



Sveriges lantbruksuniversitet

Fakulteten för veterinärmedicin och husdjursvetenskap

***Francisella tularensis* subspecies *holarctica*: The Curious Case of a Water- and Mosquito Associated Bacterium in Sweden**

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Francisella tularensis holarctica: en bakteries underliga vatten- och myggassociation i Sverige

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SUMMARY

Francisella tularensis, the causative agent of the zoonotic disease tularemia, is highly contagious and potentially fatal for a wide range of wildlife species of the northern hemisphere, also humans. Although recognized as a pathogen for over 100 years, much still remains to be elucidated concerning the ecology and transmission of the bacterium, hence this literature overview aims at compiling data regarding the aquatic association and the role of mosquitoes in transmission of *Francisella tularensis* subspecies *holarctica*, the sole subspecies in Sweden. While a linkage between the bacterium and natural waters stands beyond dispute, there is no consensus in the literature concerning its potential as a reservoir. However, two prevailing theories can be distinguished; one proposes the water association being mammal-dependent and thus merely the result of contamination from semi-aquatic mammals living in close vicinity to the water source. The other, quite contrary, suggests mammal-independence and hence that water, possibly in association with protozoa, serves as an environmental reservoir for the bacterium. Regarding transmission of the infection in Sweden, it is assumed to be mediated by mosquitoes, yet their precise role and acquisition of bacteria is not well characterized. Again, two possibilities prevail. On the one hand, mosquitoes have been proposed to acquire the bacterium while feeding on infected animals and, on the other hand, already at their larval stages. Although no agreement is reached in literature, the involvement of larvae would inevitably bridge the gap between the aquatic and terrestrial cycle of the bacterium, especially when considering that mosquito larvae invariably feed on protozoa as larvae in floodwater. Future determination of the natural reservoir of *Francisella tularensis* subspecies *holarctica*, as well as its relationship with mosquitoes, will shed new light upon its complex life cycle and thus significantly aid in prediction and prevention of new outbreaks.

SAMMANFATTNING

Francisella tularensis, en zoonotisk bakterie spridd över norra halvklotet, ger upphov till den smittsamma och potentiellt dödliga pestsjukdomen tularemi. Bakterien upptäcktes för över 100 år sedan, men trots detta kvarstår flertalet kunskapsluckor angående dess komplexa livscykel, vilken involverar såväl land- och vattenlevande djur som flertalet vektorer. Syftet med denna litteraturöversikt är att undersöka möjligheten till en eventuell vattenassocierad reservoar för *Francisella tularensis holarctica*, den enda existerande underarten i Sverige, samt myggors roll som smittspridare. Att bakterien är starkt korrelerad till vattenmiljöer är sedan länge känt, dock råder idag ingen konsensus angående vattnets potential som reservoar för bakterien, istället tycks två huvudsakliga teorier föreligga: Bakteriens förekomst i vattendrag förklaras, å ena sidan, vara en följd av kontamination och således helt beroende av frekvent tillförsel från akvatiska däggdjur, medan den, å andra sidan, tycks indikera en självständig reservoar, oberoende av däggdjur men möjligtvis kopplad till protozoer. Båda teorierna har såväl styrkor som svagheter gällande att förklara *Francisella tularensis holarcticas* epidemiologi och ekologi, och det verkar därmed rimligt att anta att de båda är av vikt i naturen men under olika förutsättningar. Angående myggors betydelse för spridning av tularemi är de sedan länge ansedda de viktigaste vektorerna i Sverige, dock råder delade meningar om hur de infekteras. Traditionellt antas de uppta bakterien vid födosök hos smittade värddjur medan ny forskning pekar på möjlighet till infektion redan som mygglarver. En sådan inkorporering av bakterien i myggans tidiga vattenlevande livsstadier innebär onekligen en önskvärd sammanlänkning av patogenens akvatiska livscykel med dess terrestriska värddjur. Ett fastställande av *Francisella tularensis holarcticas* naturliga reservoar, eller reservoarer, samt en ökad förståelse för myggors roll som bärare och spridare av bakterien är av största vikt för att förutsäga och förhindra framtida epidemier och epizootier.

INTRODUCTION

The bacterium *Francisella tularensis* (*F. tularensis*), the causative agent of the zoonosis tularemia (also known as rabbit fever and deer-fly fever), is highly virulent for humans and a wide range of animals. Being recognized as a pathogen for over 100 years and responsible for causing multi-systemic and potentially fatal disease in the northern hemisphere, including North America, Europe and Asia, surprisingly much remains to be elucidated about the bacterium. Routes of transmission are numerous and in Sweden it is assumed to be associated with mosquitoes, however there is still no consensus regarding how these vectors acquire and transmit the bacterium, nor how the bacterium resides in water environments between outbreaks. The aim of this literature study includes examining the microbiology and ecology of the infectious agent *Francisella tularensis* subspecies *holarctica*, with emphasis on mosquito vector transmission and the aquatic association of the bacterium in Sweden.

MATERIALS AND METHODS

Literature search was performed using PubMed and Web of Knowledge. The search terms used were (Tularemia OR *Francisella tularensis* OR *holarctica*) AND (water OR ecology) AND (transmission OR spread) or alternatively (vector OR mosquito). Furthermore, lists of references in review articles were scanned for appropriate research articles.

LITERATURE OVERVIEW

The infectious agent *Francisella tularensis*

Historical overview

Francisella tularensis has been recognized as a human pathogen for over 100 years. It was first discovered and described in 1911 by the surgeon and pathologist George McCoy in the publication 'A Plague-like Disease of Rodents', a report based on investigation of ground squirrels with plague-like alterations in Tulare County, California (Sjöstedt *et al.*, 1996). Soon after, McCoy and Chapin isolated the bacterium and it was given the name *Bacterium tularensis* after the site of discovery (Tärnvik & Berglund, 2003). Following the initial publication, reports from several different regions of the United States describing a similar disease were published (Sjöstedt *et al.*, 1996). However, there are reports of diseases with strong resemblance to the one described by McCoy but of much earlier date from various parts of the world. There is, for example, a description of such a disease in lemmings in Norway dated 1653. In humans, the first case was recognized in 1914, Ohio (Sjöstedt, 2007). Later on, the bacteriologist Edward Francis recovered the bacterium from blood samples of severely ill human patients and hence proposed the disease to be named tularemia (Tärnvik & Berglund, 2003). Furthermore, he suggested the involvement of blood-sucking insects in the transmission from rodent to humans. In the commemoration of his achievements the bacterium was eventually given its present name, *Francisella tularensis* (Sjöstedt, 2007). Since then, it has become obvious that the agent is not solely confined to North America but occurs in many countries of the northern hemisphere (Sjöstedt *et al.*, 1996). In Sweden, tularemia was first described in the 1930s (Ryden *et al.*, 2009).

Microbiology and taxonomy

Francisella tularensis is the causative agent of tularaemia and, due to its zoonotic potential, may be responsible for causing epidemics as well as epizootics. Phenotypic characteristics include having coccoidal morphology and being a Gram-negative, facultative aerobic and intracellular microorganism (Tärnvik, 2007). Moreover, it has the capacity of biofilm formation, an ability thought to facilitate environmental persistence (Mahajan *et al.*, 2011). Regarding taxonomy, *Francisella tularensis* is a member of the genus *Francisella*, the sole genus within the Francisellaceae family, and further divided into four subspecies (subsp.): *tularensis*, *holarctica*, *mediasiatica*, and *novicida* (Tärnvik, 2007). The classification into subspecies is based on virulence and biochemical characteristics, as well as geographical distribution (Sjöstedt, 2007).

Francisella tularensis subsp. *tularensis*, also known as Type A, is one of the most infectious pathogens known (Tärnvik, 2007), being highly virulent for humans and several animals. The infective dose in humans is as low as 10 bacteria when given as subcutaneous injection and 25 bacteria when inhaled as aerosols. It is geographically restricted to North America where it is the predominant subspecies. Furthermore, it is traditionally associated with dry environments and hence believed to have a terrestrial life cycle, depending primarily on rabbits, ticks and tabanid flies for transmission to humans (Sjöstedt, 2007).

Francisella tularensis subsp. *holarctica*, also referred to as Type B, is the other major causative agent of human tularaemia. Although there is no information regarding its infectiousness, it is, nevertheless, explicit that *holarctica* has lower virulence, causing less fulminant infections with lower mortality. It is geographically spread throughout the northern hemisphere, thus overlapping the boundaries of the Holarctic animal region, and is the sole subspecies found in Europe. Quite contrary to subspecies *tularensis*, *holarctica* is mainly associated with fresh water environments and semiaquatic rodents, such as muskrats and beavers, and hence assigned an aquatic life cycle (Sjöstedt, 2007). Moreover, its transmission to humans appears to be complex and differ between regions (Rydén *et al.*, 2012).

Francisella tularensis subsp. *mediasiatica* has not been fully explored, however, as shown by experimental studies in rabbits, its virulence is assumed to be of moderate degree, similar to that observed for subspecies *holarctica*. Subspecies *mediasiatica* has been reported merely from central Asia (Sjöstedt, 2007).

Francisella tularensis subsp. *novicida* is considered a less virulent subspecies, causing disease mainly in immune deficient individuals and is thus frequently used as model organism for the more infectious subspecies. In similarity with subspecies *holarctica*, *novicida* is closely linked to water-borne transmission (Sjöstedt, 2007), but, as opposed to the other subspecies, it has been isolated also from the southern hemisphere. Additionally, *novicida* has never been recovered from animals (Tärnvik, 2007).

As mentioned above, *Francisella tularensis* subsp. *tularensis* and *holarctica* are the predominant etiological agents of tularaemia. Clinical expression of the disease depends on

the infecting subspecies, where Type A generates more fulminant and severe infection, and the route of entrance. Based on this, five distinct forms of tularaemia have been recognized. Ulceroglandular (or glandular) tularaemia, the most frequent form accounting for more than 90% of outbreaks in Sweden, is contracted through the skin, commonly by means of vector-transmission or direct contact with infected animals. Oculoglandular tularaemia results from inoculation of the eye, e.g. by a contaminated finger. The oropharyngeal form occurs after ingestion of contaminated food or water. Respiratory tularaemia is acquired upon inhalation of aerosolized *F. tularensis*. Finally, if no port of entry can be determined, the term typhoidal tularaemia may be used. Regardless of form, the clinical picture include, apart from more specific symptoms, high fever and chills, general body ache and headache, fatigue and nausea, and frequently a dry cough (Tärnvik & Berglund, 2003).

Epidemiology of *Francisella tularensis*

Geographical distribution of tularaemia cases

Cases of tularaemia have been reported from most countries in the northern hemisphere, where the disease often occurs endemically, but not yet from the southern. Nevertheless, as mentioned above, subspecies *novicida* has been isolated from the southern hemisphere, more precisely Australia, but none of the typical clinical manifestation associated with the infection were observed. Tularaemia is regularly reported from the USA and in Canada, and occurs also in Japan and China. In Europe, more cases are reported annually from the eastern parts than from continental western parts. Although tularaemia emerges only as isolated occasions in most countries, there are some countries in which endemic regions have been known to exist for several decades, most notably Sweden, Finland and Russia. In most of these areas, the disease displays seasonality with incidence culminating during late spring, summer and early autumn (Tärnvik, 2007). In Sweden, such areas are found in northern parts of central Sweden (Norrland, Götaland and Svealand), from which probably the highest incidence in the world has been reported (Smittskyddsinstitutet, 2012). Within these regions, tularaemia occurs at particularly high frequency in restricted foci, which can range from the size of a single rodent burrow to a landscape zone. Ljusdal (Gävleborg) along the river Ljusnan compose such a focus, as does a few villages along the river Västerdalälven (Dalarna) (Rydén *et al.*, 2009). Furthermore, based on epidemiological data, tularaemia in Sweden appears to be strongly associated with water environments such as rivers, streams, lakes and ponds (Berdal *et al.*, 1996).

Routes of transmission and the role of mosquitoes as vectors

The host range of *Francisella tularensis* is broader than for any other known zoonotic disease-causing agent, comprising approximately 250 wildlife species of which the vast majority are mammals, but also a few birds, amphibians, fish and invertebrates are susceptible (Broman *et al.*, 2011). Likewise, there are several potential vectors for its transmission and a wide array of arthropods has been identified as such, including ticks, mosquitoes, tabanid flies and mites. In Sweden, only one tick species (*Ixodes ricinus*) is able to transmit tularaemia to humans, instead mosquitoes are recognized as key players (Eliasson *et al.*, 2002). The importance of mosquitoes as primary vectors was first identified when detecting infected *Aedes cinereus* during an outbreak in Sweden in 1938 (Petersen, 2009) and today some ten

species of mosquitoes have been proven naturally infected (Rydén *et al.*, 2009). Transmission is proposed to be mechanical, i.e. without multiplication or development of the bacterium, and consistently, long-term survival of *F. tularensis* in mosquitoes is not supported and thus they are capable only of transient transmission. However, how the mosquito acquires the bacterium remains to be unravelled. It has been suggested though that bacterial uptake occurs while feeding on infected animals. When disrupted, the mosquito seeks another available host for continued feeding and thus transmits the infection. Alternatively, mosquitoes have been implied to acquire the bacterium already as larvae feeding in water. Regardless of mode of acquisition, the disease is transferred also due to host-defence behaviour such as crushing an infected mosquito on the skin and subsequently scratching the bite (Petersen *et al.*, 2009).

In addition to arthropod bites, humans might contract tularaemia also by inhalation of infectious dust, often during farming activities such as piling hay contaminated by infected corpses of voles, as seen during the 1966-67 winter outbreak among farmers. Indeed, farmers are considered at-risk-persons, as are veterinarians, hunters and laboratory workers (Eliasson *et al.*, 2002). Other routes of transmission include ingestion of contaminated food or water as well as direct contact with infected animals, e.g. skinning. However, there is no human-to-human transmission (Tärnvik & Berglund, 2003).

Reservoirs

In Sweden, human tularaemia cases are highly associated with natural waters (Berdal *et al.*, 1996) as seen along the rivers Ljusnan and Västerdalälven (Rydén *et al.*, 2009). This is further supported by time and place analysis of patient data ranging over 26 year, indicating that *F. tularensis* has a reservoir in vicinity to watersheds or in the water (Rydén *et al.*, 2012). However, the natural reservoir itself remains unrevealed although semiaquatic mammals as well as watercourses are prominent candidates.

The mammal-dependent theory claims that the water association seen for *F. tularensis* in Sweden is dependent on semiaquatic mammals. According to this theory, the persistence of the causative agent in waters relies on continuous contamination from tularaemia-infected animals. In particular, lemmings and beavers might be important in maintaining the bacterium in the environment. Additionally, infected voles and water voles are also thought to be of significance and have been proven to contaminate watercourses by shedding bacteria by urine. Furthermore, outbreaks among voles lead to die-offs and corpses contaminating water, soil and mud (Bell & Stewart, 1975).

The other prevailing theory suggests mammal-independence, a feature proposed by Parker *et al.* already in 1951 after demonstrating long-term persistence of *F. tularensis* in mud and water (as reviewed by Sjöstedt, 2007). A later study of Berdal *et al.* (1996) supported this theory when proving the bacterium able to persist for months in natural waters, possibly in association with protozoa. According to Berdal *et al.*, the proposal of protozoan as bacterial hosts would explain the epidemiology of the disease, including its occurrence in restricted areas and seasonality. Furthermore, water-dwelling amoebas as natural environmental reservoirs would account for the aquatic association of tularaemia observed in Sweden and

the water-borne outbreaks seen in Norway (Berdal *et al.*, 1996). Perhaps even more important, it provides an explanation for the environmental persistence of *F.tularensis* in between outbreaks (Broman *et al.*, 2011).

DISCUSSION

The natural reservoir of *Francisella tularensis* subspecies *holarctica* and the role of mosquitoes are current issues of debate. Hence, the purpose of this literature study was to examine the microbiology and ecology of the infectious agent *Francisella tularensis* subspecies *holarctica*, with emphasis on mosquito vector transmission and the aquatic association of the bacterium in Sweden.

Over the years, it has been clearly demonstrated that natural water sources are of great importance for the dissemination of *Francisella tularensis* subspecies *holarctica*. Indeed, large tularaemia outbreaks in both Turkey and Bulgaria have been proven connected to ingestion of infected drinking water (Foley, 2010). However, as mentioned above there are discrepancies regarding whether water is a mammal-dependent or independent reservoir.

In favour of a mammal-dependent reservoir, tularaemia outbreaks have traditionally been preceded by epidemics among rodents and lagomorphs. Consequently, rodents have been used as sentinels for monitoring the environmental occurrence of *F. tularensis*. Moreover, for long a correlation between peaks in rodent populations and subsequent tularaemia epizootics has been reported. This is exemplified in the Novosibirsk region of the Russian Federation where increased density of water rat population has preceded human outbreaks, as measured from 1956 to 2000. A similar pattern has been observed in Sweden where denser populations of voles and hares have been indicative of higher number of human tularaemia cases. However, this linkage was only seen during the 1960s and the 1970s and has not been reported during more recent outbreaks in Sweden. Perhaps, this could imply a change of spreading pattern over time, as seen in Canada where water-living muskrats appear to have replaced contact with rabbits as the predominant route of transmission (Tärnvik, 2007). Nevertheless, there are other indications of mammal-dependency such as the observation of partly immune voles shedding *F. tularensis* by urine, thus contaminating waters (Bell & Stewart, 1975). Still, constant or frequent contamination of watersheds between outbreaks could only be explained by the existence of chronically infected animals. However, this is generally not the case in nature where infected rodents and lagomorphs succumb, or, alternatively, recover and eradicate the bacteria. In both cases they are rendered unable to chronically spread tularaemia (Tärnvik & Berglund, 2003). Alternatively, if carcasses were to function as continuous contaminators of water, fatal infections among rodents would need to be frequently occurring. Even so, when testing water infectivity downstream of carcasses *F. tularensis* has failed to be detected (Bell & Stewart, 1975). Taken together, it appears as if mammalian hosts can neither harbour the bacterium long-term nor explain the interepizootic periods typical of tularaemia.

In comparison, water as a mammal-independent reservoir of *F. tularensis* has been proven capable of harbouring the infectious agent also in between epidemics. This has been

investigated thoroughly through environmental studies performed by Broman *et al.* (2011). During a period of three consecutive years, natural water samples were collected from two regions where tularaemia is reoccurring in Sweden: Örebro and Ljusdal. Both these regions have an outbreak history with epidemics being reported at irregular intervals within geographically enclosed areas, as typically seen in tularaemia endemic regions. The obtained results demonstrated a persistence of subspecies *holarctica* in samples collected over the entire study length, hence proving a static presence of the bacterium in natural waters for years, also when outbreaks are not occurring. In the same study, rodents from the investigated regions were sampled and found uninfected, although water samples were positive. This further supports the idea of a mammal-independent water reservoir. What is more, it proposes the use of rodents as sentinels for the environmental surveillance of *F. tularensis* during interepizootic periods inappropriate and unreliable (Broman *et al.*, 2011).

Assuming *F. tularensis* being able to reside in natural waters, it is puzzling that only one water-borne tularaemia outbreak has occurred in Sweden. This 1973-outbreak affected only nine individuals and originated from a well of drinking water (Sjöstedt, 2007). Also, being a facultative intracellular microorganism *F. tularensis* relies on host cells for replication, hence it is reasonable to question where in water it resides. One possible explanation is that the bacterium, when released into free water, enters a viable but non-culturable state, a feature that could account for the difficulties associated with culturing *F. tularensis* from water samples. Supporting this non-culturable, but infectious state, inoculation of laboratory animals with water samples resulted in subsequent recovery of culturable bacteria (Parker *et al.*, 1951, as reviewed by Broman *et al.*, 2011). This resting state is further enhanced by whole-genome sequencing studies of *F. tularensis* recording a low metabolic capacity, as would be expected from an obligate host-dependent bacterium (Broman *et al.*, 2011). Another possible explanation for *F. tularensis* ability to reside in a water environment is the establishment of biofilm. Studies have demonstrated that the bacterium is capable of biofilm formation and persistence in natural waters and, furthermore, that the existence of *F. tularensis* in such a niche concentrates bacteria at one site and hence provide a number sufficient for infection (Mahajan *et al.*, 2011).

F. tularensis being an intracellular microorganism, it is reasonable to consider involvement of water-dwelling protozoa in the maintenance of the bacterium in aquatic environments. Studies have demonstrated the bacterium capable of infecting and propagating within *Acanthamoeba castellanii*, a characteristic shared with several other obligate intracellular pathogens, such as *Legionella pneumophila*. Additionally, in co-culture with *Acanthamoeba castellanii*, *F. tularensis* displays an increased growth rate. There are also observations of *F. tularensis* being able to invade an array of other ubiquitous protozoa and thereto improve its environmental persistence, further implicating the importance of protozoan as hosts (Abd *et al.*, 2003). Besides, it is tempting to assume also the virulence of the bacteria being enhanced when cultured in association with protozoa, as is the case with *Legionella* (Berdal *et al.*, 1996). Moreover, in nutrient rich aquatic systems, such as the tularaemia areas of Örebro and Ljusdal, protozoa have been shown to exert a predatory impact on bacteria (Auer *et al.*, 2004). Accordingly, *Francisella tularensis* subspecies *holarctica* is suggested to possess a protozoa

water-borne reservoir. As such, it could be further accumulated by mollusks, crayfish, frogs and semiaquatic rodents ingesting infected protozoa (Foley, 2010).

Supportive of the protozoa-as-host theory, mosquitoes, the main vectors and transmitters of tularaemia in Sweden, have been confirmed to invariably feed on protozoa as larvae. Hence, it is reasonable to assume mosquitoes constituting the connection between the aquatic reservoir of the bacterium and its terrestrial hosts, including humans. This is particularly applicable for the species *Aedes sticticus*, but also for larvae of other floodwater mosquitoes, which have been demonstrated to exert an extensive predation pressure on protozoa populations inhabiting temporary marshlands (Östman *et al.*, 2008). What is more, larvae collected from floodwater samples in tularaemia endemic areas in Sweden have been proven to harbour *F. tularensis* when analysed as hatched, adult mosquitoes. This implies a transstadial transmission of the bacterium from its aquatic reservoir via larvae and subsequent adult mosquitoes to various terrestrial hosts (Lundström *et al.*, 2011). In this aspect, *Aedes sticticus* is of special interest considering that it is currently increasing its geographical spread within Sweden (Schäfer & Lundström, 2009). All in all, it appears as if a mammal-independent water reservoir, possibly in association with protozoa, can adequately account for long-term environmental survival of the bacterium, also in between outbreaks. Still, it does not account for the exceedingly rare water-borne epidemics observed in Sweden.

Regarding the importance of mosquitoes as vectors, Eliasson *et al.* (2002) conducted a case-control study to identify risk factors during the 2000 tularaemia outbreak in Sweden and concluded mosquito bites to be the main one. In addition, the work of Rydén *et al.* (2012) have demonstrated that outbreaks of tularaemia in Sweden are dependent on mosquito prevalence, as established during the interval 1981 to 2007 when six out of seven outbreaks correlated with predicted mosquito highs. Interestingly, predicted peaks in mosquito prevalence preceded human tularaemia outbreaks with a delay corresponding to the incubation period of tularaemia. Taken together, this emphasizes the crucial role of mosquitoes as disease transmitters to humans in boreal forest regions (Rydén *et al.*, 2012). Perhaps, surveillance of mosquito prevalence in late summer would serve as a more appropriate and reliable method of predicting tularaemia outbreaks, than does the use of rodents as sentinels. Moreover, tularaemia is, due to its seasonality and mosquito dependency, unarguably connected to various climate factors, such as temperature. Temperature influences many factors vital for transmission, including precipitation and water temperatures, and thereby impacts mosquito larvae conditions as well as transmission to humans. In accordance, future climate changes are likely to prolong the outbreak duration (Rydén *et al.*, 2009) and lead to alterations in the geographic distribution of tularaemia in Sweden. Thus, it is likely that the disease will vanish in certain regions while emerging in others (Sjöstedt, 2007).

Concluding remarks and future directions

In conclusion, although *Francisella tularensis* subspecies *holarctica* for long have been associated with aquatic environments such as rivers, ponds and lakes, many queries and anomalies concerning its persistence in nature between outbreaks still exist. In particular, it remains to be unravelled whether water as a reservoir is mammal dependent, i.e. relies on

contamination from aquatic mammals, or mammal independent, i.e. in itself (or in association with protozoa) favouring persistence of the bacterium. Both theories have their strengths and weaknesses when it comes to fully explain the complex, and sometimes contradictory ecology of *F. tularensis*. One possibility is that they work in alliance, in which both potential reservoirs play major roles. It seems likely, however, that semi-aquatic mammals are not so much a true natural reservoir, harbouring the bacterium in between epidemics, as a contaminant source during an already existing outbreak. Certainly, they amplify the bacterium, and thus the outbreak, by urine shedding, carcass deposition and serving as mosquito hosts.

Concerning the role played by mosquitoes, their importance as vectors in boreal forest regions stands beyond doubt. As such they appear to constitute the link between the water and terrestrial cycle of the bacterium. Hence, it would be valuable to evolve effective approaches for mosquito surveillance in order to predict, and prevent, future tularaemia outbreaks, as well as developing methods for efficient mosquito control. This is perhaps of even greater importance when standing before future climate changes which will most likely affect mosquito prevalence. An important question awaiting answer is how mosquitoes acquire the bacterium. Recent research, however, points at the possibility of mosquitoes coming into contact with the bacterium already as larva, a working hypothesis that appears well balanced.

So, challenges for the near future include determining the natural reservoir, or reservoirs, of *Francisella tularensis* subspecies *holarctica* in Sweden and elucidating the mosquito-bacterium relationship. In finding answers to these queries, new light will be shed upon the complex ecology and life cycle of the bacterium, hopefully contributing significantly in the prediction and restriction of epidemic outbreaks.

LIST OF REFERENCES

- Abd, H., Johansson, T., Golovliov, I., Sandström, G., Forsman, M. (2003) Survival and growth of *Francisella tularensis* in *Acanthamoeba castellanii*. *Applied and Environmental Microbiology*, 69, 600-606
- Auer, B., Elzer, U., Arndt, H. (2004) Comparison of pelagic food webs in lakes along a trophic gradient and with seasonal aspects: influence of resource and predation. *Journal of Plankton Research*, 26, 697-709
- Bell, J. F., Stewart, S. J. (1975) Chronic shedding tularemia nephritis in rodents: possible relation to occurrence of *Francisella tularensis* in lotic waters. *Journal of Wildlife Diseases*, 11, 421-430
- Berdal, B. P., Mehl, R., Meidell, N. K., Lorentzen-Styr, A-M., Scheel, O. (1996) Field investigations of tularemia in Norway. *FEMS Immunology and Medical Microbiology*, 13, 191-195
- Broman, T., Thelaus, J., Andersson, A.-C., Bäckman, S., Wikström, P., Larsson, E., Granberg, M., Karlsson, L., Bäck, E., Eliasson, H., Mattsson, R., Sjöstedt, A., Forsman, M. (2011) Molecular detection of persistent *Francisella tularensis* subspecies *holartica* in natural waters. *International Journal of Microbiology*, 2011, 1-10
- Eliasson, H., Lindbäck, J., Pekka Nuorti, J., Arneborn, M, Giesecke, J., Tegnell, A. (2002) The 2000 tularaemia outbreak: a case-control study of risk factors in disease-endemic and emergent areas, Sweden. *Emerging Infectious Diseases*, 8, 956-960
- Lundström, J. O., Andersson, A-C., Bäckman , S., Schäfer, M. L., Forsman, M., Thelaus, J. (2011) Transstadial transmission of *Francisella tularensis holarctica* in mosquitoes, Sweden. *Emergent Infectious Diseases*, 17, 794-799
- Mahajan, U. V., Gravgaard, J., Turnbull, M., Jacobs, D. B., McNealy, T. L. (2011) Larval exposure to *Francisella tularensis* LVS affects fitness of the mosquito *Culex quinquefasciatus*. *FEMS Microbiology Ecology*, 78, 520-530
- Petersen, J. M., Mead, P. S., Schriefer, M. E. (2009) *Francisella tularensis*: an arthropod-borne pathogen. *Veterinary Research*, 40, 7
- Rydén, P., Sjöstedt, A., Johansson, A. (2009) Effects of climate change on tularaemia disease activity in Sweden. *Global Health Action* 2009. Doi: 10.3402/gha.v2i0.2063
- Rydén, P., Björk, R., Schäfer, M. L., Lundström, J. O., Petersén, B., Lindbom, A., Forsman, M., Sjöstedt, A., Johansson, A. (2012) Outbreaks of tularemia in boreal forest region depends on mosquito prevalence. *The Journal of Infectious Disease*, 205, 297-304
- Schäfer, M. L., Lundström, J. O. (2009) The present distribution and the predicted geographic expansion of the floodwater mosquito *Aedes sticticus* in Sweden. *Journal of Vector Ecology*, 34, 141-147
- Sjöstedt, A. (2007) Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. *New York Academy of Sciences*. Doi: 10.1196/annals.1409.009

- Sjöstedt, A., Tärnvik, A., Sandstöm, G. (1996) *Francisella tularensis*: Host-parasite interaction. *FEMS Immunology and Medical Microbiology*, 13, 181-184
- Smittskyddsinstitutet. Sjukdomsinformation om harpest [online](2012-06-11) Available: <http://www.smittskyddsinstitutet.se/sjukdomar/harpest/>. [2013-03-04]
- Tärnvik, A (2007) *WHO guidelines on tularaemia*. 1st ed. Geneva: WHO Press
- Tärnvik, A., Berglund, L. (2003) Tularaemia. *European Respiratory Journal*, 21, 361-373
- Östman, Ö., Lundström, J. O., Persson Vinnersten, T. Z. (2008) Effects of mosquito larvae removal with *Bacillus thuringiensis israelensis* (Bti) on natural protozoan communities. *Hydrobiologia*, 607, 231-235