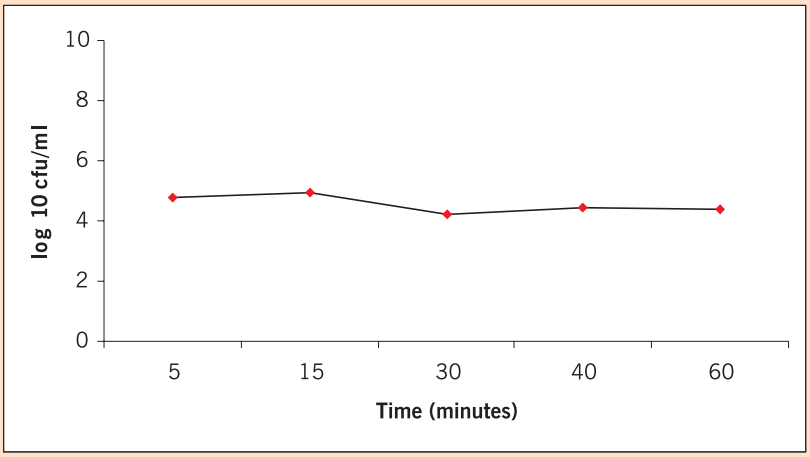
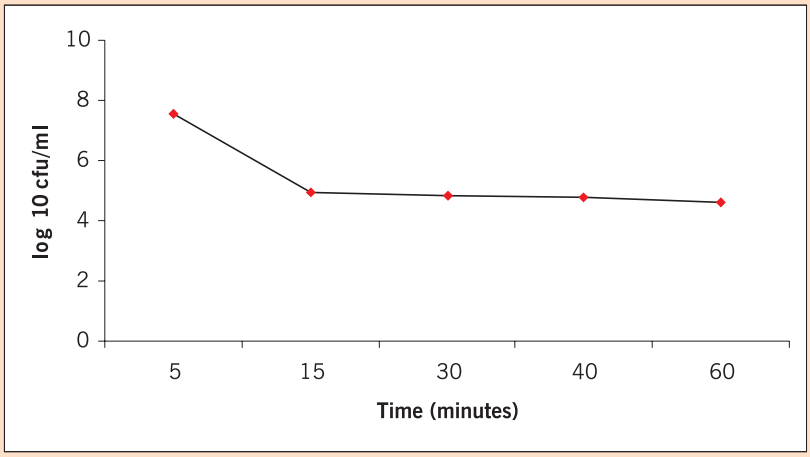


Figure 4. ***F. tularensis* subsp. *novicida* survives temperature of 65 °C.** At the indicated time points, bacteria were plated, and then CFUs were enumerated. The error bars represent the standard deviation of triplicate samples.



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