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Short Communication

Absence of *Francisella tularensis* in Finnish *Ixodes ricinus* and *Ixodes persulcatus* ticks

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A B S T R A C T

Francisella tularensis subsp. *holarctica* is the causative agent of tularaemia in Europe. Finland is a high-incidence region for tularaemia, with mosquito bites as the most common sources of infection. However, in Central and Western Europe, ticks (Acari: Ixodidae) have been suggested as the main vectors. Indeed, several studies have reported the pathogen from the locally most common human-biting tick species, *Ixodes ricinus*. In Finland, the occurrence of the pathogen in ticks has started receiving attention only recently. Here, we collate previous tick screening data from Finland regarding *F. tularensis* as well as present the results from a novel screening of roughly 15 000 *I. ricinus* and *I. persulcatus* collected from across the country.

In total, 14 878 ticks collected between 2015 and 2020 were screened for *F. tularensis* using a TaqMan-based qPCR assay targeting the *23 KDa* gene. The combined screening efforts of the current and previous studies, encompassing roughly 20 000 ticks, did not find any positive ticks. Given the negative results despite the considerable sample size, it appears that the pathogen is not circulating in local tick populations in Finland. We discuss some possible reasons for the lack of the bacterium in ticks in this high-incidence region of tularaemia.

1. Introduction

Francisella tularensis is a well-recognized bacterial pathogen of medical and veterinary interest (Sjöstedt, 2007). It is divided into four subspecies, of which *F. tularensis* subsp. *holarctica* (type B strains) cause tularaemia in Europe (Maurin and Gyuranecz, 2016). There are three major modes of transmission of *F. tularensis* subsp. *holarctica* to humans, namely direct transmission from infected animals, through contaminated water or soil, and via arthropod bites (tick, tabanid fly or mosquito) (Petersen et al., 2009; Hennebique et al., 2019).

Two different lifecycles of *F. tularensis* subsp. *holarctica* have been suggested, the so-called terrestrial and aquatic cycles (Maurin and Gyuranecz, 2016). The terrestrial cycle is dominant in Central and Western Europe, and it is suggested to involve lagomorphs and rodents as reservoir hosts, and ticks as the main vectors for pathogen transmission (Maurin and Gyuranecz, 2016). Indeed, the bacterium has been reported from the locally most common human-biting tick species, *Ixodes ricinus*, in several European countries (Milutinović et al., 2008; Gehringer et al., 2013; Tomaso et al., 2018).

In the aquatic cycle, which is dominant in Finland, the primary sources of human infections are aquatic environments (Hennebique et al., 2019). The mechanisms of *F. tularensis* persistence in water environments remain obscure, although there is evidence suggesting that amoebae likely promote the survival of these bacteria in water ecosystems (Hennebique et al., 2021). Human infections may occur via the consumption of contaminated water leading to the oropharyngeal form of tularaemia. However, in Finland tularaemia is most often a mosquito-borne infection, with the ulceroglandular and glandular forms being the predominant clinical manifestations (Jounio et al., 2010; Hennebique et al., 2019). Ticks, on the other hand, are prevalent in Finland (Laaksonen et al., 2017), tick-borne infections (especially Lyme borreliosis) are diagnosed almost throughout the country (Sajanti et al.,

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2017), and identified vertebrate reservoirs of *F. tularensis* subsp. *holarctica* (i.e. hares and rodents) are present in Finnish nature (Rossow et al., 2014; Finnish Food Authority, 2018; Hennebique et al., 2019). Therefore, it is striking that tularaemia is not recognized as a tick-borne infection in Finland.

Regarding Finnish wildlife, *F. tularensis* has previously been reported only from voles (Rodentia: Cricetidae) and hares (Lagomorpha: Leporidae) (Rossow et al., 2014; Finnish Food Authority, 2018). As mentioned, while ulceroglandular and glandular forms of tularaemia may arise from bites of tabanid flies as well, mosquitoes (Diptera: Culicidae) are considered to be the main transmitters of *F. tularensis*in Finland (Jounio et al., 2010). However, no data regarding the occurrence of the bacterium in Finnish mosquitoes has been published. Regarding ticks, the degree to which the bacterium occurs in local ticks has only started receiving attention during the past decade (Laaksonen et al., 2018; Pakanen et al., 2020; Sormunen et al., 2020a; Sormunen et al., 2020b). Considering that the local incidence of tularaemia is one of the highest reported worldwide (Sissonen et al., 2015) and that the animals identified as important reservoirs for *F. tularensis* (rodents and lagomorphs) are also common hosts for ticks (e.g. (Jaenson and Tälleklint, 1996), one might expect local ticks to frequently encounter *F. tularensis*. However, no ticks carrying the pathogen have been detected in the previous surveys, raising questions about the possible absence of tick-borne *F. tularensis* in Finland (Laaksonen et al., 2018; Pakanen et al., 2020; Sormunen et al., 2020a, 2020b). Consequently, in order to more comprehensively assess the occurrence of the pathogen in Finnish ticks, we screened an additional set of tick samples originating from across the country. In the current paper, we present novel screening results regarding the occurrence of *F. tularensis* in the two human-biting tick species present in Finland, *I. ricinus* and *I. persulcatus*. In addition to this novel data set of roughly 15 000 ticks, we collate the previously reported data to form a complete picture of the total screening effort in Finland.

2. Materials and methods

Collected ticks (Fig. 1) were identified to species and life stage using morphological keys (Filippova, 1977; Estrada-Peña et al., 2018), a Taqman-based duplex qPCR assay targeting the *ITS2* gene of *I. ricinus* and *I. persulcatus* (Sormunen et al., 2016), or both methods.

Total DNA and RNA were extracted from ticks using NucleoSpin® RNA kits and RNA/DNA buffer sets (Macherey-Nagel, Germany), following the kit protocols (NucleoSpin 96 RNA Core Kit: Rev. 05/April 2014 and RNA/DNA buffer set: Rev. 09/April 2017). DNA extracts were stored at −20 °C to await later analyses. DNA samples were screened for *F. tularensis* using a TaqMan-based qPCR assay targeting the *23* KDa gene (Skottman et al., 2007). All samples were run in duplicates. Positive and negative controls (MBC110; Vircell, Granada, Spain and ddH2O, respectively) were included in all runs. Assays were carried out in 5 μl reaction volume, including 2,5 μl SensiFAST Probe Lo-ROX Kit, 300 nM forward and reverse primers, 150 nM probe and 1 μl DNA template.

3. Results

Previously reported tick screening data from Finland includes a total of 3 866 *I. ricinus* and 1 710 *I. persulcatus*, consisting of both crowd-sourced and field-collected samples (Laaksonen et al., 2018; Pakanen et al., 2020; Sormunen et al., 2020a, 2020b) (Fig. 1). These are comprised of 1 939 adults, 1 924 nymphs and 3 larvae for *I. ricinus*, and 1 633 adults, 67 nymphs and 10 larvae for *I. persulcatus*. In the current study, we screened a further 14 878 ticks collected by crowdsourcing (Laaksonen et al., 2017) or cloth dragging between 2015 and 2020. These samples consisted of 12 562 *I. ricinus* (3 184 adults, 4 844 nymphs and 4 534 larvae) and 2 408 *I. persulcatus* (2 347 adults, 60 nymphs and 1 larva). Consequently, the total number of ticks analyzed for the presence of *F. tularensis* is currently 20 546. No positive ticks were found in either the previous or the current screenings.

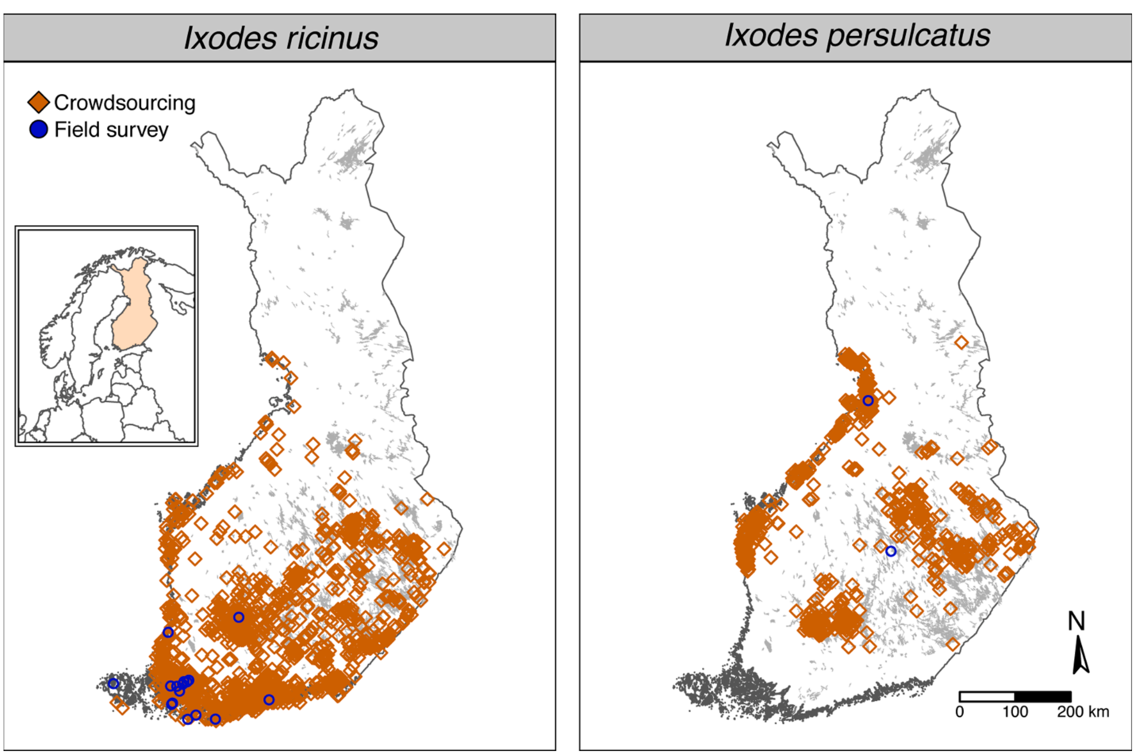

Fig. 1. Sources of study samples for *I. ricinus* and *I. persulcatus*. For *I. ricinus*, samples include 4974 crowdsourced ticks (from 1569 locations) and 11 454 field collected ticks (from 16 locations). For *I. persulcatus*, samples include 3562 crowdsourced ticks (781 locations) and 556 field collected ticks (2 locations).

4. Discussion

Despite the large volume of ticks from Finland screened for the presence of *F. tularensis*, no positive samples have been detected. As such, it appears that the bacterium is not circulating in local *I. ricinus* or *I. persulcatus* populations. In addition to these two species, the bacterium has also been reported from *Dermacentor* ticks in Europe (Gehringer et al., 2013). However, no *Dermacentor* species inhabit Finland, and *I. ricinus* and *I. persulcatus* are indeed the only tick species present that commonly bite humans. The prevalence rates reported for the bacterium in ticks in Europe have varied between 1 and 10% (Milutinović et al., 2008; Gehringer et al., 2013; Tomaso et al., 2018). Consequently, the occurrence of the pathogen at even the lowest reported prevalence rates should have resulted in over a hundred detections in the current study. This lack of *F. tularensis* positive ticks is particularly noteworthy due to the large size and wide geographical coverage of the data set (Fig. 1) - as well as the fact that Finland is considered a high-incidence region for tularaemia (Sissonen et al., 2015).

While arthropods such as ticks, tabanid flies and mosquitoes (although currently demonstrated only in Sweden and Finland) serve as vectors for *F. tularensis*, its main natural reservoirs are likely either mammals, particularly rodents and lagomorphs (for the terrestrial cycle dominant in Central and Western Europe), or a hitherto undefined aquatic reservoir (for the aquatic life cycle dominant in Northern Europe) (Maurin and Gyuranecz, 2016; Hennebique et al., 2019). Considering the complete lack of *F. tularensis* from tick samples in Finland, it may be that either a) local ticks do not come into contact with *F. tularensis* or b) ticks are unable to vector local strains of *F. tularensis*. The aquatic life cycle of *F. tularensis* subsp. *holarctica* seems to be dominant in Finland, with most infections linked to mosquito bites (Jounio et al., 2010). There exists a possibility that ticks may be excluded from the aquatic life cycle, but such exclusion would require a strict adherence to reservoirs other than vertebrates with which ticks have frequent contact. From an aquatic reservoir, *F. tularensis* could infect mosquito larvae (Hennebique et al., 2019), whereas ticks generally avoid liquid water (Krober and Guerin, 1999). From mosquito larvae, the bacterium can then be transstadially transmitted to adult mosquitoes (Lundström et al., 2011). However, such strict adherence to aquatic and/or mosquito reservoirs seems unlikely. For example, lagomorphs have previously been implicated as reservoirs in the aquatic life cycle of *F. tularensis* (Maurin and Gyuranecz, 2016). Two species of lagomorphs commonly occur in Finland, the European hare (*Lepus europaeus*) and the varying hare (*Lepus timidus*). Both of these are considered important hosts for *I. ricinus* and *I. persulcatus*, seemingly even able to upkeep tick populations on their own in the absence of other hosts (Jaenson and Tälleklint, 1996). The Finnish Food Authority reports detecting *F. tularensis* nearly annually (two years of no detections) between 2000 and 2018 in bacteriological and histological studies of wild hares from Finland, with reported prevalence rates reaching higher than 20% during three years in the period (2007, 2009 and 2016) (Finnish Food Authority, 2018). Consequently, local ticks should have plenty of chances to contract the pathogen from hares. In addition, the detection of high copy numbers of *F. tularensis* DNA from Finnish field voles (*Microtus agrestis*), a species recognized as a host for *I. ricinus*, makes the existence of such clear adherence even more unlikely (Rossow et al., 2014).

A more plausible alternative would be that the strain(s) of *F. tularensis* present in Finland - or perhaps those involved in the aquatic life cycle in general - cannot be effectively circulated by ticks, due to the bacterium being adapted to the specific vectors and reservoirs involved in the aquatic life cycle. As contact between ticks and infected animals should occur relatively commonly as discussed above, the mechanism behind the exclusion of ticks could be related to, for example, the inability of ticks to transstadially transmit the bacterium after feeding on an infected source. Clearly, both experimental studies regarding the ability of *I. ricinus* to serve as vectors for strains of *F. tularensis* involved in the aquatic life cycle common in Northern Europe as well as more thorough assessment of potential reservoirs in the Finnish wildlife are required, in order to determine the cause for the observed phenomenon.

CRediT authorship contribution statement

Jani J. Sormunen: Conceptualization, Data curation, Funding acquisition, Investigation, Supervision, Project administration, Writing – original draft. **Veli-Matti Pakanen:** Investigation, Resources, Writing – review & editing. **Riikka Elo:** Investigation, Resources, Writing – review & editing. **Satu Mäkelä:** Methodology, Validation, Investigation, Data curation. **Jukka Hytönen:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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References

Estrada-Peña, A., Mihalca, A.D., Petney, T.N., 2018. Ticks of Europe and North Africa: a Guide to Species Identification. Springer.

Filippova, N.A., 1977. Ixodid ticks of the subfamily Ixodinae - Fauna USSR. Arachnida 4, 272–283.

Finnish Food Authority, 2018. *Francisella Tularensis* in Finnish Wild Hares. https://www.ruokavirasto.fi/globalassets/teemat/zoonoosikeskus/zoonoosit/bakteerien-aiheuttamat-taudit/zoo_janisrutto_elaimet.pdf.

Gehringer, H., Schacht, E., Maylaender, N., Zeman, E., Kaysser, P., Oehme, R., Pluta, S., Splettstoesser, W.D., 2013. Presence of an emerging subclone of *Francisella tularensis holarctica* in *Ixodes ricinus* ticks from south-western Germany. Ticks. Tick. Borne. Dis. 4, 93–100.

Hennebique, A., Boisset, S., Maurin, M., 2019. Tularemia as a waterborne disease: a review. Emerg. Microbes. Infect. 8, 1027–1042.

Hennebique, A., Peyroux, J., Brunet, C., Martin, A., Henry, T., Knezevic, M., Santic, M., Boisset, S., Maurin, M., 2021. Amoebae can promote the survival of *Francisella* species in the aquatic environment. Emerg. Microbes. Infect. 1–36.

Jaenson, T.G.T., Tälleklint, L., 1996. Lyme borreliosis spirochetes in *Ixodes ricinus* (Acari: Ixodidae) and the varying hare on isolated islands in the Baltic Sea. J. Med. Entomol. 33, 339–343.

Jounio, U., Renko, M., Uhari, M., 2010. An outbreak of holarctica-type tularemia in pediatric patients. Ped. Infect. Dis. J. 29, 160–162.

Krober, T., Guerin, P.M., 1999. Ixodid ticks avoid contact with liquid water. J. Exp. Biol. 202, 1877–1883.

Laaksonen, M., Klemola, T., Feuth, E., Sormunen, J.J., Puisto, A., Mäkelä, S., Penttinen, R., Ruohomäki, K., Hänninen, J., Sääksjärvi, I.E., Vuorinen, I., Sprong, H., Hytönen, J., Vesterinen, E.J., 2018. Tick-borne pathogens in Finland: comparison of *Ixodes ricinus* and *I. persulcatus* in sympatric and parapatric areas. Parasit. Vectors. 11, 556.

Laaksonen, M., Sajanti, E., Sormunen, J.J., Penttinen, R., Hänninen, J., Ruohomäki, K., Sääksjärvi, I.E., Vesterinen, E.J., Vuorinen, I., Hytönen, J., Klemola, T., 2017. Crowdsourcing-based nationwide tick collection reveals the distribution of *Ixodes ricinus* and *I. persulcatus* and associated pathogens in Finland. Emerg. Microbes. Infect. 6, e31.

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. Emerg. Infect. Dis. 17, 794.

Maurin, M., Gyuranecz, M., 2016. Tularaemia: clinical aspects in Europe. Lancet. Infect. Dis. 16, 113–124.

Milutinovic, M., Masuzawa, T., Tomanovic, S., Radulovic, Ž., Fukui, T., Okamoto, Y., 2008. *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Francisella tularensis* and their co-infections in host-seeking *Ixodes ricinus* ticks collected in Serbia. Exp. Appl. Acarol. 45, 171–183.

Pakanen, V.M., Sormunen, J.J., Sippola, E., Blomqvist, D., Kallio, E.R., 2020. Questing abundance of adult taiga ticks *Ixodes persulcatus* and their *Borrelia* prevalence at the north-western part of their distribution. Parasit. Vectors. 13, 1–9.

Petersen, J.M., Mead, P.S., Schriefer, M.E., 2009. *Francisella tularensis*: an arthropod-borne pathogen. Vet. Res. 40, 1.

Rossow, H., Sissonen, S., Koskela, K.A., Kinnunen, P.M., Hemmilä, H., Niemimaa, J., Huitu, O., Kuusi, M., Vapalahti, O., Henttonen, H., 2014. Detection of Francisella tularensis in voles in Finland. Vector. Borne. Zoonotic. Dis. 14, 193–198.

Sajanti, E., Virtanen, M., Helve, O., Kuusi, M., Lyytikäinen, O., Hytönen, J., Sane, J., 2017. Lyme Borreliosis in Finland, 1995–2014. Emerg. Infect. Dis. 23, 1282–1288.

Sissonen, S., Rossow, H., Karlsson, E., Hemmilä, H., Henttonen, H., Isomursu, M., Kinnunen, P.M., Pelkola, K., Pelkonen, S., Tarkka, E., 2015. Phylogeography of *Francisella tularensis* subspecies *holarctica* in Finland, 1993–2011. Infect. Dis. 47, 701–706.
Sjöstedt, A., 2007. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. Ann. N. Y. Acad. Sci. 1105, 1–29.
Sormunen, J.J., Penttinen, R., Klemola, T., Hänninen, J., Vuorinen, I., Laaksonen, M., Sääksjärvi, I.E., Ruohomäki, K., Vesterinen, E.J., 2016. Tick-borne bacterial pathogens in southwestern Finland. Parasit. Vectors. 9, 1–10.
Sormunen, J.J., Andersson, T., Aspi, J., Bäck, J., Cederberg, T., Haavisto, N., Halonen, H., Hänninen, J., Inkinen, J., Kulha, N., Laaksonen, M., Loehr, J., Mäkelä, S., Mäkinen, K., Norkko, J., Paavola, R., Pajala, P., Petäjä, T., Puisto, A., Sippola, E., Snickars, M., Sundell, J., Tanski, N., Uotila, A., Vesilahti, E.M., Vesterinen, E.J., Vuorenmaa, S., Ylönen, H., Ylönen, J., Klemola, T., 2020a. Monitoring of ticks and tick-borne pathogens through a nationwide research station network in Finland. Ticks. Tick. Borne. Dis. 11, 101449.
Sormunen, J.J., Kulha, N., Klemola, T., Mäkelä, S., Vesilahti, E.M., Vesterinen, E.J., 2020b. Enhanced threat of tick-borne infections within cities? Assessing public health risks due to ticks in urban green spaces in Helsinki, Finland. Zoonoses. Public. Health. 67, 823–839.
Tomaso, H., Otto, P., Peters, M., Süss, J., Kargler, A., Schamoni, H., Zuchantke, E., Hotzel, H., 2018. *Francisella tularensis* and other bacteria in hares and ticks in North Rhine-Westphalia (Germany). Ticks. Tick. Borne. Dis. 9, 325–329.