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QUESTING TICKS, HIDDEN CAUSES

Tracking changes in *Ixodes ricinus* populations and associated pathogens in southwestern Finland

Jani J. Sormunen

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"One thing is for certain: there is no stopping them; the ~~ants~~ ticks will soon be here.
And I, for one, welcome our new ~~insect~~ arthropod overlords.
I'd like to remind them that as a trusted TV personality, I could be helpful in rounding up others to toil in their underground ~~sugar caves~~ blood refineries."

- Kent Brockman, *The Simpsons* (modified)

ABSTRACT

Ticks (Acari: Ixodidae) and tick-borne diseases, especially Lyme borreliosis caused by *Borrelia burgdorferi* s.l. spirochetes and tick-borne encephalitis (TBE) caused by the TBE-virus (TBEV), are a growing problem in northern Europe and Russia. Surveys conducted in Russia, Sweden, and Norway have revealed an increase in tick abundance and a northwards shift in their distribution over the past few decades. In the southern parts of Finland, *Ixodes ricinus* is the primary vector for *Borrelia*, TBEV, and several other tick-borne pathogens (TBP). Despite their central role in the zoonotic transmission of severe diseases, ecological data of *I. ricinus* populations in Finland are almost non-existent. Less than 20 research papers focusing on *I. ricinus* have been published from Finland between 1961 and 2017, with only a few of them touching upon any ecological aspects of the ticks themselves. As such, no scientific data regarding changes in tick and TBP occurrence has been available prior to recent years. However, citizen science surveys mapping the distribution of *I. ricinus* in 2014 and 2015, as well as the numbers of Lyme borreliosis cases that have been increasing since the mid-90's, suggest that changes similar to those observed in neighboring countries are likewise taking place in Finland.

In this thesis, I have focused on several of the neglected aspects of tick-related research in Finland, in an effort to determine the current status of tick populations in southwestern Finland and whether changes similar to those observed elsewhere in the northern parts of Europe can be observed there. Among others, study themes included changes in the abundance and distribution of *I. ricinus*, changes in the prevalence rates and occurrence of several TBPs, as well as long-term monitoring of spatial changes in the diversity and occurrence of TBPs.

I found that *I. ricinus* numbers in southwestern Finland have increased over the past few decades in all study sites with reference data available, with the highest densities being observed in 2017, the last year of studies for this thesis. Ticks were commonly found from all coastal areas in southwestern Finland, with particularly high densities being observed on islands in the Archipelago Sea. Similarly, in 2017 ticks were detected from several urban and suburban study sites in the city of Turku, some of which were surveyed but found lacking ticks in 2013. Regarding pathogens, higher prevalence of *B. burgdorferi* s.l. was observed than in reference studies from 2000, and its geographical range had also expanded. Disconcertingly, particularly high *Borrelia* prevalence rates were observed in tick populations in urbanized areas around Turku. Altogether seven different pathogen groups were detected from southwestern Finland, including several species that had not been reported from Finland before. Furthermore, the probable emergence of two pathogens was observed on Seili Island during the study, highlighting that ticks are not proliferating alone: tick-borne pathogens are as well.

To conclude, the results of this thesis confirm that tick densities are increasing, tick-borne pathogens are becoming more common, and both ticks and TBPs are spreading in southwestern Finland. Particularly alarming is the detection of high numbers of ticks and TBPs in the sphere of influence for hundreds of thousands of people annually, in urban and suburban areas within and around Turku. Vigilance is required from both citizens and medical professionals, in order to prevent and detect harmful tick-borne infections. Action should be taken to inform citizens of noticeable tick risk also in urbanized areas. Unfortunately, no decrease in the number of ticks is likely to occur in the southern parts of Finland in the foreseeable future, so further increasing tick awareness is the best course of action for minimizing their negative impact.

TIIVISTELMÄ

Puutiaiset (Acari: Ixodidae) ja niiden levittämät taudit, erityisesti *Borrelia* -bakteerien aiheuttama borrelioosi ja puutiaisaivokuumeviruksen aiheuttama puutiaisaivotulehdus, ovat kasvava ongelma pohjoisessa Euroopassa ja Venäjällä. Ruotsissa, Norjassa ja Venäjällä tehdyt tutkimukset ovat osoittaneet, että puutiaisten määrät ovat lisääntyneet ja niiden levinneisyysalue siirtynyt pohjoisemmaksi viime vuosikymmenten aikana. Eteläisessä Suomessa puutiainen (*Ixodes ricinus*) on puutiaisvälitteisten taudinaiheuttajien pääasiallinen kantaja. Vaikka puutiaiset toimivat lukuisten vakavien tautien levittäjinä, ei niiden ekologiaa ole Suomessa aiemmin juuri tutkittu: vuosien 1961–2017 välillä Suomesta on julkaistu alle kaksikymmentä puutiaista käsittelevää tieteellistä tutkimusta. Näistä valtaosa keskittyy niiden kantamien taudinaiheuttajien esiintymiseen, ja vain muutama käsittelee millään tapaa itse puutiaisen ekologiaa. Näin ollen tieteellistä näyttöä puutiaisten ja niiden kantamien taudinaiheuttajien määrien muutoksista ei ole ollut saatavilla. Vuosina 2014 ja 2015 toteutettujen, puutiaisten levinneisyyttä kartoittaneiden kansalaiskeräysten tulokset sekä vuosittain raportoitujen borrelioositapausten lisääntyminen 90-luvun puolivälistä saakka viittaavat kuitenkin siihen, että naapurimaissa havaitut muutokset koskevat myös Suomea.

Väitöskirjatyössäni keskityin selvittämään Lounais-Suomen puutiaispopulaation nykyistä tilaa, sekä arvioimaan, onko puutiais- ja taudinaiheuttajapopulaatioissa havaittavissa samansuuntaisia muutoksia kuin muissa Pohjoismaissa ja Venäjällä. Tutkittuihin teemoihin lukeutuivat mm. muutokset puutiaisten määrissä ja esiintymisessä, muutokset puutiaisten kantamien taudinaiheuttajien määrissä ja esiintymisessä, sekä pitkän aikavälin muutokset taudinaiheuttajien paikallisessa esiintymisessä.

Tutkimuksissani havaitsin, että puutiaismäärät ovat viime vuosikymmenten aikana kasvaneet kaikilla niillä tutkimusalueilla, joilta vertailuaineistoa on olemassa. Alueilla, joilla tutkimuksia tehtiin useamman vuoden ajan, puutiaismäärät olivat korkeimmillaan viimeisimpänä tutkimusvuotena, 2017. Löysin puutiaisia kaikilta saarilla ja merenrannan tuntumassa sijainneilta tutkimusalueilta, ja korkeimmat puutiaismäärät havaitsin Saaristomeren saarilta. Puutiaisia havaittiin runsaasti myös Turun kaupunki- ja esikaupunkialueilta. Löysin *Borrelia* -bakteereita kantavia puutiaisia niin ikään yleisemmin ja useammalta eri paikalta kuin 2000-luvun alkuun sijoittuvissa vertailututkimuksissa. Hieman huolestuttavasti näitä bakteereita tavattiin erityisen yleisinä Turun kaupunkialueen puutiaisilta. Kaiken kaikkiaan tutkimuksissa löydettiin seitsemää eri taudinaiheuttajaa, joista osa oli Suomelle uusia lajeja. Seilin saaren seurantatutkimuksissa havaitsin lisäksi kahden taudinaiheuttajan ilmaantumisen saarelle tutkimukseni kuluessa, mikä osaltaan tukee havaintoa, että taudinaiheuttajien esiintymisessä Suomessa on tapahtumassa samanlaisia muutoksia kuin naapurimaissa.

Väitöskirjani tulokset osoittavat, että puutiaisten ja niiden välittämien taudinaiheuttajien määrät sekä levinneisyysalueet ovat kasvaneet Lounais-Suomessa 2000-luvulla. Erityisen huolestuttavia ovat havainnot korkeista puutiais- ja taudinaiheuttajamääristä myös Turun kaupunkialueella, jossa asuu ja vierailee satoja tuhansia ihmisiä vuosittain. Tämän väitöskirjan tulokset korostavat, että tietoisuutta puutiaisista ja taudinaiheuttajista tulisi yhä lisätä kansalaisten ja terveydenhuollon ammattilaisten parissa – erityisesti kaupunkialueilla, joissa ihmiset eivät välttämättä ajattele olevansa alttiina tartunnoille. Puutiaisten määrät ovat tuskin vähenemässä lähitulevaisuudessa, joten puutiaistietoisuuden lisääminen tulee jatkossakin olemaan tärkeässä asemassa niiden aiheuttaman haitan minimoimisessa.

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LIST OF ORIGINAL PUBLICATIONS

This thesis consists of the following publications and manuscripts, which are referred to in the text by their Roman numerals:

I Sormunen JJ, Klemola T, Vesterinen EJ, Vuorinen I, Hytönen J, Hänninen J, Ruohomäki K, Sääksjärvi IE, Tonteri E & Penttinen R: Assessing the abundance, seasonal questing activity, and *Borrelia* and tick-borne encephalitis virus (TBEV) prevalence of *Ixodes ricinus* ticks in a Lyme borreliosis endemic area in Southwest Finland. *Ticks and Tick-borne Diseases*. 2016, 7:208-215.

II Sormunen JJ, Penttinen R, Klemola T, Hänninen J, Vuorinen I, Laaksonen M, Sääksjärvi IE, Ruohomäki K & Vesterinen EJ: Tick-borne bacterial pathogens in southwestern Finland. *Parasites & Vectors*. 2016, 9:168

III Sormunen JJ, Klemola T, Hänninen J, Mäkelä S, Vuorinen I, Penttinen R, Sääksjärvi IE & Vesterinen EJ: The importance of study duration and spatial scale in pathogen detection – Evidence from a tick-infested island. *Emerging Microbes & Infections*. In press.

IV Klemola T, Sormunen JJ, Mojzer J, Mäkelä S & Vesterinen EJ: Increased tick abundance and diversity of prevalent tick-borne pathogens in a Finnish city. *Manuscript*.

Contributions to the original articles.

	I	II	III	IV
Original idea	JJS, TK, EJV, IV, JHä, IES, RP	JJS, TK, EJV, IV JHä, IES, RP	JJS, TK, EJV, IV, JHä, IES, RP	JJS, TK, EJV
Field work	JJS	JJS	JJS	JM
Laboratory work	JJS, EJV	JJS, EJV	JJS, EJV, SM	JM, EJV, JJS
Data analysis	JJS, TK	JJS, EJV	JJS, TK, EJV	TK, SM, JM, JJS
Writing	JJS, TK, EJV, IV, JHy, JHä, KR, IES, ET, RP	JJS, RP, TK, JHä, IV, IES, KR, EJV, ML	JJS, TK, JHä, SM, IES, EJV	TK, JJS, JM, SM, EJV

1. INTRODUCTION

Zoonoses are infectious diseases that can be naturally transmitted between animals and humans. A recently published global survey of 335 emerging infectious disease events indicated that over 60% of emerging diseases are zoonoses and that the amount of zoonoses is increasing over time (Jones *et al.* 2008). Many of these zoonotic diseases are caused by pathogens vectored by arthropods. Among the most well-known arthropod-transmitted diseases are malaria and dengue fever, both vectored by mosquitos, affecting millions of humans and causing hundreds of thousands of deaths annually (Murray *et al.* 2012; Bhatt *et al.* 2013). In addition to mosquitos, also another group of arthropods, ticks (Acari: Ixodida), are considered particularly important vectors for both human and companion animal infections (Jongejan & Uilenberg 2004). For example, approximately 30,000 new Lyme borreliosis cases, caused by *Borrelia burgdorferi* s.l. spirochetes vectored by hard ticks, are reported annually from the United States and 85,000 from Europe, though these numbers are estimated to be rather coarse underestimations (Lindgren & Jaenson 2006; Smith *et al.* 2006; Sajanti *et al.* 2017).

In northern Europe and western Russia, Lyme borreliosis and tick-borne encephalitis (TBE) caused by the tick-borne encephalitis virus (TBEV) are among the most common and severe zoonotic infections frequently affecting humans (Gritsun *et al.* 2003; Hubálek 2009; Rizzoli *et al.* 2011; Pettersson *et al.* 2014). However, in addition to the agents of these two severe diseases, humans bitten by ticks in the northern parts of Europe may also be infected with several other bacterial and/or protozoan pathogens, which may present themselves through various clinical manifestations. Indeed, the diversity of pathogens transmitted by ticks is greater than that of any other group of blood-feeding arthropods, including mosquitos (Pfäffle *et al.* 2013). Furthermore, an individual tick may carry several different pathogens, potentially infecting bitten humans with two or more pathogens simultaneously. Such co-infections with different pathogens may result in unpredictable and more severe disease cases (Krause *et al.* 1996; Swanson *et al.* 2006).

1.1. Hard ticks (Acari: Ixodidae)

Ticks (Acari: Ixodida) are an order of parasitic, blood-feeding arthropods of the class Arachnida, closely related to mites, and more distantly to spiders and scorpions. The order Ixodida is divided into three families: hard ticks (Ixodidae), soft ticks (Argasidae), and the family Nuttalliellidae, comprised of a single species. Hard ticks are differentiated from soft ticks by the hard scutum (or shield) on the upper surface of the body (Figure 1), as well as their prominent mouthparts (hypostome and palps), which can clearly be seen from above and below (Figure 2) (Hillyard 1996). Hard ticks are the largest family in the order, comprising of approximately 700 species over 14 genera, with *Ixodes* forming the largest genus with roughly 250 species (Guglielmone *et al.* 2010).

Figure 1. Microscope photograph of an adult *Ixodes ricinus* female. The scutum can be seen as the darker area covering the anterior parts of the upper surface of the body (marked by yellow arrow). Photo by Maija Laaksonen.

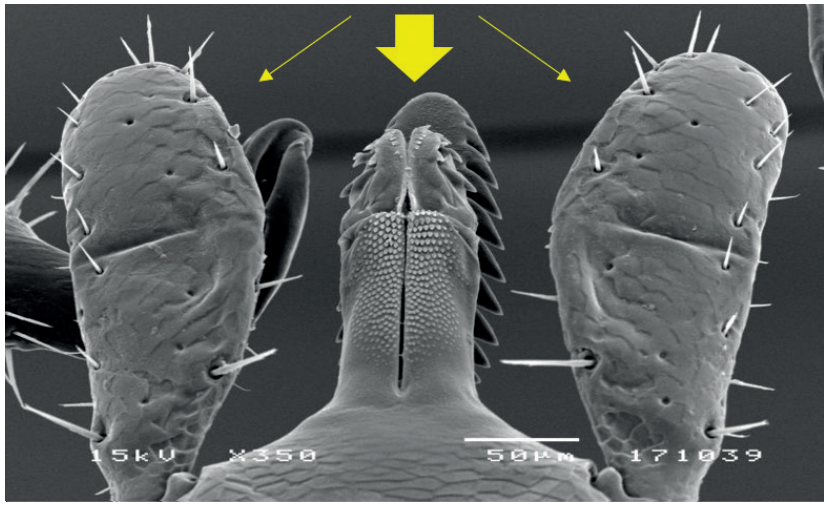

Figure 2. A scanning electron microscope photograph showing the hypostome (middle arrow) and palps (side arrows) of an adult *Ixodes ricinus*. Photo by Ritva Penttinen.

Six species of *Ixodes* ticks likely endemic to Finland have been identified: *I. ricinus*, *I. persulcatus*, *I. trianguliceps*, *I. lividus*, *I. arboricola,* and *I. uriae* (Saikku *et al.* 1971; Ulmanen 1972; Ulmanen *et al.* 1977; Laaksonen *et al.* 2017). The latter four of these are endophilic tick species inhabiting – and thus usually only encountered in – animal nests, burrows, tree holes etc., where they mostly feed on animals

living in or visiting the respective space (Hillyard 1996). *Ixodes ricinus* and *I. persulcatus* on the other hand are exophilic species, actively seeking blood meal hosts when weather conditions are suitable, by climbing on and clinging to plant stems and leafs (or on top of litter, in case no suitable plants are available) to await passing animals in an activity known as questing (Hillyard 1996). Unlike their endophilic counterparts, which are often specialists focusing on one or a few host species, these exophilic species are opportunistic generalists, readily attaching to and feeding on nearly any terrestrial vertebrate that signals its suitability to act as a host by expressing certain odors, emitting body heat and gasses such as CO_2, and/or causing physical disturbance of the environment (Anderson 1989; Estrada-Peña *et al.* 2018). Due to this non-specificity, *I. ricinus* and *I. persulcatus* readily attach to and bite humans, potentially transmitting various pathogenic agents in the process. While both *I. ricinus* and *I. persulcatus* are present in Finland, only *I. ricinus* has been found from southwestern Finland, where the studies of this thesis are focused (Mäkinen *et al.* 2003; Wilhelmsson *et al.* 2013; Sormunen *et al.* 2016; Laaksonen *et al.* 2017) (**I-II**).

1.2. Study species – the sheep tick, *Ixodes ricinus* (L.)

Ixodes ricinus ticks are relatively small animals, with larvae typically <1 mm, nymphs around 1-2 mm, and adults 2-4 mm in length, prior to engorgement (Figure 3). Nymphs and adults have four pairs of legs, as typical for arachnids. However, larvae only have three pairs, and only develop the fourth pair when they molt to nymphs. The scutum covers the upper frontal parts of the ticks in all life stages apart from adult males, for which it covers the entire upper body. Whereas year-round activity is possible for *I. ricinus* in, for example, southern Italy (Dantas-Torres & Otranto 2013), in northern Europe ticks overwinter during the coldest months of the year. Following the winter, tick activity resumes again in the early spring, when daily temperatures exceed ~7°C (Gray *et al.* 2009; Sonenshine & Roe 2014). However, these activation temperatures also depend on tick life stage and size. For example, in the United Kingdom, the threshold for larval activity has been measured as ~10°C (Randolph 2004b). In the southern parts of Finland, the first reports of tick activity from citizens have often been received in mid-March and the last reports in October-November in recent years, resulting in activity periods of 7-9 months in these regions.

The complete life cycle of *I. ricinus* typically takes from two to six years in the northern parts of Europe, and involves three active life stages in addition to the sessile egg stage: larvae, nymph, and adult (Hillyard 1996; Sonenshine & Roe 2014) (Figure 3). Each of these active life stages needs to seek a host, attach to it and feed on its blood, and finally drop off from the host to digest the meal (Sonenshine & Roe 2014). The three hosts required to complete the life cycle of *I. ricinus* can be of varying species composition, and, unlike for some endophilic tick species, are indeed typically not the same species. *Ixodes ricinus* is a true generalist, feeding (or attempting to feed) on practically any warm-blooded terrestrial vertebrate they come into contact with (Anderson 1989; Estrada-Peña & de la Fuente 2016).

Once on the host, ticks may wander around for several hours in search of a good attachment spot (Estrada-Peña *et al.* 2018). All life stages feed only once, and in the process engorge themselves with the blood of their host, increasing their body size and mass by multiples. Feeding is usually a long process for all developmental stages, lasting approximately 2-6 days for larvae, 3-8 days for nymphs, and 6-12 days for adult females (Estrada-Peña *et al.* 2018). After feeding, ticks detach and drop off the host to molt to the next life stage, or to lay a large quantity of eggs in one large batch (adult females) (Hillyard 1996). Adult females die after oviposition; adult males wander around copulating until they have spent all their energy reserves. While the non-discriminating feeding habits of *I. ricinus* are the cause for Europe-wide medical concern, the time they spend on hosts is actually quite limited. In fact, at least 90% of their lifetime is spent off host (Hillyard 1996; Sonenshine & Roe 2014). When not searching for a host (due to the tick having already fed, undergoing development, experiencing suboptimal weather conditions for questing, rehydrating, or overwintering), *I. ricinus* are typically found under the cover of litter or moss in the ground layer.

Figure 3. Life stages of *Ixodes ricinus*, from left to right: larvae, nymph, and adult (male and female). Sizes are approximate. Photos by Maija Laaksonen.

Distribution in nature

The distribution of *I. ricinus* in the environment is somewhat dependent on tick life stage. The juveline life stages, particularly larvae, are often found in aggregated clusters in nature (Nilsson & Lundqvist 1978). This is largely a consequence of adult females ovipositioning their eggs in a single large batch and the limited horizontal mobility expressed by *I. ricinus* in general (Milne 1950; Healy & Bourke 2008). As such, larvae hatch in a cluster within leaf litter, moss, or the top layers of soil, and seldom venture far from their place of birth, resulting in questing larvae often being discovered in aggregates.

The different life stages of *I. ricinus* tend to quest at different heights in vegetation, where this is possible (i.e. vegetation is present). Larvae man the lowest parts of plants and the ground, nymphs ascend to somewhat higher positions, and adults ascend to the highest parts of plants to reach the larger host animals they prefer (Mejlon & Jaenson 1997; Sonenshine & Roe 2014). This behavior has an effect on the host animal species typically encountered and parasitized by different life stages, although other factors, such as the thickness of skin in larger animals, may also limit the ability of larvae to utilize them.

The most commonly reported host animals of *I. ricinus* larvae are voles, mice, shrews, and certain ground-feeding birds – species that themselves mostly inhabit the field and ground layers, or forage there for food (Mejlon & Jaenson 1997). Following the blood meal, the engorged larva detaches from the host, drops off, and molts to the next life stage, the nymph. If the larvae happened to feed on a vole, mouse, or shrew, chances are that the drop-off point for the tick is somewhat proximate to the initial point of attachment, as small rodents and shrews typically only travel limited distances (Viitala & Hoffmeyer ; Norrdahl & Korpimäki 1995). While larvae feeding on birds may disperse great distances, the utilization of small rodents and insectivores as main hosts of larvae leads to nymphs also being found somewhat aggregated in nature, as all the larvae feeding on a specific vole, mouse, or shrew must drop off somewhere along its limited range of movement before developing to nymphs.

The distribution of *I. ricinus* is further dispersed following the nymph and adult blood meals. The most common host animals for *I. ricinus* nymphs and adults in northern Europe are mammals like hares, deer, raccoon dogs (*Nyctereutes procyonoides*), foxes (*Vulpes vulpes*) and birds like thrushes – animals with much greater movement ranges than those of small rodents and shrews. The same mechanics apply as for larvae, but the range of dispersal is far greater, resulting in adult ticks and tick egg clusters (after the blood meal of adult females) being more randomly distributed.

Besides the clustering factors related to oviposition and host animals, abiotic factors affect the occurrence of ticks in nature as well. Particularly, hard ticks are relatively sensitive to desiccation. This is a problem for *I. ricinus*, which ascend on vegetation in search of a host. When on the vegetation, the relative humidity (RH) of the environment often drops below the comfort threshold of the species (somewhere between 86–96% RH) (Lees 1946; Kahl & Alidousti 1997), resulting in a transpiratory loss of water. Furthermore, movement of the tick when questing further increases water loss, as water is lost also through respiratory exchange via the tracheal system (Knülle & Rudolph 1982). To combat these water loss mechanisms, *I. ricinus* must acquire water from their environment. However, ticks do not drink water, but instead extract water vapor from the atmosphere – preferably at as saturated an environment as they can find (Lees 1946; Kahl & Alidousti 1997). Therefore, high temperatures or low humidity, or a combination of both, force ticks to seek shelter and moisture replenishment from the cooler and more humid microhabitats under vegetation, ground floor litter, and/or moss (Sonenshine & Roe 2014). As such, suitable shelters offered by ground and field layers are a requirement for the continued survival of any *I. ricinus* population. In addition, ticks also require such shelters for development, as well as overwintering in areas where year-round activity is not possible. A lack of suitable shelters renders certain biotopes such as rocky hills and mowed lawns unsuitable for tick inhabitation. In general, also

other dry areas subject to direct sunlight and drying winds are suboptimal for *I. ricinus*, but even in such areas, safe havens suitable for tick inhabitation may – and often do – exist.

1.3. Source of infamy – Tick-borne pathogens (TBPs)

Ticks have acquired worldwide infamy as the vectors for numerous pathogens, some of which have the potential to cause severe diseases in humans and companion animals. For most TBPs reported from Europe, enzootic cycles involve warm-blooded reservoir hosts that serve as amplification platforms, while *I. ricinus* only serve as vectors transmitting the pathogen from host to host (ideally from reservoir host to reservoir host) (Dantas-Torres *et al.* 2012). Different pathogen species (may) have different reservoir hosts. For example, for *B. burgdorferi* s.l., several small rodents (mice, voles, and squirrels), shrews, hares, passerine birds, and some larger mammals such as raccoon dogs (*N. procyonoides*) have been reported as reservoir hosts in Europe (Lane *et al.* 1991; Tälleklint & Jaenson 1994; Pisanu *et al.* 2014; Wodecka *et al.* 2016). Elks (*Alces alces*), roe deer (*Capreolus capreolus*), and white-tailed deer (*Odocoileus virginianus*), while having an important role in the upkeep and growth of tick populations, do not appear to have reservoir competence for *B. burgdorferi* s.l. (Telford *et al.* 1988; Tälleklint & Jaenson 1994; Rosen *et al.* 2014). On the other hand, some species, such as humans and some of our companion animals, have no role in either tick or TBP population upkeep, and are therefore considered incidental dead-end hosts (Kurtenbach *et al.* 2006). Contacts between ticks and humans seldom serve the interests of either side – humans may get infected with TBPs through tick bites, whereas ticks attached to humans seldom make it back to the nature to continue their life cycles.

Ticks themselves do not usually hatch from eggs already infected, but rather acquire the various bacteria, protozoa, and viruses from the respective reservoir hosts of each pathogen species during blood meals. Consequently, higher pathogen prevalence rates are often observed for adults, who have already fed twice previously, than for nymphs, who have only fed once (see e.g. Strnad *et al.* 2017). Although the mechanisms and particulars of pathogen transmission may differ between pathogen species to some degree, the most common and typical cycle is depicted in Figure 4.

While horizontal transmission between ticks and host animals is the most common transmission route, vertical transmission (from mother to offspring) has also been shown for some TBPs, although never as the sole transmission route. The most notable examples of these are *B. miyamotoi* and some *Rickettsia* and *Babesia* species, for which transovarial transmission may have a significant part in population upkeep (Burgdorfer & Brinton 1975; Socolovschi *et al.* 2009; Gray *et al.* 2010; Wagemakers *et al.* 2015). *Borrelia burgdorferi* s.l. and TBEV seem unable to transfer through this route, and the rare cases where Lyme borreliosis spirochetes have been detected from unfed tick larvae are expected to have been caused by other means, for example through interrupted feeding on an infected host, forcing the larva back to questing after acquiring the pathogen (Richter *et al.* 2012; Wagemakers *et al.* 2015).

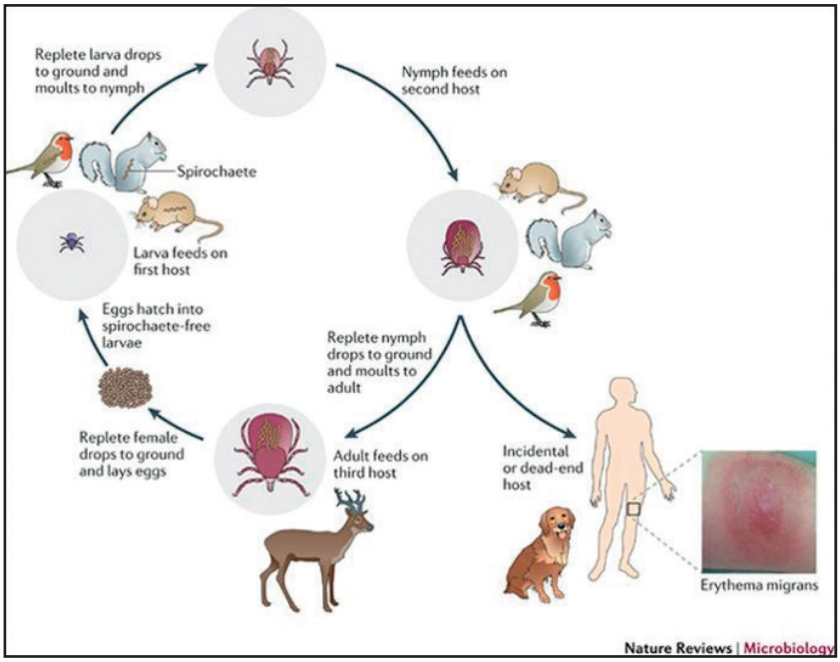

Figure 4. The enzootic cycle of *Borrelia burgdorferi* s.l. spirochetes. Ticks usually acquire the bacteria from reservoir animals when feeding as larvae or nymphs. Despite adults carrying the bacteria, they are usually not transmitted to tick eggs, resulting in larvae hatching uninfected. Reprinted with permission from Springer Nature (Radolf *et al.* 2012)

As the enzootic transmission cycles of vector-borne pathogens are inherently ecological processes, involving the participation of a minimum of three species: a pathogen, a vector, and a host (in the case of ticks and TBPs usually various hosts), the ecology and epidemiology of not only vectors, but also those of the pathogens themselves have been the subjects of considerable interest among researchers (Norman *et al.* 1999; Ostfeld & Keesing 2000a, b; Keesing *et al.* 2006; Hartemink *et al.* 2008; Lloyd-Smith *et al.* 2009; Keesing *et al.* 2010; Randolph & Dobson 2012; Bouchard *et al.* 2013; Pfäffle *et al.* 2015). Amidst the various topics of the subject, the concept of 'dilution effect' is probably the most discussed and debated. Briefly, the concept of dilution effect predicts that infection rates of a pathogen decrease with increasing diversity of host communities, as increasing numbers of non-competent (in regard to pathogen transmission) hosts dilute the rates of pathogen transmission between the vectors and more competent hosts (Ostfeld & Keesing 2000a, b). While some data, mostly originating from studies conducted in North America, seem to support the existence of such a diluting effect, a critical review article by Randolph & Dobson (2012) questioned many of the conclusions drawn in these studies,

concluding that dilution or amplification of a pathogen in relation to biodiversity is likely more dependent on the specific composition of the local host community than on biodiversity in itself. A recent study conducted in Belgium also claims "low probability of a dilution effect" in regard to one species of Lyme borreliosis causing *Borrelia*, following extensive field surveys over 19 different study localities (Ruyts *et al.* 2018).

The difficulties related to assessing the existence of diluting effects can be exemplified by considering *I. ricinus* as a vector of *Borrelia* and roe deer and white-tailed deer as hosts. As stated above, these two deer species seem to be non-competent hosts for *Borrelia* spirochetes (Telford *et al.* 1988; Tälleklint & Jaenson 1994; Rosen *et al.* 2014). Consequently, as the deer are unable to infect ticks feeding on them with *Borrelia,* a high density of deer should, in theory, reduce (dilute) the prevalence of the bacteria in the local tick population. However, at the same time, deer are important maintenance hosts for the tick vectors themselves, with their ready availability leading to increased tick abundance (Rizzoli *et al.* 2009; Dobson *et al.* 2011; Dobson & Randolph 2011). Some sources have estimated that the increase in vector abundance due to the increased availability of blood meal sources is more than enough to offset any decrease in prevalence due to a dilution effect (Rand *et al.* 2004; Dobson & Randolph 2011; Randolph & Dobson 2012). As such, the realized impact on the density of *Borrelia*-infected ticks resulting from either of these deer species arriving (increasing diversity) or leaving (decreasing diversity) an area is not straightforwardly discernible. Similar counteracting effects can likely also be identified regarding many other hosts used by different vectors, further increasing the difficulty of assessing dilution effects. This is especially true for *I. ricinus*, which are generalists feeding on hundreds of different host species, all with unique affinities for transmitting different pathogens and with different inter- and intraspecific interactions with each other (and their environment). Consequently, whether changes in biodiversity result in pathogen dilution or amplification is indeed likely to differ between localities, as concluded by Randolph & Dobson (2012). In the future, surveys specifically tailored to study the ecology and epidemiology of tick-borne pathogens in different areas around Europe are needed to comprehensively assess potential dilution or amplification effects, as well to identify different scenarios determining which of these takes place.

Overview of TBP's and associated diseases screened from Finnish ticks

Bacteria of the *B. burgdorferi* s.l. group are the most commonly reported and important tick-borne pathogens in Europe (Stanek *et al.* 2012). These spirochetes are the causative agents of Lyme borreliosis, a severe zoonotic disease that infects tens of thousands of people annually. In Europe, an estimated 85,000 Lyme borreliosis cases are diagnosed annually, with an increase in case numbers reported from several countries over the past few decades (Lindgren & Jaenson 2006; Smith *et al.* 2006; Sajanti *et al.* 2017). Several *B. burgdorferi* s.l. genospecies have been reported from *I. ricinus* in Europe, most of which have been shown to cause Lyme borreliosis (Rudenko *et al.* 2011) and have known reservoir hosts

(Piesman & Gern 2004). Overall, the distribution of *B. burgdorferi* s.l. seems to largely coincide with *I. ricinus* distribution, at least in northern Europe (Gustafson *et al.* 1995; Laaksonen *et al.* 2017). In Finland, the annual numbers of microbiologically confirmed cases of Lyme borreliosis (tracked by the National Infectious Diseases Register, NIDR) have been increasing since the mid-90s, from 345 reported cases in 1995 (incidence 7/100,000 population) to 1,679 in 2014 (31/100,000) (Sajanti *et al.* 2017). However, clinically diagnosed cases are not included in these numbers. Sajanti *et al.* (2017) estimated that an additional ~3000 cases of Lyme borreliosis are annually diagnosed clinically, resulting in a total estimation of 6440 cases in 2014 (118/100,000 population) (Sajanti *et al.* 2017). The highest incidence rates of borreliosis in Finland are reported from coastal areas, particularly in southern Finland. Unsurprisingly, these are also areas where ticks appear to be abundant (Laaksonen *et al.* 2017) (**II & IV**).

More recently, a species of *Borrelia* not belonging to the *B. burgdorferi* s.l. species complex has been reported from hard ticks in Europe. This species, *B. miyamotoi*, belongs to the relapsing fever group of *Borrelia*, which are usually transmitted by soft ticks (Argasidae). Following the initial detection and description of the pathogen from hard ticks (*Ixodes ovatus*) in Japan (Fukunaga *et al.* 1995), reports of *B. miyamotoi* or relapsing fever -like bacteria in *Ixodes* ticks have been published from over a dozen European countries (Geller *et al.* 2012; Wilhelmsson *et al.* 2013; Kjelland *et al.* 2015; Wagemakers *et al.* 2015; Siński *et al.* 2016; Laaksonen *et al.* 2017) (**II-IV**). Whereas Lyme borreliosis cases are relatively common and well documented, human patient cases linked to *B. miyamotoi* have only been reported recently (Chowdri *et al.* 2013; Gugliotta *et al.* 2013; Hovius *et al.* 2013; Krause *et al.* 2013), with the initial report coming from Russia in 2011 (Platonov *et al.* 2011). While the pathogen has been detected from a small percentage of *I. ricinus* and *I. persulcatus* from Finland, Sweden, and Norway, no human infections have yet been identified from the respective countries (Siński *et al.* 2016; Laaksonen *et al.* 2017).

Anaplasma phagocytophilum is the agent of an emerging tick-borne disease, human granulocytic anaplasmosis (HGA). Following the reorganization of the Anaplasmataceae family, several species from the genus *Ehrlichia* were re-described as members of *Anaplasma*, including the *Ehrlichia phagocytophilum* genogroup (now collectively named *Anaplasma phagocytophilum*), which contained the agents of human granulocytic ehrlichiosis (now human granulocytic anaplasmosis, HGA) (Dumler *et al.* 2001). The first case of HGA was reported in Wisconsin, USA, in 1990 (Chen *et al.* 1994). In Europe, confirmed cases of HGA are rare, with less than 100 confirmed patient cases reported since 1995 (Brouqui *et al.* 1995; Blanco & Oteo 2002; Edouard *et al.* 2012; Dugat *et al.* 2015). No patient cases have been reported from Finland thus far. Despite there being a relatively low number of diagnosed cases from Europe overall, seroepidemiological studies have revealed antibody prevalences of up to 28% in humans in some areas of Europe (median 6.2% from 35 published reports) (Strle 2004; Dumler *et al.* 2005). This implies that infections are more common than they appear, but apparently frequently asymptomatic or mild enough to not require medical attention. Indeed, different genetic variants of *A. phagocytophilum* circulate in different tick and host animal species in the wild, and may differ in their pathogenicity (Massung *et al.* 2003; Rymaszewska & Grenda 2008; Doudier *et al.* 2010; Portillo *et al.* 2010; Dugat *et al.* 2017).

The bacterial genus *Rickettsia* is traditionally classified into two groups: the spotted fever group (SFG) and the typhus group (Parola *et al.* 2005). While species from both groups have been found from hard ticks, particularly SFG *Rickettsia* are frequently reported from *I. ricinus* in Europe (Sprong *et al.* 2009; Lommano *et al.* 2012; Overzier *et al.* 2013b; Castro *et al.* 2015). Thus far, three *Rickettsia* species have been reported from ticks in the northern parts of Europe: *R. helvetica* from Finland (Laaksonen *et al.* 2017) (**II-IV**), Sweden (Nilsson *et al.* 1997; Nilsson *et al.* 1999; Severinsson *et al.* 2010; Wallménius *et al.* 2012), Norway (Quarsten *et al.* 2015), and Estonia (Katargina *et al.* 2015), and *R. monacensis* and *Candidatus* R. tarasevichiae from Finnish and Estonian tick populations (Katargina *et al.* 2015; Laaksonen *et al.* 2017; Laaksonen *et al.* 2018) (**II-III**). Various patient cases related to *R. helvetica* have been reported since 1999, although the clinical picture of the infection appears to still be debated among scientists (Parola *et al.* 2005; Parola *et al.* 2013). For *R. monacensis* and *Ca.* R. tarasevichiae, patient cases have been reported from Spain, Italy, and China (Jado *et al.* 2007; Madeddu *et al.* 2012; Jia *et al.* 2013). No cases of rickettsiosis have been reported from Finland to date.

Bartonella is a genus of gram-negative intracellular bacteria, several species of which are considered to have the potential for human infection (Billeter *et al.* 2008). The role of *I. ricinus* as vectors for *Bartonella* spp. has recently begun to receive increasing scientific attention (Morozova *et al.* 2004; Podsiadly *et al.* 2007; Billeter *et al.* 2008; Cotté *et al.* 2008; Dietrich *et al.* 2010; Schorn *et al.* 2011; Wormser & Pritt 2015). However, while DNA of various *Bartonella* spp. has been found from ticks, actual vector competence of ticks has only been shown for a few (Cotté *et al.* 2008; Reis *et al.* 2011). Likewise, as far as I am aware, no patient cases of human bartonellosis have been incontrovertibly linked to tick bites. As such, the status of *Bartonella* as zoonotic pathogens transmitted by ticks in northern Europe remains undetermined (Telford & Wormser 2010).

Candidatus Neoehrlichia mikurensis is a candidate status species of gram-negative cocci belonging to the family Anaplasmataceae, closely related to *A. phagocytophilum*. The bacterium was first isolated from wild rats (*Rattus norvegicus*) and *I. ovatus* in Japan in 2004 (Kawahara *et al.* 2004). The first case of human infection was reported from Sweden in 2010, in a patient with recurrent fever episodes (Welinder-Olsson *et al.* 2010). This was swiftly followed by reports of patient cases from Switzerland and Germany later that same year (Fehr *et al.* 2010; Loewenich *et al.* 2010). Since these initial case reports, more than a dozen cases of neoehrlichiosis have been published from Europe (Silaghi *et al.* 2016). *Candidatus* N. mikurensis has been detected from *I. ricinus* in several European countries, including nearby Sweden, Norway, and Estonia (Silaghi *et al.* 2016). Furthermore, it has only recently been detected from Finnish ticks for the first time (**III-IV**) (Laaksonen *et al.* 2018). Despite its presence in ticks, no patient cases have been reported from Finland.

Babesia is a genus of pathogenic protozoa causing babesiosis, best known as a disease of livestock, horses, and dogs (Kjemtrup & Conrad 2000; Bock *et al.* 2004). The first case of human babesiosis was reported from a farmer living in former Yugoslavia in 1957 (Skrabalo & Deanovic 1957). While more than 100 species of *Babesia* infect wildlife and livestock, human infections are associated with only a few species, namely *B. microti*, *B. divergens*, and *B. venatorum* (formerly *Babesia* sp. EU1), with the

latter two behind most cases reported from Europe (Herwaldt *et al.* 2003; Häselbarth *et al.* 2007; Vannier & Krause 2009; Martinot *et al.* 2011). While human babesiosis cases have mostly been linked to immunocompromised patients, more recent reports have identified immunocompetent persons also suffering from the disease (Martinot *et al.* 2011). Cases of human babesiosis are altogether quite rare in Europe, with around 50 reported cases in total (Vannier & Krause 2009). As far as I am aware, the only case of human babesiosis from Finland is a fatal infection reported in 2010 (Haapasalo *et al.* 2010). Interestingly, the patient in question suffered from a co-infection of *B. divergens* and *Borrelia* sp., likely acquired through tick bite. Indeed, reports suggest that concurrent infections with both these pathogens may cause more severe diseases (Krause *et al.* 1996).

The tick-borne encephalitis virus is a tick-borne flavivirus causing tick-borne encephalitis. Thousands of TBE cases are reported annually from Europe, and the incidence rate seems to be increasing in many European countries, including Finland (Lindquist & Vapalahti 2008; Amicizia *et al.* 2013; Tonteri *et al.* 2015). Overall, the virus is today reported to be endemic in 27 European countries (Amicizia *et al.* 2013). Three known subtypes of the virus circulate in *I. ricinus* and *I. persulcatus* populations, the European (Eur-TBEV), Siberian (Sib-TBEV), and Far-Eastern (FE-TBEV) subtypes. While both the European and Siberian subtypes are present in Finland (Jääskeläinen *et al.* 2006; Jääskeläinen *et al.* 2010; Jääskeläinen *et al.* 2011; Jääskeläinen *et al.* 2016; Laaksonen *et al.* 2017), only Eur-TBEV has been identified from *I. ricinus* in southwestern Finland. All in all, more than 70% of human TBEV infections are estimated to be symptomless or subclinical (Gritsun *et al.* 2003). However, in the rare cases where the disease proceeds to the second phase (central nervous system infection), severe symptoms and sequelae are possible. Different rates of mortality have been reported for different TBEV subtypes, with Eur-TBEV infections generally being milder (mortality 0–2%) and Sib-TBEV infections more severe (0–8%) (Gritsun *et al.* 2003). The prevalence of the virus in *I. ricinus* is relatively low, between 0.1–5% in the northern parts of Europe, and the occurrence of TBEV is extremely patchy (Pettersson *et al.* 2014). In Finland, 60–80 TBE cases have been reported annually from 2015 to 2017 (incidence ~1–1.4/100,000 population), and a generally increasing trend in case numbers can be observed since 1995 (NIDR). However, these case numbers do not precisely reflect the prevalence of the virus in Finnish tick populations, as a vaccination campaign against TBEV covering the entire populace of Åland Islands, the area previously contributing most disease cases in Finland, was started in 2006. As such, the recent rise in TBE cases can mostly be attributed to increased prevalence in other parts of Finland (or increased human contact with ticks therein).

1.4. Why the need for tick-related studies?

Despite the severity of certain tick-borne diseases and the crucial role *I. ricinus* have in their zoonotic transmission in Europe, studies focusing on Finnish *I. ricinus* are quite rare. In fact, less than 20 studies concerning the species have been published from Finland between 1961 and 2017 (Öhman 1961; Nuorteva & Hoogstraal 1963; Oker-Blom *et al.* 1964; Saikku *et al.* 1971; Saikku & Brummer-

Korvenkontio 1975; Junttila *et al.* 1994; Junttila *et al.* 1999; Han *et al.* 2001; Mäkinen *et al.* 2003; Jääskeläinen *et al.* 2010; Bugmyrin *et al.* 2012; Wilhelmsson *et al.* 2013; Hokynar *et al.* 2016; Jääskeläinen *et al.* 2016; Sormunen *et al.* 2016; Cayol *et al.* 2017; Laaksonen *et al.* 2017) (**I-II**). Most of these are focused on the prevalence of certain pathogens in the ticks, and only a few touch upon any ecological aspects of the ticks or tick populations themselves. Therefore, aspects such as seasonal tick questing activity patterns, changes in tick abundance, preferred biotopes of ticks, and/or factors limiting *I. ricinus* distribution have long been neglected in regard to Finnish populations, and researchers have had to rely on research data from countries with similar climates and environments for reference, namely neighboring Sweden, Norway, and western Russia.

Luckily, the past decade or so has seen renewed activity in *I. ricinus* studies in Finland. Among the first updated major aspects regarding the ecology of Finnish *I. ricinus* was their geographical distribution in the country. The first nationwide distribution mapping of *I. ricinus* was published in 1961 (Öhman 1961). In this study, Christina Öhman contacted veterinaries and nature enthusiasts via letter, and asked for tick sightings. Based on these answers, the first distribution map was drawn on the municipality level. This distribution map (or derivatives of it) was referred to for nearly 60 years before a new distribution map was created in 2017, when the results of a nationwide crowdsourcing campaign studying *I. ricinus* and *I. persulcatus* distribution in Finland were released (Laaksonen *et al.* 2017). Over the 57 years separating these two surveys (data collected in 1958 and 2015, respectively), *I. ricinus* distribution appears to have shifted some 100-200 km northwards. Furthermore, apart from the southernmost parts of Finland, *I. ricinus* are now accompanied by *I. persulcatus* in most locations around the country (Laaksonen *et al.* 2017).

Many other tick-related aspects also long remained unstudied. For example, the occurrence of any tick-borne pathogens apart from *B. burgdorferi* s.l. and TBEV in Finnish ticks received very little attention from researchers prior to 2010s, with only a few published studies in which any of them were screened (Mäkinen *et al.* 2003; Alekseev *et al.* 2007). Following the increased screening activity of recent years, seven tick-borne pathogens new to Finnish *I. ricinus* have now been reported between 2013 and 2018 (Wilhelmsson *et al.* 2013; Sormunen *et al.* 2016; Laaksonen *et al.* 2018) (**II-IV**). Likewise, changes in the tick populations themselves were not addressed prior to the last few years. This led to a situation where the increasing number of human diseases cases (the National Infectious Diseases Register maintained by the National Institute for Health and Welfare keeps track of Lyme borreliosis and TBE cases) and reports of citizens were for a long time the only indicators of potentially increasing tick abundance. Recently, studies from southwestern Finland have shown higher tick densities in 2013-2017 than in 2000 for several study locations (Mäkinen *et al.* 2003) (**II, IV**). While this indicates increasing tick numbers in southwestern Finland, the occurrence of ticks is highly patchy and dependent on local environmental conditions. Therefore, further studies across Finland are required to determine whether this constitutes a nationwide trend. Unfortunately, as mentioned before, only a few studies concerning *I. ricinus* exist from Finland, and even fewer that report local tick densities. As such, very limited prior tick density data is available.

It seems clear that ticks are becoming more and more of a nuisance and threat to Finnish citizens. It is imperative that we have up-to-date data on ticks and tick-borne pathogens in order to minimize their negative impact on human welfare. Likewise, it is important that we have access to national data, as such data describes local tick and TBP trends better than corresponding data from neighboring countries. In an effort to lead the way for future surveys and produce more comprehensive data for Finnish *I. ricinus*, the studies in this thesis focus on several of the neglected aspects of tick-related research in Finland, as presented below.

2. STUDY THEMES

The studies of this thesis cover a range of themes related to *I. ricinus* and associated pathogens in southwestern Finland. These themes are studied and discussed over the chapters included in the thesis as follows:

1. ***The abundance of I. ricinus in southwestern Finland***. Studies conducted in other countries in northern Europe have revealed increasing tick abundance particularly in well-established tick areas. Chapters **I, II & IV** assess whether similar changes can be detected in the tick populations in southwestern Finland, a well-known and established tick area.

2. ***Prevalence and diversity of tick-borne pathogens in southwestern Finland***. The emergence and increasing prevalence and species diversity of tick-borne pathogens have been documented elsewhere in Europe in the 21st century, but similar studies have not been conducted in Finland. Chapters **I-IV** are concerned with the current prevalence and species diversity of tick-borne pathogens in southwestern Finland, as well as long-term monitoring of spatial changes in the diversity and occurrence of TBPs (**III**).

3. ***Differences in the occurrence of I. ricinus and associated pathogens***. The occurrence of ticks and tick-borne pathogens is dependent on local host animal populations and other environmental variables, and thus not expected to be equal among different areas. Chapters **I-IV** assess tick densities and pathogen prevalence over different areas, study sites, and biotopes.

4. ***Human tick risk in urban and suburban areas***. The realization of human tick risk requires contact between infected ticks and humans. The activity of humans is highest in urban and suburban areas, where ticks are not necessarily thought to present a danger by citizens. However, if ticks carrying pathogens can naturally inhabit such areas, these "urbanized" ticks may present an even greater problem for healthcare than their counterparts in the wilderness. In chapters **II & IV** study is focused on tick populations and associated pathogens in urban and suburban areas in the city of Turku.

3. MATERIALS AND METHODS

3.1. Field collections of *I. ricinus*

The study material of this thesis consists of *I. ricinus* collected by cloth dragging from different areas around southwestern Finland between 2012 and 2017 (Figure 5). All ticks found during field studies were collected and preserved for molecular analyses of TBPs and tick species, although not all samples were analyzed in the end due to temporal and financial restraints (some ticks also managed to escape or otherwise disappear before they could be analyzed). Estimates of tick densities were calculated for each study area separately. Field collection methods, times and places of sampling, and molecular analyses of ticks are described in more detail below.

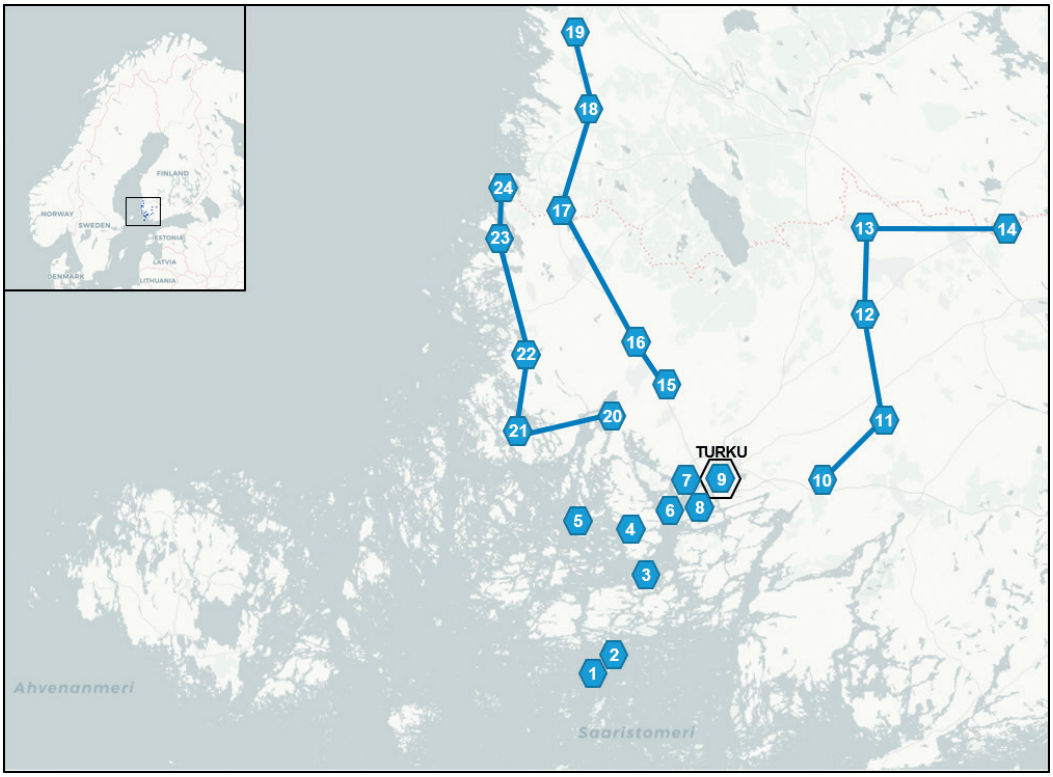

Figure 5. Field collection sites in southwestern Finland: [1]Boskär, [2]Berghamn, [3]Seili, [4]Maisaari, [5]Pähkinäinen, [6]Vepsä, [7]Ruissalo, [8]Hirvensalo, [9]Turku city (detailed info in **IV**: Figure 1), [10-14]Northwestern line ([10]Paimio, [11]Marttila, [12]Matku, [13]Alastaro, [14]Mellilä), [15-19]Northern line ([15]Nousiainen, [16]Laitila, [17]Kodisjoki, [18]Eurajoki, [19]Luvia), and [20-24]Coastal line ([20]Askainen, [21]Kustavi, [22]Lokalahti, [23]Pyhämaa, [24]Pyhäranta). ©OpenStreenMap

3.1.1. Cloth dragging and flagging

The standard methods used for field collections of *I. ricinus* are known as (cloth/blanket) dragging and flagging. In both these methods, a cloth or blanket is dragged over vegetation over a predetermined area (e.g. 10 meters) or for a predetermined time (e.g. 30 minutes). The cloth simulates a passing host and actively host-seeking ticks attach to it. White cloths are typically used in such collections, as the detection of ticks is easiest from these. No additional attractants are usually used whilst applying these methods for scientific study; however, CO_2 and other natural compounds, which are known to stimulate ticks, are radiated from the users. As such, whilst these are not usually documented, the orientation of the user as well as the direction of the wind can have some (minor) impact on the function of the methods.

In dragging, the cloth is typically dragged behind the user, brushing over ground floor vegetation (Figure 6). The cloths used are often 1 × 1 m in size, allowing for simple assessments of tick densities over square meters. Some form of weight is often sowed to the trailing edge of the cloth, in order to ease the passage of the cloth and press down vegetation, as well as to prevent it from catching wind. In flagging, a cloth is swept back and front (or sideways) over vegetation next to (or in front of) the user whilst they walk. Ticks attached to the cloth can be calculated and collected with tweezers, should the need arise. Tick densities are estimated based on the choice of measure: either as ticks/area (usually 100 m^2 or 1000 m^2) or as ticks/time unit (often 1 hour). While both estimation methods are used, the use of an objective reference frame (area) is preferable to time, since different people may have different speeds of dragging/flagging and tick collection (Estrada-Peña *et al.* 2018). Depending on the study, densities can be calculated for each or some tick life stages separately, or for all together. In the studies of this thesis, tick densities were estimated as ticks per 100 or 1000 m^2 (interchangeable values), and densities were calculated separately for all life stages (total tick densities were however also calculated).

3.1.2. Sampling sites and times

For the studies of this thesis, host-seeking *I. ricinus* were collected from southwestern Finland by cloth dragging between 2012 and 2017. Collections were carried out in four specific study groups: Seili Island (2012-2017), Turku Archipelago and Turku city areas (2013), southwestern Finland (2014), and Turku city areas (2017). These study clusters were comprised of a total of 50 individual study sites. In southwestern Finland (2014), a total of 15 study locations were combined into three study lines: the 'Coastal line' (comprised of study locations in Askainen, Kustavi, Lokalahti, Pyhämaa, Pyhäranta), the 'Northern line' (Nousiainen, Laitila, Kodisjoki, Eurajoki, Luvia), and the 'Northwestern line' (Alastaro, Matku, Mellilä, Marttila, Paimio) (Figure 5). In some localities in the city of Turku, studies were conducted in both 2013 and 2017.On Seili Island (2012-2017) and the Turku archipelago and Turku city areas (2013), fixed 50 m study transects were used for sampling. In these transects, dragging was conducted every other or every third week from May to September, apart from Seili in 2012, when

dragging was conducted every week. The total number of 50 m transects was 15 for Seili and 24 for Turku Archipelago and Turku city areas. The whole length of each transect was dragged once per sampling session, in three approximately equal portions (depending on terrain). The cloth was checked after each portion, and attached ticks were calculated and removed.

In studies in southwestern Finland (2014) and Turku city areas (2017), tick sampling was conducted at non-specific locations in the chosen study area. Dragging was done in 10 m sections, chosen spontaneously. After each 10 m section, the cloth was checked and attached ticks calculated and removed. A minimum of ten 10 m drags (for a total of 100 m) were made at each location during each sampling session. Sampling was conducted from May to August (Southwestern Finland; 2014) or May to July (Turku city areas; 2017) at varying frequencies, from weekly sampling to sampling every third week. Chapters **I-IV** provide exact sampling schedules of all these study groups.

All ticks attached to the cloth were collected using tweezers. Ticks were stored in 1.5 mL Eppendorf-tubes either alive or in 90-96% ethanol, and subsequently delivered to the University of Turku Zoological Museum for deep freezing (-80°C). Morphological species identification for most samples was done prior to DNA/RNA extraction, based on the guidelines provided by Hillyard (1996).

Figure 6. Cloth dragging on Seili Island in 2012.

3.1.3 Sampling on Seili Island (2012-2017) and Turku city areas (2017)

Seili (2012-2017)

On Seili, five different biotopes commonly found on the island were chosen for sampling: coniferous forest, deciduous forest, alder thicket, meadow, and pasture. Three fixed study transects were placed in each biotope – for a total of fifteen transects – in 2012 and subsequently dragged annually until 2017. The three transects of the same biotope were located in different areas, so that they were spatially separate. The biotope and location data from study transects in Seili were used in the analysis of tick seasonal questing activity patterns (**I**) and the spatial scope of pathogen occurrence (**III**).

The biotopes were classified as follows: coniferous forests were forests dominated by *Picea abies* and *Pinus sylvestris*, with field layers clearly dominated by *Vaccinium myrtillus* and ground layers by mosses and needle litter. Deciduous forests were forests dominated by *Betula pendula*, *Betula pubescens*, and *Corylus avellana*, with field layers dominated by grasses and ground layers with patches of mosses, *V. myrtillus*, and leaf litter. Alder thickets were forested wetlands dominated by *Alnus glutinosa* with diverse field layers mostly dominated by *Filipendula ulmaria* and ground layers of mixed litter. Meadows were treeless areas with field layers of true grasses, mostly *Dactylis* sp., *Festuca* sp., and *Deschampsia* sp. Pastures were fenced grazing areas with flora similar to meadows. Each of the chosen pasture areas was grazed annually by cattle.

Turku (2017)

In Turku city in 2017, tick sampling was conducted in ten distinct localities between late May and early July. Five of the areas ("main study areas") were sampled four times in weekly or biweekly intervals, whereas another five ("secondary areas") were visited only twice (**IV**: Figure 1).

Two of the study areas were suburban islands (Ruissalo and Hirvensalo, a main and secondary study area, respectively) that were also surveyed in earlier city investigations (Mäkinen *et al.* 2003) (**II**). The eight other study areas were located in the mainland. Urban city parks, yards and vegetation-flanked walkways in the grid-planned city center of Turku were divided to the eastern and western sides according to the Aura River (**IV**: Figure 1), forming a main study area each. One main study area was established in the main campus area of the University of Turku, while the fifth one was close to housing estates in Koroinen/Halinen. Two city parks, Urheilupuisto and Samppalinna, in the eastern side of the city, were also sampled in the earlier studies.

Besides Hirvensalo Island, suburban areas Katariina, Kuninkoja, Littoinen and Luolavuori were visited twice during the sampling period and thus classified as secondary areas. Although all these localities also contain walking/jogging/nature trails for recreational purposes, the main study area Ruissalo is by far the most popular district of the city for many kinds of outdoor activities, such as walking, jogging, biking, golf, berry picking, bird watching, swimming, camping, and music festivals, and is annually visited by hundreds of thousands of people. All the five main and one secondary (Hirvensalo) study areas consisted

of 3–5 nearby but separate study sites (**IV**: Figure 1). In the other four secondary areas, only one site was studied.

3.2. Laboratory analyses

DNA and RNA were extracted from frozen tick samples between 2013 and 2017 using NucleoSpin®TriPrep–kits (**I**) or NucleoSpin® RNA kits and RNA/DNA buffer sets (**II-IV**) (Macherey-Nagel, Germany), following the kit protocols (TriPrep: Rev. 04, Feb 2012; RNA: Rev. 16/May 2014; RNA/DNA buffer set: Rev.08/May 2014). RNA extracts were stored at -80 °C for later use. DNA extracts were stored at -20 °C.

Real-time quantitative PCR (henceforth abbreviated qPCR) assays were carried out to screen tick DNA/RNA samples for several well-known or putative tick-borne pathogens. DNA samples were screened for bacterial pathogens *Borrelia burgdorferi* sensu lato (**I-IV**), *Borrelia miyamotoi* (**I-IV**), *Rickettsia* spp. (**II-IV**), *Anaplasma phagocytophilum* (**III-IV**), *Candidatus* Neoehrlichia mikurensis (**II-IV**), and *Bartonella* spp. (**II-IV**). Furthermore, DNA samples were screened for protozoan pathogens *Babesia* spp. (**III-IV**). Finally, RNA samples were screened for TBEV (**I, III-IV**).

Analyses regarding *Borrelia* spp. were carried out on individual DNA samples. For screening of *Rickettsia*, *A. phagocytophilum*, *Ca.* N. mikurensis, *Bartonella, Babesia*, and TBEV, aliquots of original DNA/RNA samples were pooled (ten samples per pool, 5 µl of each sample) due to low expected prevalence, as well as to make the procedure faster and more cost-efficient. Original, separate DNA/RNA samples were re-analyzed as needed when a pooled sample was found positive.

All samples were analyzed in three replicate reactions carried out on 384-well plates. At least three blank water samples were used as negative controls in each assay, to account for reagent contamination. In addition, commercial pathogen samples, DNA extracted from cultivated pathogen strains, or confirmed positive samples were used as positive controls in each qPCR assay to account for errors in reaction setup and technical aspects where applicable. The thermal cycling profile used for analyses of DNA samples was 95°C for 5 minutes, then 50 cycles of 95°C for 10 sec and 60°C for 30 sec (annealing/extension temperature was 58°C in assays involving *Rickettsia*). For RNA samples, thermal cycling profile was 48°C for 10 minutes (reverse transcription) and 95°C for 2 minutes, then 50 cycles of 95°C for 5 sec and 60°C for 30 sec. Thermal cycling was carried out at Finnish Microarray and Sequencing Centre (FMSC, Turku, Finland). All qPCR results were analyzed using QuantStudio™ 12 K Flex Software v.1.2.2 (LT1). Samples were considered positive only when successful amplification was detected in all three replicate reactions. The primers and probes used in qPCR screening and the mastermix contents of assays are described in their respective chapters.

Pathogen species determination

For DNA samples found positive for *Rickettsia*, *Babesia*, and *A. phagocytophilum* using qPCR, further pathogen species determination was done by conventional PCR and DNA barcoding. Barcoding was also performed for *Borrelia* -positive samples in chapter **I**.

Genospecies identification for samples positive for *B. burgdorferi* s.l. was performed by conventional PCR using primers targeting the *Borrelia* flagellin gene (**I**). PCR was carried out in 12.5 µL reaction volume, containing 1 µL of DNA extract and 500 nM forward and reverse primers. Thermal cycling was performed with the following program: 95°Cfor 1 min, then 35 cycles of 95°C for 15 s, 50°C for 15 s, 72°C for 10 s. Furthermore, samples positive for *B. miyamotoi* were sequenced using primers targeting *B. miyamotoi* 16S rRNA (**I**). These reactions were carried out using the same primer concentrations and total reaction and DNA template volumes as for *B. burgdorferi* s.l. PCR. Thermal cycling profile was also identical, apart from the annealing temperature being 55 °C.

For samples found positive for *Rickettsia* and *A. phagocytophilum*, primers targeting *Rickettsia gltA* gene (**II-IV**) and 16S rRNA of *Anaplasma* (**III-IV**) were used. For both these pathogens, PCR was carried out in 12.5 µl reaction volume, containing 3 µl of DNA template and 500 nM forward and reverse primers. Thermal cycling profiles were 95°C for 3 min, then 35 cycles of 95°C for 20 sec, 61(*Rickettsia*)/62 (*Anaplasma*)°C for 30 sec, and 72°C for 1 min.

Finally, for samples positive for *Babesia*, primers targeting 18S rRNA of *Babesia* were used (**III-IV**). *Babesia* PCR was carried out in 12 µl reaction volume, containing 4 µl of DNA template and 400 nM forward and reverse primers. Thermal cycling profile was 95°C for 3 min, then 50 cycles of 95°C for 30 sec, 58°C for 20 sec, and 72°C for 1 min.

Water samples were used as blank controls in each PCR batch. Successful PCR products were purified by mixing 1 µl EXO I enzyme, 1 µl rSAP enzyme, 3 µl of ddH2O, and 5 µl of PCR product, after which the samples were first incubated 5 min at 37°C and then heated 10 min at 80°C. Purified samples were sent to Macrogen Inc. Europe (The Netherlands) for sequencing. The sequences were trimmed using Geneious version 6 and run through BLAST (www.ncbi.nlm.nih.gov/BLAST/). The trimmed sequences were then further compared to reference sequences of corresponding species, downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/), to ascertain species using the software Geneious Pro R6

Tick species identification

For some tick samples, laboratory analyses were carried out either to ascertain morphological identification or to perform identification for samples not morphologically identified. The main objective was to differentiate between two similar looking species, *I. ricinus* and *I. persulcatus*.

For samples in the first chapter (**I**), tick species determination was accomplished by DNA barcoding using PCR primers targeting the *COI* gene of *I. ricinus*. Polymerase chain reaction (PCR) was carried

out in 12.5 µL reaction volume, containing 1 µL of DNA template and 500 nM of both primers. Thermal cycling was performed with the following program: 95°C for 1 min, then 35 cycles of 95°C for 15 s, 50°C for 15 s, 72°C for 10 s. A water sample was used as a blank control in each PCR batch. Successful PCR products were purified by mixing 1 µL EXO I enzyme, 1 µL rSAP enzyme, 3 µL of ddH2O, and 5 µL of PCR product. The samples were then incubated 5 min at 37°C, heated 10 min at 80°C, and sequenced by Macrogen Europe (The Netherlands).

For some samples (**II-IV**), tick species was determined in a species-specific duplex qPCR assay. Primers targeting the *Ixodes* spp. *ITS2* gene were designed to amplify genus specific segments (presented in chapter **II**). Species-specific probes were designed to match either of the tick species (*I. persulcatus* and *I. ricinus*). The newly designed primers and probes were tested by amplifying hundreds of sequenced *I. ricinus* and *I. persulcatus* DNA samples from earlier studies. Furthermore, DNA samples of *I. ricinus* and *I. persulcatus* confirmed by sequencing were used as positive controls in each assay.

3.3. Statistical analyses

Detailed descriptions of the used statistical methods are given in original chapters (**I, III-IV**). Briefly, tick abundance (count data) and pathogen occurrence (binomial) data were analyzed by generalized linear (mixed) models using negative binomial (**I**) and binary error distributions (**III-IV**), respectively. Explanatory factors generally included sampling-site and -time related factors like biotope, location, and sampling month or year. When appropriate, random effects were included to account for obvious dependency structures in the data. All the models were run with the GLIMMIX procedure of SAS v. 9.4.

4. RESULTS

Altogether 13838 *I. ricinus* were collected during approximately 79 km of cloth dragging between 2012 and 2017. Collected ticks consisted of 374 adults, 4441 nymphs, and 9023 larvae. Highly varying tick densities were observed between study localities and years (Table 1). In general, tick densities were highest on islands in the Archipelago Sea, whereas lower densities were observed when moving inland. The highest larvae and nymph densities were measured in Boskär in 2013, whereas the highest adult densities were observed in Seili in 2017. For study sites with data from two or more years (Seili, Ruissalo, Hirvensalo, Samppalinna & Urheilupuisto), tick densities were highest in the last year of study, 2017.

A total of 8788 *I. ricinus* were screened for the listed pathogens. These consisted of 262 adults, 3067 nymphs, and 5459 larvae. All bacterial and protozoan pathogens apart from *Bartonella* spp. were detected from at least two study sites and during at least three years (Table 2). Tick-borne encephalitis virus was only detected from Seili Island in 2016-17 (**III**).

Table 1. Collected ticks and tick densities in the major study areas of this thesis.

Study area (**manuscript**)	Year	Distance dragged (m)	Collected ticks by life stage[a]				Tick densities (ticks/100m^2)			
			L	N	A	Tot	L	N	A	Tot
Seili (**I-III**)	2012	11250	1356	540	44	1940	12.1	4.8	0.4	17.3
	2013	6000	476	311	24	811	7.9	5.2	0.4	13.5
	2014	5250	1030	352	20	1402	19.6	6.7	0.4	26.7
	2015	5250	559	268	38	865	10.6	5.1	0.7	16.4
	2016	5250	1006	626	44	1676	19.2	11.9	0.8	31.9
	2017	6000	1941	1438	117	3499	32.4	24	1.95	58.35
	2018	7450	2924	877	140	3941	39.2	11.8	1.9	52.9
Boskär (**II**)	2013	600	2220[b]	205	11	2436[b]	370	34.2	1.8	406[b]
Berghamn (**II**)	2013	400	55	31	2	88	13.75	7.75	0.5	22
Vepsä (**II**)	2013	1050	4	7	8	19	0.4	0.7	0.8	1.9
Maisaari (**II**)	2013	1050	4	18	2	24	0.4	1.7	0.2	2.3
Pähkinäinen (**II**)	2013	1050	196	96	5	297	18.7	9.1	0.5	28.3
Ruissalo (**II, IV**)	2013	1600	0	5	6	11	0	0.3	0.4	0.7
	2017	3330	58	396	20	474	1.74	11.89	0.6	14.23
Hirvensalo (**II, IV**)	2013	900	1	19	0	20	0.1	2.1	0	2.2
	2017	1070	0	19	6	25	0	1.78	0.57	2.34
Samppalinna (**II, IV**)	2013	800	0	0	0	0	0	0	0	0
	2017	1150	0	2	0	2	0	0.17	0	0.17
Urheilupuisto (**II, IV**)	2013	800	0	0	0	0	0	0	0	0
	2017	1300	0	1	2	3	0	0.08	0.15	0.23
Coastal line (**II**)	2014	2400	3	42	3	48	0.1	1.8	0.1	2
Northern line (**II**)	2014	2400	0	3	0	3	0	0.13	0	0.13
Northwestern l. (**II**)	2014	2400	0	0	0	0	0	0	0	0
Turku, east (**IV**)	2017	5030	0	7	3	10	0	0.14	0.06	0.2
Turku, west (**IV**)	2017	5080	1	7	1	9	0.02	0.14	0.02	0.18
University area (**IV**)	2017	4050	113	35	16	164	2.79	0.86	0.39	4.05
Koroinen/Halinen (**IV**)	2017	3570	0	13	4	17	0	0.36	0.12	0.48
Total (2012-2017)	-	79030	9023	4441	374	13838	11.4	5.6	0.5	17.5

[a]Abbreviations: L = larvae; N = nymphs; A = adults, Tot = Total.

[b]In Boskär, larval densities were approximated due to extremely high numbers of larvae (occasionally 500–600 larvae per 50 m drag).

Table 2. Bacterial and protozoan pathogens detected from tick samples from different areas around southwestern Finland.

Study area (chapters)	No. tick samples[a]			Number samples positive (prevalence/MIR) for:																	
				B. burgdorferi s.l.			*B. miyamotoi*			*Rickettsia*			*A. phagocytophilum*			*Babesia*			*Ca.*N. mikurensis		
	Ad	N	L	Ad	N	L[b]	Ad	N	L[b]	Ad	N	L[b]	Ad	N	L[b]	Ad	N	L[b]	Ad	N	L[b]
Seili Island (**I-III**)	182	1932	4521	46 (25.3)	373 (19.3)	0	2 (1.1)	13 (0.7)	3 (0.1)	6 (3.3)	36 (1.9)	37 (0.8)	18 (9.9)	65 (3.4)	0	2 (1.1)	25 (1.3)	14 (0.3)	0	37 (1.9)	0
Boskär (**II**)	11	149	711	1 (9.1)	43 (30.9)	0	0	1 (0.67)	6 (0.8)	0	1 (0.67)	1 (0.1)	-	-	-	-	-	-	-	-	-
Berghamn (**II**)	1	19	39	0	1 (5.3)	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
Vepsä (**II**)	4	5	0	2 (50)	0	-	0	0	-	0	0	-	-	-	-	-	-	-	-	-	-
Maisaari (**II**)	2	16	0	1 (50)	3 (18.8)	-	0	0	-	0	0	-	-	-	-	-	-	-	-	-	-
Pähkinäinen (**II**)	5	83	180	2 (40)	16 (19.3)	0	0	1 (1.2)	0	1 (20)	0	0	-	-	-	-	-	-	-	-	-
Ruissalo (**II, IV**)	23	310	3	11 (47.8)	118 (38.1)	1 (33)	1 (4.3)	2 (0.6)	0	5 (21.7)	30 (9.7)	1 (33)	2 (8.7)	11 (3.5)	0	0	4 (1.3)	1 (33)	0	8 (2.6)	0
Hirvensalo (**II, IV**)	5	28	0	3 (60)	10 (35.7)	-	0	0	-	0	0	-	0	1 (3.6)	-	0	0	-	0	0	-
Coastal line (**II**)	4	23	0	2 (50)	2 (8.7)	-	0	0	-	0	0	-	-	-	-	-	-	-	-	-	-
Northern line (**II**)	0	3	0	-	0	-	-	0	-	-	0	-	-	-	-	-	-	-	-	-	-
Northwestern line (**II**)	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Turku, east (**IV**)	3	6	0	0	0	-	0	0	-	0	1 (16.7)	-	0	0	-	0	0	-	0	0	-
Turku, west (**IV**)	1	8	0	0	1 (12.5)	-	0	0	-	0	2 (25)	-	0	1 (12.5)	-	0	1 (12.5)	-	0	0	-
University (**IV**)	17	36	5	10 (58.8)	10 (27.8)	1 (20)	0	0	0	2 (11.8)	9 (25)	1 (20)	0	0	0	0	0	0	0	0	0
Koroinen/Halinen (**IV**)	4	12	0	2 (50)	3 (25)	-	0	0	-	0	0	-	0	0	-	0	0	-	0	0	-
Total	262	2630	5459	80 (30.5)	580 (22.1)	2 (0.04)	2 (0.8)	17 (0.6)	9 (0.2)	14 (5.3)	79 (3)	40 (0.7)	20 (7.6)	78 (3)	0	2 (0.8)	30 (1.1)	15 (0.3)	0	45 (1.7)	0

[a]Larvae were studied in pools containing 1-111 (years 2012-2014) or 1-24 (2015-2017) larvae.

[b]Estimates for larvae are mimimum infection rates (MIR).

4.1. Biotope preferences and seasonal questing activity patterns of *I. ricinus* (I)

In order to study their biotope preferences and questing activity patterns, I collected total of 1940 *I. ricinus* from Seili Island in 2012. Larvae were the most numerous life stage collected with 1356 individuals (70% of all collected ticks), followed by nymphs (540 individuals; 28% of collected ticks), and adults (44 individuals; 2% of collected ticks). Tick density estimates also varied among the different biotopes on the island, with transects in coniferous forests having the highest densities and transects in meadows/pastures having the lowest densities (**I:** Table 2). Despite some fluctuation between biotopes and life stages, in general, the weekly overall densities of ticks rose throughout the study. The highest densities of ticks were observed during the last two weeks of field studies, in late August and early September (**I**: Figure 1).

Statistical analysis of the seasonal questing activity of *I. ricinus* was conducted separately for larvae and for adults + nymphs together. For both these groups, there was much variation in the number of individuals observed among biotopes and transects (Figure 7). For larvae, the cubic time trend ($week^3$) had a significant interaction with the biotope, indicating different questing activity patterns in different biotopes (**I:** Table 3a). Bimodal activity patterns were observed for larvae in some study transects in coniferous forest, deciduous forest, and alder thicket, while unimodal patterns were observed for two pasture transects (Figure 7a-e). None of the weather-related explanatory factors remained in the model for larvae once the progress of the season was already taken into account by week numbers. For nymphs and adults, the cubic time trend was relatively weak, although bimodal activity patterns were displayed for several transects (Figure 7f-j). Relative humidity had a positive effect on the number of adults and nymphs observed (**I:** Table 3b).

Borrelia were detected from a total of 41 DNA samples (11 adults and 30 nymph pools). For adults, *Borrelia* prevalence was 25% (11/44 samples). For nymphs, minimum infection rate was 5.6% (30/540; nymphs were analyzed in pools). Five species/genospecies of *Borrelia* were detected from the samples: *B. afzelii* (63.4% of positive samples), *B. garinii* (14.6%), *B. burgdorferi* s.s. (7.3%), *B. miyamotoi* (4.9%), and *B. valaisiana* (2.4%). All larval samples were negative for *Borrelia*. Likewise, all RNA samples were negative for TBEV.

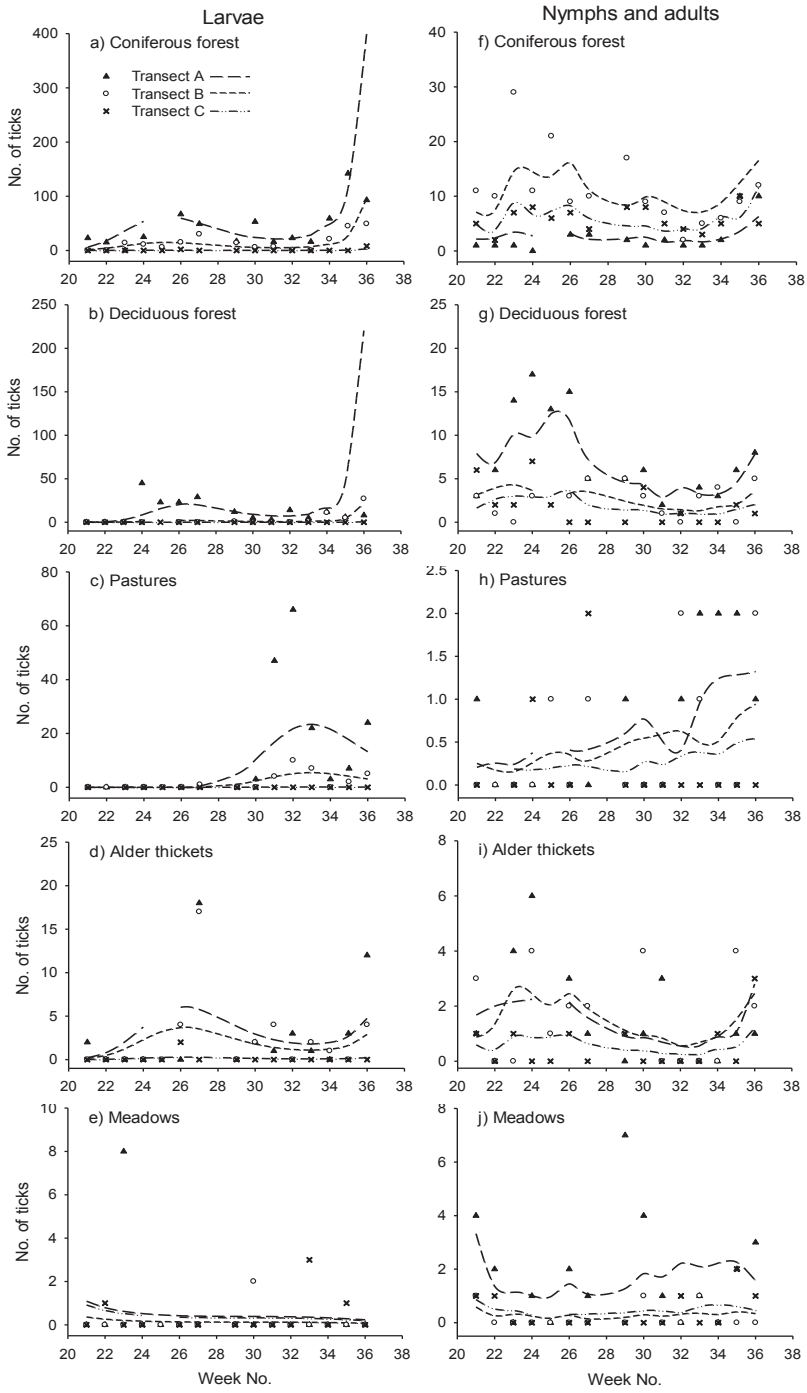

Figure 7. Sampling data (symbols) and third-order polynomials predicting tick numbers (lines) in different biotopes: numbers of larvae (a-e) and numbers of nymphal and adult ticks (f-j). Due to high variation even within biotopes, the prediction is made for each study transect separately (three transects per biotope). Note different scales of y-axes, highlighting largely differing tick numbers among biotopes and development stages.

4.2. Revisiting southwestern Finland – Changes in local tick and TBP populations? (II)

In order to assess possible changes in *I. ricinus* populations and associated TBPs in southwestern Finland, I collected a total of 3158 ticks from 25 different study sites between 2013 and 2014. The tick densities and *B. burgdorferi* s.l. and *A. phagocytophilum* prevalence of ten of these study sites had been assessed in a previous study in 2000 (Mäkinen *et al.* 2003). The collected samples consisted of 87 adult ticks (2.8% of all samples), 1132 nymphs (35.8%), and 1939 larvae (61.4%). Highly varying tick densities were observed among the different study sites (Table 1). Densities were higher in 2013-14 than in 2000 at every study site that yielded ticks (Table 3). A generally decreasing trend in tick density estimates was observed when moving towards the mainland from islands in the outer parts of the Archipelago Sea, as well as when moving inland from the coast (Table 2; Figure 5).

Five different pathogens or pathogen groups were screened from DNA samples of the collected ticks: *B. burgdorferi* s.l., *B. miyamotoi*, *Rickettsia* spp., *Bartonella* spp., and *Ca.* N. mikurensis. Three of them were detected, while two (*Ca.* N. mikurensis and *Bartonella*) were absent. Out of the three detected pathogens, *B. burgdorferi* s.l. were the most common with 217 positive samples. The prevalence of *B. burgdorferi* s.l. was 23.5% for adults (18/87) and 18.5% for nymphs (181/979). All larvae were negative for the pathogen.

Rickettsia and *B. miyamotoi* were detected in 21 and 16 DNA samples, respectively. *Rickettsia* prevalence was 5.1% for adults and 1.1% for nymphs. *Borrelia miyamotoi* was detected with a prevalence of 1% for adults and 0.5% for nymphs. *Rickettsia* and *B. miyamotoi* were also detected from larvae pools (4 and 6 pools, respectively). These accounted for minimum infection rates of 0.2% for *Rickettsia* and 0.3% for *B. miyamotoi* in larvae. Furthermore, 20 *Rickettsia* samples were sequenced in order to verify species. Sixteen samples were successfully identified as *R. helvetica* and three as *R. monacensis*, forming the first reports of both these pathogens from Finnish ticks.

Regarding differences in *B. burgdorferi* s.l. prevalence in 2000 and 2013, comparisons were hampered by low sample sizes for several study sites in one or both studies. For sites with adequately large sample sizes (Boskär, Berghamn, Seili, Pähkinäinen), higher prevalence was observed in 2013 (Table 3).

Table 3. Tick densities and prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum* in sampling sites for which previous data was available. Note that tick densities and prevalence are calculated for nymphs and adults together (no larvae), as was done by Mäkinen *et al.* (2003).

Study locality	**Tick density (ticks/100 m^2)**			**Samples analyzed**			***B. burgdorferi* s.l. positive samples (prevalence)**			***A. phagocytophilum* positive samples (prevalence)**		
Year	2000[a]	2013	2017	2000[a]	2013	2017	2000[a]	2013	2017	2000[a]	2013	2017
Seili	0.28	5.6	26	69	319	514	8 (11.6)	54 (16.9)	129 (25.1)	0	13 (4.1)	20 (3.9)
Ruissalo	0.23	0.6	14.2	14	10	326	0	0	131 (40.2)	0	0	13 (4)
Hirvensalo	0.01	2.2	2.34	1	9	24	0	1 (11.1)	12 (50)	0	0	1 (4.2)
Boskär	7.3	36	-	272	160	-	13 (4.8)	47 (29.4)	-	0	13 (8.1)	-
Berghamn	0.93	8.3	-	66	20	-	0	1 (5)	-	0	3 (15)	-
Pähkinäinen	0.82	9.6	-	22	88	-	2 (9.1)	18 (20.5)	-	0	2 (2.3)	-
Maisaari	0.06	1.6	-	2	18	-	0	4 (22.2)	-	0	0	-
Vepsä	0.2	1.4	-	8	9	-	0	2 (22.2)	-	0	0	-
Samppalinna	0	0	0.2	0	0	1	-	-	0	-	-	0
Urheilupuisto	0	0	0.2	0	0	3	-	-	0	-	-	0

[a]Data from Mäkinen *et al.* (2003).

4.3. Longitudinal and spatial patterns of tick-borne pathogen occurrence (III)

In an effort to study longitudinal and spatial patterns in the occurrence of TBPs and the possible emergence of novel pathogens, 7070 ticks collected from Seili Island between 2012 and 2017 were screened for a total of eight different bacterial, protozoan, and viral zoonotic pathogens. These samples consisted of 182 adults (2.6% of all samples), 2370 nymphs (33.5%), and 4518 larvae (63.9%). Seven pathogens/pathogen groups were detected from Seili: *B. burgdorferi* s.l., *B. miyamotoi*, *Rickettsia* spp., *A. phagocytophilum*, *Ca.* N. mikurensis, TBEV, and *Babesia* spp. The former five of the listed pathogens/pathogen groups were detected from samples annually (**III:** Table 1). *Borrelia burgdorferi* s.l. and *A. phagocytophilum* were the most commonly detected pathogens, with prevalence rates of 19.3%

and 3.4% for nymphs, and 25.3% and 9.9% for adults, respectively (**III:** Table 1). Both pathogens were found from nymphs and adults annually. No positive samples were detected among larvae.

Rickettsia, *Babesia*, *C.* N. mikurensis, *B. miyamotoi*, and TBEV were detected less frequently, with respective prevalence rates of 1.9%, 1.3%, 1.9%, 0.7%, and 0.4% for nymphs, and 3.3%, 1.1%, 0%, 1.1%, and 0% for adults (**III:** Table 1). *Rickettsia, Babesia*, and *B. miyamotoi* were also detected from larvae pools, with minimum infection rates of 0.8%, 0.3%, and 0.1%, respectively (**III:** Table 2). *Candidatus* N. mikurensis was only detected in 2015-2017 and TBEV in 2016-2017, suggesting that emergence of these pathogens may have occurred during the latter years of the study. Some *Rickettsia* (n=41) and *Babesia* (n=22) samples were sequenced to determine species. Two species of *Rickettsia* were detected: *R. helvetica* (39/41 samples) and *R. monacensis* (2/41). All *Babesia* samples were identified as *B. venatorum*, forming the first report of the pathogen from Finnish ticks.

Statistical analyses were carried out to determine whether differences in the probability of a nymph to be positive for *B. burgdorferi* s.l., *A. phagocytophilum*, or *Rickettsia* exist among biotopes or transects. Following analysis, significant differences in prevalence estimates between biotopes were detected for *B. burgdorferi* s.l. and *A. phagocytophilum* (Figure 8). Furthermore, differences among transects in similar biotopes were detected for *B. burgdorferi* s.l. and *A. phagocytophilum* in coniferous forest, and *B. burgdorferi* s.l., *Rickettsia*, and *Ca.* N. mikurensis in deciduous forest (Figure 9).

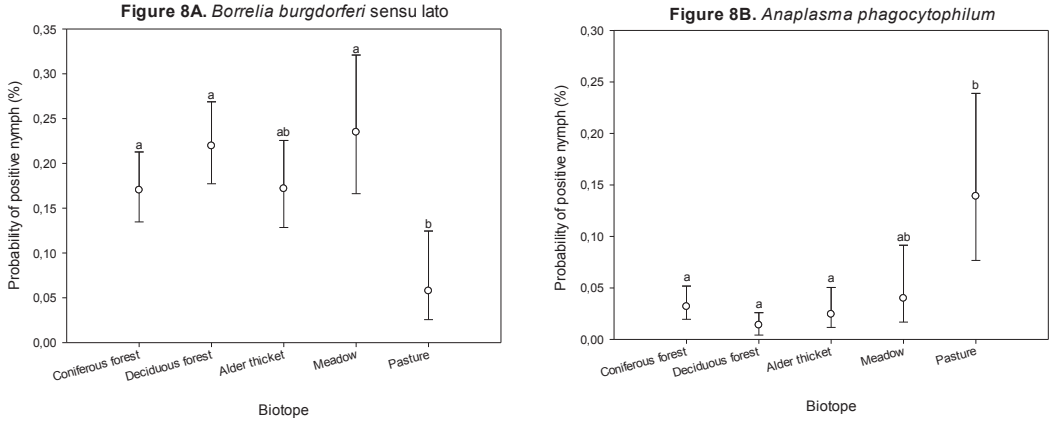

Figure 8. Estimated probabilities (with 95% confidence limits) of nymph samples being positive for *B. burgdorferi* s.l. and *A. phagocytophilum* across biotopes in Seili, as predicted by the GLMM. Different assigned letters denote statistically significant differences between biotope classes ($p<0.05$).

Figure 9a. *B. burgdorferi* s.l. in deciduous forest

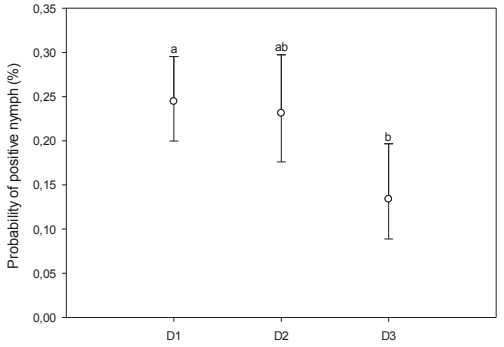

Figure 9b. *Rickettsia* in deciduous forest

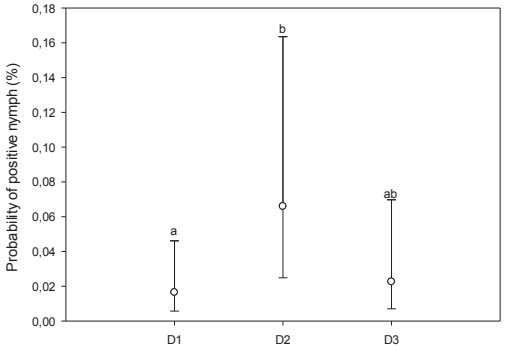

Figure 9c. *Candidatus* N. mikurensis in deciduous forest

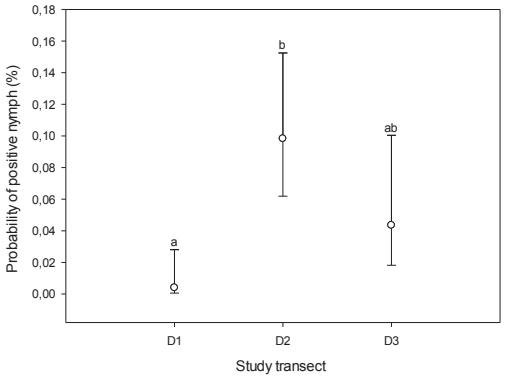

Figure 9d. *B. burgdorferi* s.l. in coniferous forest

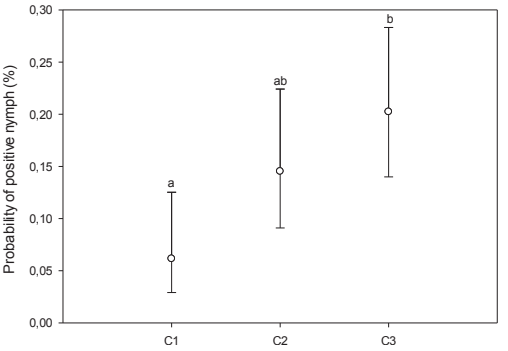

Figure 9e. *A. phagocytophilum* in coniferous forest

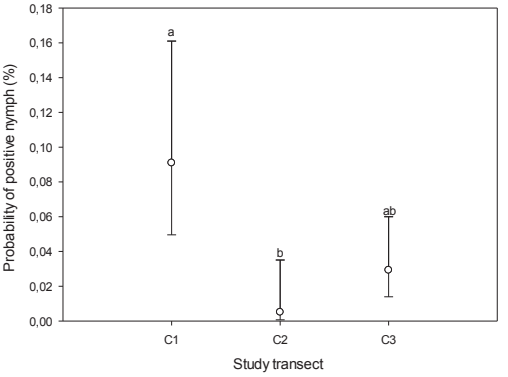

Figure 9. Estimated probabilities (with 95% confidence limits) of nymph samples being positive for *B. burgdorferi* s.l. and *A. phagocytophilum* within biotopes (across transects), as predicted by the GLMM. Different transects were assigned matching letters when no statistically significant differences between them could be identified ($p>0.05$). Mismatching letters denote statistically significant differences between transects with different letters ($p<0.05$).

4.4. The urban tick – *I. ricinus* presence in urban and suburban areas of Turku (IV)

To determine whether *I. ricinus* are commonly present in different urban and suburban areas in the city of Turku, cloth dragging was conducted at different study sites around the city between May-June 2017 (**IV**: Figure 1). This resulted in a total of 706 ticks collected from both urban and suburban areas around the city. Most of these were nymphs (481; 68.1% of all samples) and larvae (172; 24.4%), whereas adults were relatively rare (53; 7.5%). Tick densities varied greatly among different study sites (**IV:** Table 3). In general, the suburban islands Ruissalo and Hirvensalo had higher tick densities than mainland study areas, although the mainland University study area formed an exception, displaying relatively high tick densities (mostly due to high amounts of larvae being found there) (Table 1). Interestingly, ticks were also found from two city parks, Urheilupuisto and Samppalinna, which were previously found not to harbor ticks in 2000 and 2013 (Mäkinen *et al.* 2003) (**II**). All in all, ticks were found from each of the five major study areas, located in both suburban and urban parts of the city.

A total of 449 DNA samples extracted from 8 larvae, 388 nymphs, and 53 adults were screened for the presence of bacterial pathogens *B. burgdorferi* s.l., *B. miyamotoi*, *Rickettsia* spp., *A. phagocytophilum*, *Ca.* N. mikurensis, and *Bartonella* spp., as well as protozoan pathogens *Babesia* spp. Furthermore, a separate subset of 157 RNA samples (3 larvae, 147 nymphs, and 7 adults) was analyzed for the presence of TBEV. Altogether half (222/449) of the DNA samples were determined to carry at least one pathogen.

Borrelia burgdorferi s.l. were the most common pathogens detected, with 47.2% (25/53) of adults and 36.6% (142/388) of nymphs harboring the bacteria. On the genospecies level, *B. afzelii* was identified from 24.6% (35/142) of positive nymph samples, *B. garinii* from 19% (27/142), and *B. burgdorferi* s.s. from 23.2% (33/142); for positive adult samples, corresponding numbers were 16% (4/25), 4% (1/25), and 28% (7/25), respectively. The remaining samples could not be identified to the genospecies level. The probability of a nymph being positive for *B. burgdorferi* s.l. was higher [0.388 (95% CI: 0.366–0.443)] in suburban island samples than in mainland samples [0.258 (95% CI: 0.166–0.376)]. This difference was primarily caused by *B. garinii*, which was absent on the mainland but frequently (8.4%, 27/322) occurred in island samples.

Rickettsia spp. were also relatively common, with 11.1% (43/388) of nymphs and 11.3% (6/53) of adults being positive. One larva was also positive for the pathogen. For 39 of the positive samples, the species was identified as *R. helvetica* by sequencing. Contrary to *B. burgdorferi* s.l., the probability of a nymph being positive for *Rickettsia* was much lower on suburban islands [0.093 (95% CI: 0.066–0.131)] than on the more urbanized mainland [0.197 (95% CI: 0.118–0.311)].

The remaining bacterial and protozoan pathogens were detected more infrequently in samples. *Babesia* spp. and *Ca.* N. mikurensis were detected in 1.3% (5/388) and 2.3% (9/388) nymphs, respectively. Out of the *Babesia* positive samples, four were successfully sequenced and identified as *B. venatorum*. *Anaplasma phagocytophilum* was detected in 4.1% (16/388) of nymphs and 5.7% (3/53) of adults, and *B. miyamotoi* in 0.5% (2/388) of nymphs and 1.9% (1/53) of adults. The probability of a nymph to be

positive for *A. phagocytophilum* did not differ between island [0.037 (95% CI: 0.021–0.065)] and mainland [0.061 (95% CI: 0.023–0.151)] samples. All samples were found to be negative for *Bartonella* spp. and TBEV.

5. DISCUSSION

In this work I have successfully combined a diverse set of methods – ecological, molecular, and statistical – to assess changes in *I. ricinus* tick populations and associated pathogens in southwestern Finland. In the course of these studies, I confirmed the presence of several putative and dangerous pathogens in the study area and reported the occurrence of several TBPs for the first time from Finnish ticks. In addition, on Seili Island I observed the likely emergence of pathogens novel to the study area, highlighting that changes in the distributions of TBPs are also ongoing. Finally, I found that both tick and TBP numbers and the areas of their occurrence have increased in southwestern Finland in the 21st century. In the following sections I will discuss all these findings and the themes presented in the beginning in more depth, based on data gathered during the work for this thesis.

5.1. *Ixodes ricinus* occurrence in southwestern Finland

Overall, *I. ricinus* were found from 16/17 main study areas/groups in southwestern Finland, although at highly varying densities (Table 1). Consequently, the entire area appears to be highly endemic for *I. ricinus*, as suggested earlier by the (relatively) high amounts of diagnosed cases of tick-borne infections, as well as the results from the few tick-related studies conducted there (Mäkinen *et al.* 2003; Sormunen *et al.* 2016) (**I-II**). However, the highly varying tick densities, as well as the absence of ticks in certain study sites, also highlight the patchy distribution of ticks in different environments even in highly similar climates, and consequently the small spatial grain of their occurrence.

In general, the highest tick densities were observed on islands the furthest away from the mainland (Boskär, Berghamn & Seili), whereas islands on the whole had higher tick densities than mainland areas (Table 1; Figure 5). On the mainland, tick densities decreased when moving inland from the coast, with the only major study area from where ticks were not found being the 'Northwestern line' (**II)**, comprised of five inland study sites (Figure 5). Interestingly, in the study sites of the 'Northwestern line', abiotic factors were deemed to be suitable for tick inhabitation, despite the lack of *I. ricinus* found: there was ample litter or mosses, canopy cover, and moisture. However, in most of these study sites, I observed a pronounced absence of signs of host animals during cloth dragging: no droppings, no animal pathways or burrows, no signs of animal feeding. Consequently, if the host animals necessary for tick population upkeep are absent, so are, in all likelihood, the ticks themselves. These otherwise undocumented observations from the field highlight the problem with predicting tick or TBP occurrence solely based on abiotic and vegetation-related factors (Sonenshine & Roe 2014).

The proximity of large water bodies, the Baltic Sea in the case of southwestern Finland, appears to positively affect tick occurrence. A recent citizen science survey mapping *I. ricinus* and *I. persulcatus* distribution in Finland also suggested clustering along the seashore, as well as near large lakes in the

central, inland parts of Finland (although the method of data collection likely influences these results as well) (Laaksonen *et al.* 2017). Furthermore, similar observations have been made in other tick-related studies conducted in northern Europe (Jore *et al.* 2011; Jaenson *et al.* 2012). While the exact reasons for this phenomenon have not been thoroughly explored, they are likely linked to the regulating effects of large water bodies on the temperature and humidity of nearby areas, the tendency of water bodies to either isolate or aggregate host animal populations, or, more than likely, both of these factors together.

The distribution and density data I have gathered for this thesis forms a relatively comprehensive documentation of the current *I. ricinus* situation in southwestern Finland, particularly in the proximity of the city of Turku. Overall, 50 study sites were surveyed for the presence of ticks, effectively at least doubling the amount of localities with published *I. ricinus* density data from Finland. Likewise, for several study sites, data from two or more years in the 21st century now exists, enabling further longitudinal studies in the future. Prior to the data presented here, no published studies measuring tick densities over two or more years from the same location existed, making scientific assessments of changes in Finnish tick populations challenging. While there now exists a good base for continued monitoring in southwestern Finland, further studies in other parts of Finland are still required to form a complete picture of the nationwide (as well as regional) increases or decreases taking place in *I. ricinus* populations. A good start in this regard is the collaboration project started in 2014 between Finnish research stations (RESTAT) and the University of Turku tick project, annually monitoring tick occurrence and densities at research stations all over Finland.

Preferred biotopes and seasonal questing activity (I)

In chapter **I**, I studied the questing activity patterns and biotope preferences of *I. ricinus* on Seili Island. Different activity patterns were observed for different tick life stages, biotopes, and study transects. Coniferous forests displayed the highest tick density estimates, followed by deciduous forests. Meadows had the lowest overall densities. In general, forested areas had higher tick density estimates than open areas. Two pasture transects displayed surprising larvae peaks in July-August (Figure 7c).

The tick biotope distribution observed on Seili Island conforms well to other studies of *I. ricinus* conducted in the northern parts of Europe, with forested areas having the highest densities, wet areas such as alder thickets and marshes having intermediate densities, and open areas such as meadows, heaths, and pastures having the lowest densities (Mejlon & Jaenson 1993; Walker *et al.* 2001; Lindström & Jaenson 2003; Mäkinen *et al.* 2003). It is worth noting, however, that blanket dragging estimates tick questing activity at the time of sampling rather than absolute tick abundance. It is possible that tick numbers are in reality more similar between forested and open areas, but that other factors, such as direct sunlight or lack of shelter from drying winds in the open areas during daytime, force ticks to quest at different times, for example during nights in open areas (when dragging is generally not conducted).

Indeed, observations suggesting the possibility of tick diurnal patterns being, at least partly, short-term behavioral responses to local (micro)climatic conditions have been reported (Lees & Milne 1951; Randolph & Storey 1999; Perret *et al.* 2003; Sonenshine & Roe 2014). Particularly, high saturation deficit, which is a measure of atmospheric drying power derived from temperature and relative humidity, appears to trigger the descent of ticks from questing positions on the vegetation, leading to more ticks being found during nighttime than noon during hot and dry seasons (Randolph & Storey 1999; Perret *et al.* 2000; Sonenshine & Roe 2014).

While the presence and availability of suitable host animals may be the major determinant of tick occurrence in any given area, environmental conditions and suitable off-host habitats are major factors affecting the length of questing periods and overall survival of individual ticks (Perret *et al.* 2000; Herrmann & Gern 2010). In support of this, I discovered that relative humidity measured at the time of dragging had a positive effect on the number of host-seeking nymphs and adults found. However, no such connection was observed for larvae. The reasons for this remain undetermined, but may be related to the aggregated nature of larval distribution: one usually finds either many larvae or none. A large difference in larvae numbers between transects and dragging weeks in the analysis can obscure some more subtle factors affecting tick questing activity. While host animal abundance may be the major factor determining *I. ricinus* population size and overall occurrence on the island, these results support the notion that environmental conditions are important in determining where and when tick activity peaks occur (Jore *et al.* 2011; Gilbert *et al.* 2012; Jaenson *et al.* 2012).

In the statistical analysis of tick seasonal questing activity, there was some indication of bimodal occurrences for both larvae and adults+nymphs, particularly in transects with high tick densities, i.e., in forested biotopes (Figure 7). However, in some transects there were no clear activity patterns, and in pastures, larvae exhibited mostly unimodal questing activity. However, the sample sizes for some transects may have been too low for the analysis to discern any definite questing activity patterns. Furthermore, the seasonal questing activity patterns of *I. ricinus* are highly variable in relation to tick life stage and environmental conditions. Unimodal and bimodal activity patterns have both been reported from tick populations in northern Europe (Milne 1945; Nilsson 1988; Craine *et al.* 1995; Gray 2008). Consequently, while the diapause and hibernation -mediated onset of questing by different tick cohorts may be the major factor affecting seasonal questing activity patterns of ticks (Randolph *et al.* 2002), it seems that activity is likewise highly affected by local and current (micro)climatic conditions, and that no universal rules determining localized annual seasonal modality exist. Subsequently, no firm statements can be made regarding any recurring *I. ricinus* seasonal questing activity patterns on the island from this data (as has been shown by later surveys in Seili in 2013-17; unpublished data), nor the observed patterns expected to depict trends in other parts of southwestern Finland.

5.2. Occurrence of TBP's in southwestern Finland

Altogether seven pathogens or pathogen groups were detected from *I. ricinus* collected from different areas around southwestern Finland (ten if *B. burgdorferi* s.l. genospecies are counted separately). The study area with the most detected TBPs was Seili Island, from whence all seven (or ten) pathogens were detected. However, an overwhelmingly large number of samples were collected from Seili for analysis, so direct comparisons of pathogen diversity between Seili and other study areas are not justifiable. Interestingly though, the same pathogens apart from TBEV that were detected from Seili were also detected from Ruissalo, from where only a total of 336 samples were analyzed. *Bartonella* spp. were the only pathogen group not detected from any of the analyzed samples. *Bartonella* were also recently screened from approximately 3500 *I. ricinus* or *I. persulcatus* samples from all around Finland (Laaksonen *et al.* 2018), but no positive samples were found in that study either. As such, it would appear that this pathogen is not present in Finnish ticks. In the future, more comprehensive analyses for the whole pathogen data set (**I-IV**) from southwestern Finland are planned, with the aim of discovering whether any quantifiable environmental factors associated with different prevalence rates can be identified, and whether any spatially consistent correlations between and within the different pathogens exist.

Borrelia burgdorferi (s.l.) were the most commonly detected pathogens (Table 2). Similar results have been reported in several studies concerning the prevalence of various pathogens found from *I. ricinus* in Europe (Christova *et al.* 2003; Pichon *et al.* 2006; Skarphédinsson *et al.* 2007; Cotté *et al.* 2009; Richter & Matuschka 2011). Furthermore, the life stage distribution of infected ticks conforms to results reported from all over Europe (Hubálek & Halouzka 1997; Strnad *et al.* 2017), with adults having noticeably higher prevalence than nymphs. The prevalence rates observed for nymphs and adults in southwestern Finland ranged between 5.3–31.8% for nymphs and 9.1–58.8% for adults, although adult prevalence estimates may be inflated by low sample sizes in some cases. For many study areas, prevalence rates were higher than those commonly reported from Scandinavia (12.9±2.2% for nymphs and 21±4% for adults) (Strnad *et al.* 2017), as well as those observed for approximately 3500 tick samples collected from all over Finland in 2015 (14% for nymphs and 17% for adults) (Laaksonen *et al.* 2017). Out of the roughly 9000 larvae analyzed, two were found to carry *B. burgdorferi* s.l. Since prevalence was high in adults and nymphs collected from the same study localities, this finding supports the notion that transovarial transmission of *B. burgdorferi* s.l. from adult females to larvae is at most an exceedingly rare occurrence (Richter *et al.* 2012). In this particular case, the positive detections might have resulted from contamination during analysis or, for example, from interrupted feeding on an infected host, forcing the larvae back to questing. In conclusion, many localities in southwestern Finland display high *B. burgdorferi* s.l. prevalence compared to other areas in northern Europe, suggesting that local conditions are particularly suitable for the circulation of the pathogen.

Borrelia miyamotoi was detected from four islands and from all tick life stages, including nine larvae pools. The prevalence rates observed for adults and nymphs, ranging from 0.6–4.3%, were higher than those recently reported for *I. ricinus* in other parts of Finland (Wilhelmsson *et al.* 2013; Laaksonen *et al.* 2017), but in general conform to surveys conducted elsewhere in Europe, which have reported modest prevalence rates (0.17–3.8%) (Geller *et al.* 2012; Burri *et al.* 2014; Hansford *et al.* 2015; Kjelland *et al.* 2015; Quarsten *et al.* 2015). However, higher rates have also been observed elsewhere (Platonov *et al.* 2011; Crowder *et al.* 2014; Cochez *et al.* 2015), suggesting that *B. miyamotoi* can attain higher prevalence given suitable conditions and time. As such, foci with low prevalence could be either recently infested or in ecologically suboptimal areas. In samples from Seili, *B. miyamotoi* has been detected annually from 2012 to 2017 (**III:** Table 1), but prevalence has remained low. Nevertheless, these consecutive findings suggest that Seili is a locality endemic for the spirochete. Whether the low prevalence observed is due to recent arrival or suboptimal ecological conditions on the island, or some completely different influencing factor, remains to be determined. Regarding the detection of positive larvae pools, transovarial transmission of *B. miyamotoi* has been demonstrated previously (Wagemakers *et al.* 2015). However, whether infected larvae are able to transmit *B. miyamotoi* to humans or other animals is unclear.

Following *B. burgdorferi* s.l., *Rickettsia* spp. were the second most common pathogens detected (prevalence range 0.67–25% for nymphs, 3.3–21.7% for adults). There seems to be great variation in *Rickettsia* prevalence across European tick populations, ranging from low to high values in a similar fashion to those reported here (1.9–58% for adults; 1.12–18% for nymphs; 1.7–16% in studies where adults and nymphs were combined) (Nilsson *et al.* 1997; Nilsson *et al.* 1999; Christova *et al.* 2003; Hartelt *et al.* 2004; Halos *et al.* 2006; Cotté *et al.* 2009; Kantsø *et al.* 2010). While *Rickettsia* spp. have been reported from ticks in many European countries, they had not been screened from Finnish ticks prior to the study in chapter **II**. Furthermore, the only species that had been reported from Nordic countries was *R. helvetica* (*Ca.* R. tarasevichiae has only recently been reported from Finnish *I. persulcatus*) (Laaksonen *et al.* 2018), whereas in my studies not only *R. helvetica*, but also *R. monacensis* was detected. While the presence of *R. helvetica* was expected based on the studies conducted in Sweden, Norway, and Estonia (Nilsson *et al.* 1997; Nilsson *et al.* 1999; Severinsson *et al.* 2010; Katargina *et al.* 2015; Quarsten *et al.* 2015), the detection of *R. monacensis* was somewhat unexpected. This species has been reported from ticks in nearby Poland and Estonia, and very recently from other parts of Finland (Rymaszewska & Piotrowski 2013; Katargina *et al.* 2015; Laaksonen *et al.* 2018), but no prior findings have been reported from questing ticks in the northernmost parts of Europe. While no patient cases linked with *R. monacensis* (or *R. helvetica*) have been reported from Finland, reports from Spain and Italy suggests that it is, as is *R. helvetica*, capable of human infection (Jado *et al.* 2007; Madeddu *et al.* 2012). *Rickettsia* were also the most common pathogens detected from *I. ricinus* larvae (40 positive pools; Table 2). Indeed, as with *B. miyamotoi*, vertical transmission of at least some species of the genus has been demonstrated (Burgdorfer & Brinton 1975; Burgdorfer *et al.* 1979; Sprong *et al.* 2009).

Anaplasma phagocytophilum was detected from *I. ricinus* nymphs and adults in three different study sites (Table 2). Relatively similar prevalence rates were observed for the two study sites with high sample sizes (Seili & Ruissalo), with nymph prevalence rates 3.4% and 3.5%, and adult prevalence rates 9.9%

and 8.7%, respectively. Two studies conducted in the early 2000s previously screened the pathogen from Finnish *I. ricinus* and *I. persulcatus*, but no ticks were found to carry the pathogen back then (Mäkinen *et al.* 2003; Alekseev *et al.* 2007). A more recent survey of ticks collected from all around Finland in 2015 revealed a lower total prevalence of 0.6% (Laaksonen *et al.* 2018). All in all, an overall median prevalence of 3% has been reported for the pathogen from *I. ricinus* in Europe, but with pronounced differences between countries, study areas, and tick life stages (Strle 2004; Dumler *et al.* 2005; Stuen 2007; Severinsson *et al.* 2010). Indeed, similar variability in occurrence seems to exist within southwestern Finland as well: while the prevalence rates observed in study sites from where positive samples were found were relatively high, most study sites, even in close proximity, displayed a complete lack of the pathogen (Table 2; Figure 5).

The protozoan pathogen *B. venatorum* (previously *Babesia* sp. EU1) was detected from the same study sites as *A. phagocytophilum*, apart from Hirvensalo, forming the first report of the pathogen from Finnish ticks. Of the genus *Babesia*, *B. microti* had previously been reported from Finnish *I. persulcatus* in 2007 (Alekseev *et al.* 2007) and *B. divergens* from *I. ricinus* in a more recent survey in 2018 (Laaksonen *et al.* 2018). Prevalence rates of *Babesia* reported from European *I. ricinus* typically range between 0.3–4.7% for nymphs and 0.6–3.6% for adults, but rates as high as 8–12% for nymphs have also been reported (Stańczak *et al.* 2004; Casati *et al.* 2006; Siński *et al.* 2006; Katargina *et al.* 2011; Schorn *et al.* 2011; Øines *et al.* 2012; Overzier *et al.* 2013a; Overzier *et al.* 2013b; Karlsson & Andersson 2016). The rates observed in southwestern Finland were on the low end of the reported range, apart from two study areas which had high prevalence for some tick life stages, possibly due to inflated estimates caused by low sample sizes (Table 2). *Babesia venatorum* was the second most commonly detected pathogen from larvae pools with 15 detections. Indeed, transovarial transmission has been established for at least some *Babesia* species (Gray *et al.* 2010). While the medical interest in rare tick-borne pathogens may in many cases be eclipsed by the causative agents of Lyme borreliosis and TBE, *Babesia* likely warrants additional surveillance. This is because co-infections of *Babesia* and the most common tick-borne pathogen, *B. burgdorferi* s.l., appear to be able to cause more severe diseases in humans (Krause *et al.* 1996), and their co-occurrence in ticks is possible (e.g. **III:** Table 3). In fact, a rare fatal case of babesiosis has been reported from a co-infected man in Finland (Haapasalo *et al.* 2010).

Candidatus Neoehrlichia mikurensis was detected from two islands, Ruissalo near the city of Turku and Seili in the Archipelago Sea. In Seili, the pathogen was detected only during the last three years of the six year study (2015-2017). As such, these results suggest that emergence of the pathogen may have occurred in the islands tick population during the past few years. In the northern parts of Europe, *Ca*. N. mikurensis has been reported from ticks and/or host animals in nearby Russia, Sweden, Norway, and Estonia (Silaghi *et al.* 2016; Portillo *et al.* 2018). The overall prevalence observed for *Ca*. N. mikurensis here was somewhat low (1.7% for nymphs; absent in adults) compared to other European studies (Silaghi *et al.* 2016; Portillo *et al.* 2018). However, at least for Seili, from where comprehensive longitudinal data is available, this might be explained by the potentially recent emergence of the pathogen. Interestingly, nearly half (21/45) of all *Ca.* N. mikurensis detections were co-infections with *B. burgdorferi* s.l. (e.g. **III:** Table 3). Similar trends regarding the co-occurrence of these two pathogens have been detected in

other studies as well (Andersson *et al.* 2013; Glatz *et al.* 2014; Kjelland *et al.* 2018; Laaksonen *et al.* 2018), and the finding seems to be linked to wild rodents (especially the bank vole, *Myodes glareolus*) as common reservoir hosts for both pathogens (Andersson & Råberg 2011; Andersson *et al.* 2013). On the medical front, reports of human neoehrlichiosis cases have started to emerge from Europe during the past few years. No cases have thus far been reported from Finland, but this novel pathogen warrants close inspection by both researchers and healthcare professionals, particularly because of its tendency for co-occurrence with *B. burgdorferi* s.l., and the potentially amplified and/or abnormal symptoms such co-infections may manifest in (Swanson *et al.* 2006).

Tick-borne encephalitis virus was detected only on Seili and only during the two last years of study there (2016-17; **III** Table 1). As a high amount of samples from Seili were screened prior to the initial detection in 2016, these results suggest that the virus emerged on the island during our studies between 2012 and 2017. The fact that TBEV was not detected elsewhere is somewhat surprising, as southwestern Finland as a whole (including the Åland Islands) is considered a TBEV risk area, which still constitutes a major region for human TBE cases in Finland despite active vaccination campaigns there (26.3–75% of annually diagnosed TBEV cases in Finland between 1995–2017; NIDR) (Tonteri *et al.* 2015). However, the prevalence of TBEV in Finnish *I. ricinus* is typically quite low (0.5–2%) (Jääskeläinen *et al.* 2006; Jääskeläinen *et al.* 2010; Jääskeläinen *et al.* 2016), which may explain why the virus was only found from Seili, from where nearly 2000 nymphs were analyzed. Overall, TBEV has been reported from *I. ricinus* in many countries in northern Europe, with prevalence rates comparable to those observed in Finland, although higher rates have also been reported (Pettersson *et al.* 2014). The possible emergence of this dangerous virus in the *I. ricinus* population on Seili Island serves as an example of the constantly shifting distributions of vector-borne pathogens of medical importance, likely driven – or at least accelerated – by the changing climate (Gray *et al.* 2009).

Small spatial scale of pathogen occurrence

In chapter **III**, I surveyed the occurrence of various TBPs in different biotopes and specific study transects on Seili Island. Differences in nymph prevalence estimates for the two most common TBPs on the island, *B. burgdorferi* s.l. and *A. phagocytophilum,* were detected between both biotopes and study transects (Figure 8 & 9). For *Rickettsia*, the models were not statistically significant, signaling that no differences could be determined from the available data. On the biotope level, the highest prevalence of *B. burgdorferi* s.l. in nymphs was detected in deciduous forests and meadows, whereas pastures exhibited the lowest prevalence. However, in contrast to having the lowest *B. burgdorferi* s.l. prevalence, pastures displayed the highest prevalence of *A. phagocytophilum* in nymphs, while forested habitats (deciduous/coniferous forests and alder thickets) had much lower prevalence. Regarding specific study transects, I was unfortunately unable to analyze all biotopes, mostly due to the manner of sampling leading to highly varying sample sizes across biotopes and transects. However, where such analyses

could be carried out, they revealed differences in *B. burgdorferi* s.l. and *A. phagocytophilum* prevalence among transects in coniferous forest, as well as between *B. burgdorferi* s.l., *Rickettsia*, and *Ca*. N. mikurensis prevalence among transect in deciduous forest. A trend similar to that observed on the biotope level was observed on the transect level in coniferous forests: one transect had lower *Borrelia* prevalence, but contrasting higher *A. phagocytophilum* prevalence, than the other two transects (Figure 9).

These results should not be interpreted as depicting any major trends in the occurrence of pathogens in different biotopes, but rather as indication that even within the confines of a small island, not only biotopes, but even individual areas (i.e. transects) within a biotope can differ from each other in regard to pathogen occurrence. As such, they highlight the small scale at which local biotope, microclimate, and/or host animal communities may potentially affect the spatial distribution of pathogens. In a recent study, *Boehnke et al.* found that humidity can vary not only between open areas and forests, but also within different layers of a forest, directly affecting questing conditions for ticks (Boehnke *et al.* 2017). Indeed, the same is likely true for various other variables affecting tick questing and living conditions in different biotope types and structures [e.g. ground floor temperatures in shaded forests vs. open meadows (biotope type), different levels of canopy cover and wind shelter based on tree density (forest structure)] (Geiger *et al.* 2009). In this regard, the biotope classification applied in the current study, as well as similar classifications that are often used in tick related studies, provide only rough estimations of *in situ* conditions for ticks and associated pathogens. Consequently, such classifications may fail to identify important differences influencing tick and pathogen occurrence across areas. Further focus should be placed on more precise measurements of abiotic and biotic factors associated with the realized occurrence of ticks and tick-borne pathogens. By closely examining and identifying variables specifically affecting the occurrence of each individual pathogen (or pathogen group), we may gain further insight into the factors driving their spread and circulation in tick populations. Consequently, such data could lead to both more precise mapping of tick and pathogen occurrence as well as more accurate predictive models, which are required in order to prevent human infections.

5.3. Temporal changes in tick and TBP populations

In the past few decades, several changes in *I. ricinus* populations and associated pathogens have been observed in the northern parts of Europe. In Finland, a countrywide citizen science survey conducted in 2015 revealed that *I. ricinus* distribution has shifted some 100-200 km northwards since the previous mapping in the late 50's, and that *I. persulcatus* now co-inhabit most areas of the country (Öhman 1961; Laaksonen *et al.* 2017). Furthermore, while longitudinal studies regarding ticks are rare in Finland, and therefore scientific reports of changes in their occurrence and abundance have not existed prior to the studies presented here, numerous reports of increasing tick numbers from citizens in the 21st century have hinted at increasing tick abundance. Likewise, the numbers of clinically diagnosed cases of Lyme borreliosis and TBE that have been constantly increasing likewise suggest increasing human contact with ticks. Similar observations have been made in neighboring Sweden, where surveys of long-term changes

in the geographical distribution and abundance of *I. ricinus* have revealed a northwards shift in geographical distribution and an increase in abundance in established tick areas over the past few decades (Tälleklint & Jaenson 1998; Jaenson & Lindgren 2011; Jaenson *et al.* 2012). Furthermore, *I. ricinus* are now found further north and at higher altitudes in Norway than reported in historical records (Jore *et al.* 2011).

These changes in Fennoscandia have largely been attributed to various effects of climate change (Lindgren *et al.* 2000; Jaenson *et al.* 2012). Climate change can affect *I. ricinus* distribution and abundance through several means: by facilitating changes (mostly increases) in the abundance or behavior of important host animals such as roe deer (*C. capreolus*), white-tailed deer (*O. virginianus*), and raccoon dogs (*N. procyonoides*) (Mysterud & Østbye 2006; Rizzoli *et al.* 2009; Dobson & Randolph 2011; Jaenson & Lindgren 2011; Jaenson *et al.* 2012); through milder winters and extended spring and autumn seasons in the northern hemisphere, allowing for longer tick activity seasons (Gray *et al.* 2009; Medlock *et al.* 2013); and through faster tick developmental rates due to higher average temperatures, potentially accelerating tick life cycles (Campbell 1948; Jaenson & Lindgren 2011; Sonenshine & Roe 2014). The degree to which any of these factors specifically influence the observed changes has not been determined. In fact, they likely impact tick populations differently in different areas.

5.3.1. Recent changes in tick and TBP populations in SW Finland

Ixodes ricinus densities and *B. burgdorferi* s.l. and *A. phagocytophilum* occurrence were previously investigated in ten of the study sites presented here by Mäkinen *et al.* (2003) (Table 3). Regarding tick density estimates, higher values were observed in all study sites in 2013-2017 than in 2000. For two city parks in Turku (Samppalinna & Urheilupuisto), no ticks were detected in 2000 or 2013 (**II**), but were found in 2017 (**IV**). Furthermore, in study sites with several years of data (Seili, Ruissalo, Hirvensalo, Urheilupuisto, Samppalinna), the last year of study (2017) displayed the highest tick density estimates.

A similar trend was observed for the two TBPs available for comparison, *B. burgdorferi* s.l. and *A. phagocytophilum*. In 2000, *B. burgdorferi* s.l. were detected from four out of the ten study sites, whereas in 2013-2017 they were detected from eight out of ten sites. The only two sites without positive samples in 2017 were city parks Samppalinna and Urheilupuisto, from where very few samples were analyzed in total (1 and 3, respectively). While sample sizes were in some cases too low for reliable prevalence estimates, higher rates were generally observed in all study sites in 2013-2017.

For *A. phagocytophilum*, all samples analyzed in 2000 were found negative, whereas the bacterium was found from three sites in the studies of this thesis. Furthermore, it was reported from three additional study areas (Boskär, Berghamn & Pähkinäinen) in 2016 (Sormunen *et al.* 2016). As such, these data suggest that the pathogen has emerged in southwestern Finland sometime between the studies conducted in 2000 and 2012-2017.

All in all, these results are in line with results from neighboring Sweden, where the abundance and *Borrelia* prevalence of *I. ricinus* has reportedly increased in established tick areas over the past few decades. It would appear that some changes in the environment are increasing tick numbers in northern Europe, as well as amplifying and/or shifting the distribution of at least some pathogens. However, the degree to which these changes are caused specifically by global warming is still uncertain (Randolph 2004a; Gray *et al.* 2009). These observed trends are somewhat disconcerting, as increases in the amount of vectors available (ticks) as well as the rate at which they carry pathogens are both factors that increase human tick risk. Should similar trends be taking place in other established tick areas in Finland as well, further healthcare problems caused by TBPs are unfortunately likely.

Further longitudinal observations of tick-borne pathogens from Seili Island

On Seili Island, *I. ricinus* were screened for all the listed TBPs from 2012 to 2017. Overall, five out of seven pathogens were detected annually (**III**: Table 1). A further two (*Ca.* N. mikurensis & TBEV) were detected annually after their initial discoveries in 2015 and 2016, respectively. While the occurrence of the observed pathogens seems relatively stable on the island, some annual fluctuations in prevalence were noticeable for all of them, without any apparent or distinct patterns or trends (**III:** Table 1). Interestingly, *Babesia* and *Rickettsia* were only seldom detected from adult ticks, despite adults typically displaying higher prevalence rates. Furthermore, for *Ca.* N. mikurensis and TBEV, no positive adults were detected at all, despite several positive nymphs. Whether the cause for the absence (or low prevalence) of these pathogens in adults is chance, faster host finding by adults carrying the pathogens, low sample sizes, or some environmental factors on the island causing higher mortality for carrier nymphs/adults remains unknown.

Three pathogens were detected from larvae in Seili: *R. helvetica*, *B. venatorum*, and *B. miyamotoi*. The former two were detected in all but one year, whereas *B. miyamotoi* was only detected during the last two years of the study (**III**: Table 2). These results suggest, to some extent, that transovarial transmission may be an important part of *R. helvetica* and *B. venatorum* circulation in nature, whereas for *B. miyamotoi* its effect seems lesser. However, the somewhat lower overall prevalence of *B. miyamotoi* may affect this observation. Furthermore, some unknown factors may reduce the proportion of larvae found by cloth dragging carrying the pathogen in nature, such as faster host animal finding due to increased activity or higher resistance to desiccation of the larvae, which may be caused by the pathogens inhabiting the ticks (Herrmann & Gern 2015).

5.4. Tick risk in urban areas of Turku

The risk that ticks pose to humans is dependent on two factors: the likelihood to encounter a tick (tick abundance × human activity) and the likelihood of the encountered tick to be infected (tick pathogen prevalence) (Sonenshine & Roe 2014). Therefore, a highly infected and abundant tick population on an isolated island does not inherently form a tick risk area from a medical point of view. Likewise, even relatively low tick densities and TBP prevalence can be an important source of human pathogen exposure in urban and suburban recreational areas, and possibly even in maintained city parks, due to the high numbers of possible contacts between humans and ticks (Rizzoli *et al.* 2014; Paul *et al.* 2016). In this context, it is somewhat alarming that several investigations conducted in urbanized areas (reviewed by Rizzoli *et al.* 2014), for example in Warsaw (Poland), Helsinki (Finland), Košice (Slovakia), and near Paris (France), have indicated well-established tick populations with corresponding, or even higher, prevalence of tick-borne pathogens when compared to endemic, more natural areas nearby (Junttila *et al.* 1999; Pangrácová *et al.* 2013; Paul *et al.* 2016; Kowalec *et al.* 2017). Indeed, results from chapter **IV** show similar trends for urban and suburban areas in the city of Turku.

In the studies of urban and suburban areas in the city of Turku in 2017, *I. ricinus* were detected from all major study sites (Table 2). In general, tick densities were higher on the two suburban islands (Ruissalo & Hirvensalo) than in urban and suburban mainland areas, although the University study area provided higher density estimates than Hirvensalo. Whenever nymphs were found, adult ticks were also detected, although at much lower densities. Tick larvae were likewise found from Ruissalo, the western side of the city, and the University study area. These findings suggest that full life cycles of *I. ricinus* are carried out at urbanized areas all around the city of Turku. Whereas several major host animals for nymphs and adults can only be found consistently from Ruissalo and Hirvensalo (such as roe deer, white-tailed deer, and raccoon dogs), a high diversity of other hosts are available for ticks in both urban and suburban habitats, such as birds, rodents (*Microtus agrestis, Myodes glareolus, Apodemus flavicollis*), hedgehogs (*Erinaceus europaeus*), shrews (*Sorex* spp.), European hares (*Lepus europaeus*), and even predators like red foxes (*Vulpes vulpes*) and mustelids (*Mustela erminea* & *M. nivalis*). Although the rich mammalian and avian fauna within Turku is thoroughly addressed in some years and at a general level (e.g. (Lappalainen & Vuorisalo 1996; Laine & Lehikoinen 2013; Tirri & Vösa 2015)), directly connecting tick observations of the summer 2017 to specific host animal abundances that prevailed in specific tick sampling areas at that time (or during some years prior to the collection, as tick life cycles may take several years) was deemed impossible.

In addition to the ticks themselves, several TBPs were detected from both urban and suburban study areas. Most pathogens were detected approximately equally from suburban and urban study sites (**IV:** Table 3). However, *B. burgdorferi* s.l. were detected more often from suburban island samples, mostly due to one genospecies, *B. garinii*, which was present in 8% of island nymphs but absent in mainland ticks. While the exact reasons for this phenomenon cannot be determined from this data, the observation may be linked to songbirds as favored reservoir hosts of *B. garinii* (Kurtenbach *et al.* 2002; Taragel'ová

et al. 2008; Jahfari *et al.* 2017). Overall, the densities of most species of birds are higher in the more natural, semi-urban environments of Ruissalo and Hirvensalo than in the maintained and structured environments of the city proper. Along with dozens of other species (Laine & Lehikoinen 2013), commonly reported bird hosts of ticks, thrushes (*Turdus* spp.) and European robins (*Erithacus rubecula*) (Taragel'ová *et al.* 2008; Marsot *et al.* 2012; Heylen *et al.* 2017), are abundant nesting birds on the suburban islands. While the same species also inhabit more urban areas of Turku, such as the University campus area (Tirri & Vösa 2015), their importance as hosts might be lesser there because of, for example, more patchily distributed habitats and because of the tendency of some thrushes to forage on maintained park lawns in urban areas, which are not suitable for tick population upkeep. Hence, reduced contact with the needed reservoir hosts, for whatever reason, may inhibit the establishment of *B. garinii* in urban city environments. In any case, this observation serves as yet another interesting example of the small spatial grain at which the occurrence of pathogens may vary.

Interestingly, in contrast to *B. garinii*, *Rickettsia* (mostly *R. helvetica*) were detected considerably more often from urban mainland nymphs than their islandic counterparts. As discussed above, differences in available host animals and the spatially restricted habitats available in more structured urban areas may influence this. As high nymphal *Rickettsia* prevalence is likely, to a large extent, a consequence of a higher proportion of tick larvae feeding on infected reservoir hosts, the higher infection rate is expected to be caused by differences in larval host utilization. Unfortunately, comprehensive data on the occurrence of larval hosts (rodents, shrews, hedgehogs, birds) does not exist for the study areas. However, some commensal rodent species, such as rats, may well be more abundant near human dwellings than in the wilderness. Furthermore, in urban habitats, the space available for ground-dwelling animals is often more restricted than in larger, more natural forest areas (in this case, Ruissalo and Hirvensalo), which may consequently cause higher relative densities of host animals in the patchy areas suitable for urban inhabitation.

In conclusion, taking into account the high numbers of humans visiting these urban and suburban areas (for example, hundreds of thousands of people annually visit Ruissalo alone), the relatively high tick abundance observed particularly in suburban but also in urban areas, and *B. burgdorferi* s.l. and *Rickettsia* prevalence rates that are in some cases even higher than those observed in more natural environments in southwestern Finland, it would appear that urban ticks may potentially form a larger threat to human welfare in the Turku region than previously thought. Further efforts should be made by officials to increase tick awareness even in urban areas, where people may generally assume they are safe from tick bites and tick-borne diseases.

6. CONCLUSIONS

This thesis, in conjunction with other recent publications (Sormunen *et al.* 2016; Laaksonen *et al.* 2017), reveals that changes similar to those observed in neighboring Sweden and Norway, likely mediated by the direct and indirect effects of climate change, are taking place in the *I. ricinus* populations in southwestern Finland (Tälleklint & Jaenson 1998; Jaenson *et al.* 2012). The numbers of ticks are increasing, as observed in several study sites around the area (**I-II, IV**). Likewise, ticks seem to be present at more and more locations, as exemplified by the high amounts of *I. ricinus* being found from areas around the city of Turku, as well as the detection of ticks in 2017 from two urban city parks that were previously found not to harbor ticks in 2000 and 2013 (**II, IV**) (Mäkinen *et al.* 2003). Similar shifts seem to be taking place regarding tick-borne pathogens: higher prevalence rates were measured in many study sites - and Lyme borreliosis spirochetes detected altogether from more sites - in recent years than before (**I-IV**) (Mäkinen *et al.* 2003). Finally, long-term studies conducted on Seili observed the possible emergence of two tick-borne pathogens on the island, highlighting that not only those of ticks, but also the distributions of pathogens are experiencing shifts (**III**).

The increase in tick abundance and distribution, as well as higher prevalence rates of TBPs in ticks, directly increases human tick risk (Sonenshine & Roe 2014). Whereas ticks have in previous years often been categorized as a threat mostly to people in a line of work that exposes them to contact (for example, forestry) and people commonly enjoying outdoor activities, the studies in different urban and suburban areas of Turku revealed that this is not the case, at least anymore (**IV**). This trend of ticks occupying more urban and suburban habitats may also be seen in the increasing numbers of Lyme borreliosis cases since the mid-90's – such an increase in patient cases can likely not only be attributed to increasing tick numbers in traditional tick-endemic areas, but also to increased contact with ticks for many people that have previously seldom encountered them (Sajanti *et al.* 2017). It therefore seems likely that people not belonging to the traditional risk groups are also getting infected more often, possibly due to ticks inhabiting habitats in closer proximity to areas of dense human inhabitation, i.e. urban and suburban areas in cities and towns. In order to potentially reduce tick risk in such areas of high human activity, more studies in urban environments are required, as too little is known about the dynamics of tick populations living in urban areas, where habitats are more restricted and from where many important host animals are missing (Rizzoli *et al.* 2014).

In addition to tick numbers and the prevalence of Lyme borreliosis spirochetes, also the overall diversity of pathogens carried by Finnish ticks seems to be increasing, although it is difficult to assess how much of this newly detected diversity can be attributed to the lack of earlier studies. In the chapters of this thesis, I report for the first time the occurrence of five tick-borne pathogens new to Finnish ticks (**I-IV**). While some of these had never been screened previously, for some (mainly *A. phagocytophilum*), there was enough data to suggest recent emergence in the tick populations of southwestern Finland (**III-IV**) (Mäkinen *et al.* 2003; Sormunen *et al.* 2016). Furthermore, an additional three novel pathogen species have recently been reported from Finnish *I. ricinus* and *I. persulcatus* in a study by the University of

Turku tick project (Laaksonen *et al.* 2018). As patient cases linked to most of these newly detected TBPs have been focused on immunocompromised persons, their medical importance may be expected to be relatively low compared to Lyme borreliosis and TBE. Increased vigilance is nevertheless required from medical professionals in order to identify potential disease cases, particularly as co-infections with different TBPs may cause more severe and abnormal symptoms (Krause *et al.* 1996; Swanson *et al.* 2006).

Finally, a theme observed throughout the chapters of this thesis is the small spatial grain of tick and TBP occurrence. Regarding the occurrence of *I. ricinus*, we observed differences not only between mainland and island study sites in southwestern Finland (**II, IV**), but also different areas within the city of Turku (**II, IV**), and even between different biotopes and transects (i.e. specific areas within biotopes) on a small island (Seili Island, surface area 1.6 km^2) (**I**). The same is true regarding the occurrence of TBPs: differences were observed between mainland and island sites (**II, IV**), different areas of Turku (**II, IV**), and different biotopes and study transects on Seili (**III**). Apart from TBE, against which a preventive vaccine exists, the best way to prevent tick-borne infections is to avoid tick risk areas. However, mapping tick and TBP occurrence over vast geographical areas by cloth dragging is virtually impossible. Therefore, researchers have started to focus on wide-scale GIS-based predictive mapping of risk areas (Rizzoli *et al.* 2002; Medlock *et al.* 2008; Boehnke *et al.* 2015; Vourc'h *et al.* 2016). However, considering that the spatial grain of tick and TBP occurrence appears to be exceedingly small, mapping done with inadequate data of local environmental factors (including host animal populations) can be misleading, potentially even subjecting more people to infections if the results of these models are made public (for example, by inadvertently assigning high risk areas as low risk areas) (Estrada-Peña *et al.* 2015; Boehnke *et al.* 2017). As such, increased effort should be placed on more precise measurements and reporting of the various environmental factors associated with observed tick and TBP populations, in order for us to be able to more accurately predict and model tick risk areas and, consequently, prevent human infections.

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