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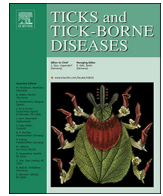
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Original article

Monitoring of ticks and tick-borne pathogens through a nationwide research station network in Finland

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ABSTRACT

In 2015 a long-term, nationwide tick and tick-borne pathogen (TBP) monitoring project was started by the Finnish Tick Project and the Finnish Research Station network (RESTAT), with the goal of producing temporally and geographically extensive data regarding exophilic ticks in Finland. In the current study, we present results from the first four years of this collaboration.

Ticks were collected by cloth dragging from 11 research stations across Finland in May–September 2015–2018 (2012–2018 in Seili). Collected ticks were screened for twelve different pathogens by qPCR: *Borrelia afzelii*, *Borrelia garinii*, *Borrelia valaisiana*, *Borrelia burgdorferi* sensu stricto, *Borrelia miyamotoi*, *Babesia* spp., *Anaplasma phagocytophilum*, *Rickettsia* spp., *Candidatus Neoehrlichia mikurensis*, *Francisella tularensis*, *Bartonella* spp. and tick-borne encephalitis virus (TBEV).

Altogether 15 067 *Ixodes ricinus* and 46 *Ixodes persulcatus* were collected during 68 km of dragging. Field collections revealed different seasonal activity patterns for the two species. The activity of *I. persulcatus* adults (only one nymph detected) was unimodal, with activity only in May–July, whereas *Ixodes ricinus* was active from May to September, with activity peaks in September (nymphs) or July–August (adults). Overall, tick densities were higher during the latter years of the study. *Borrelia burgdorferi* sensu lato were the most common pathogens detected, with $48.9 \pm 8.4\%$ (95% CI) of adults and $25.3 \pm 4.4\%$ of nymphs carrying the bacteria. No samples positive for *F. tularensis*, *Bartonella* or TBEV were detected.

This collaboration project involving the extensive Finnish Research Station network has ensured enduring and spatially extensive, long-term tick data collection to the foreseeable future.

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1. Introduction

Ticks and tick-borne pathogens (TBPs) constitute a growing threat to public health in Europe. In Northern Europe, TBPs are transmitted to humans mainly by two generalist tick species, the castor bean tick (*Ixodes ricinus*) and the taiga tick (*Ixodes persulcatus*) (Laaksonen et al., 2017; Parola and Raoult, 2001; Petney et al., 2012; Süss, 2011; Zygtiene, 2011). The geographical distributions of these species have increased over the past few decades in Fennoscandia, a geographical region encompassing Finland, Norway, Sweden and regions of Russia in the Kola Peninsula and Karelia (Bugmyrin et al., 2019; Jaenson et al., 2012; Jore et al., 2011; Laaksonen et al., 2017). Furthermore, increases in tick abundance have also been observed particularly in the southernmost parts of Fennoscandia, although in Russian Karelia, the abundance of *I. persulcatus* has recently been observed to be declining, following an increase at the start of the millennium (Bugmyrin et al., 2019; Jaenson et al., 2012; Sormunen, 2018; Sormunen et al., 2016a,2016b). In Finland, increases in tick abundance have been accompanied by a rise in human tick-borne diseases (TBDs): the annual numbers of clinically diagnosed cases of Lyme borreliosis have been increasing since the mid 1990's (Sajanti et al., 2017).

As there appears to be little that can realistically be done to cull tick populations using existing methodology (Beaujean et al., 2016; Ostfeld et al., 2006; Stafford III et al., 2017; Van Buskirk and Ostfeld, 1995), increasing citizens' awareness regarding ticks and tick-borne pathogens is particularly important in preventing TBDs (Beaujean et al., 2016; Butler et al., 2016; Zöldi et al., 2017). In order to efficiently target intervention campaigns aimed at reducing human disease cases, information of gaps in citizens' knowledge is required. However, also data on the particular properties of local tick populations is vital, as the activity patterns and abundance of ticks are dependent on prevailing environmental conditions (Gray, 2008; Sirotkin and Korenberg, 2018; Uspensky, 2016). Consequently, differences in abundance or activity may occur especially between far-apart areas, as ticks face different environments and host animal populations. These differences, in all likelihood, also affect local tick bite risk (the chance to get infected by a TBD) and its seasonal patterns, highlighting the importance of localized knowledge regarding ticks. Furthermore, in order to be able to accurately predict future changes in tick bite risk, long-term data of tick and TBP populations are needed (Bugmyrin et al., 2019). Such longitudinal data has thus far largely been missing from Finland, where few tick-related studies have been conducted prior to the current decade (Sormunen, 2018).

Observing tick abundance and phenology requires field collections, targeting either the ticks themselves (questing ticks) or their host animals (feeding ticks) (Bugmyrin et al., 2019; Cayol et al., 2017; Nilsson, 1988). Naturally, for long-term studies, such collections also have to be conducted frequently, preferably annually. Furthermore, in order to observe tick-related phenomena across vast geographical ranges, several study localities are needed to form a comprehensive view. While organizing an effort of this magnitude is possible for individual projects or project collaborations, these are often only as long-term as the project's funding. Therefore, to ensure enduring data collection, such sampling schemes would be best undertaken by instances backed by national governments.

In Finland, government-supported Universities uphold research stations across the nation (Fig. 1). These stations are optimal bases for wide-scale and long-term tick sampling schemes, as they are occupied by researchers or technical staff throughout the year, and thus also during the tick activity period. Consequently, in 2012, a long-term research regarding ticks and tick-borne pathogens was started on Seili Island, which hosts the Archipelago Research Institute of the University of Turku (Sormunen et al., 2018; Sormunen et al., 2016a,2016b). In 2015, ten other research stations joined in collaboration, forming a nationwide network of data collection points for long-term tick monitoring (Fig. 1).

In the present study, we introduce the nationwide, long-term sampling scheme organized by the Finnish Tick Project and Finnish research stations (RESTAT; www.researchstations.fi). Furthermore, we present results regarding tick activity and abundance, as well as tick-borne pathogen prevalence and diversity, from the first four years (2015–2018; 2012–2018 for Seili) of this ongoing research collaboration.

2. Materials and methods

2.1. Start of the collaboration

Field sampling of ticks was harmonized between research stations in a joint field campaign in the spring of 2015, when tick researchers from the Finnish Tick Project participated in a research station meeting, in which personnel from all the Finnish research stations participate (see Technical Appendix for research station georeference data). Tick collection was demonstrated to research station personnel, and identical sampling kits were distributed to all stations.

2.2. Field collections

Ticks were collected by cloth dragging from May to September in 2015–2018 (2012–2018 for Seili; 2015–2016 for Muddusjärvi) at eleven participating research stations: Seili, Husö, Tvärminne, Konnevesi, Lammi, Hyytiälä, Perämeri, Värriö, Oulanka, Kevo and Muddusjärvi (Fig. 1; see Table 1 for research station names). Dragging was conducted once every three weeks, with a minimum of 100 m² dragged in 10 m² subsections during each session (50 m² in Husö due to high tick abundance). Dragging locations were not fixed (apart from Seili, as explained below), but rather the locations for each 10 m drag were chosen separately during each dragging session, based on the operator's assessment of suitable tick habitats in the nearby environments. The environments surrounding research stations differ widely, but in general, patches of coniferous, deciduous or mixed forests were chosen for sampling (Fig. 1). The drags consisted of white 1 × 1 m linen cloths attached to wooden poles. Ticks were collected from the cloth with tweezers after each 10 m drag and stored in Eppendorf-tubes filled with 70% ethanol. The tubes were then sent to the Zoological Museum at the University of Turku for morphological species and life stage identification (Estrada-Peña et al., 2018; Hillyard, 1996) and laboratory analysis of pathogens.

On Seili Island, ticks have been collected more comprehensively since 2012. In Seili, a total of fifteen fixed 50 m study transects were dragged (in three 16–17 m sections) 1–3 times each month, for a total of 750–2250 m² of dragging per month (Sormunen et al., 2016a,2016b).

2.3. Laboratory analysis

Total DNA and RNA was extracted from a subset of collected ticks using NucleoSpin® RNA kits and RNA/DNA buffer sets (Macherey-Nagel, Germany), following the kit protocols (NucleoSpin 96 RNA Core Kit: Rev. 05/April 2014 and RNA/DNA buffer set: Rev. 09/April 2017). RNA extracts were stored at –80 °C for later analyses. DNA extracts were stored at –20 °C.

DNA samples were screened for bacterial pathogens *Borrelia burgdorferi* sensu lato (henceforth abbreviated BBSL; including separate screening for *Borrelia afzelii*, *Borrelia garinii*, *Borrelia valaisiana*, and *Borrelia burgdorferi* sensu stricto), *Borrelia miyamotoi*, *Anaplasma phagocytophilum*, *Rickettsia* spp., *Candidatus* Neoehrlichia mikurensis, *Francisella tularensis* and *Bartonella* spp., and for protozoan parasites *Babesia* spp. Furthermore, RNA samples were screened for tick-borne encephalitis virus (TBEV). Analyses regarding *Borrelia* were carried out on individual DNA samples. For the screening of all other pathogens, samples were analyzed in pools (12 samples per pool, 5 µl of each

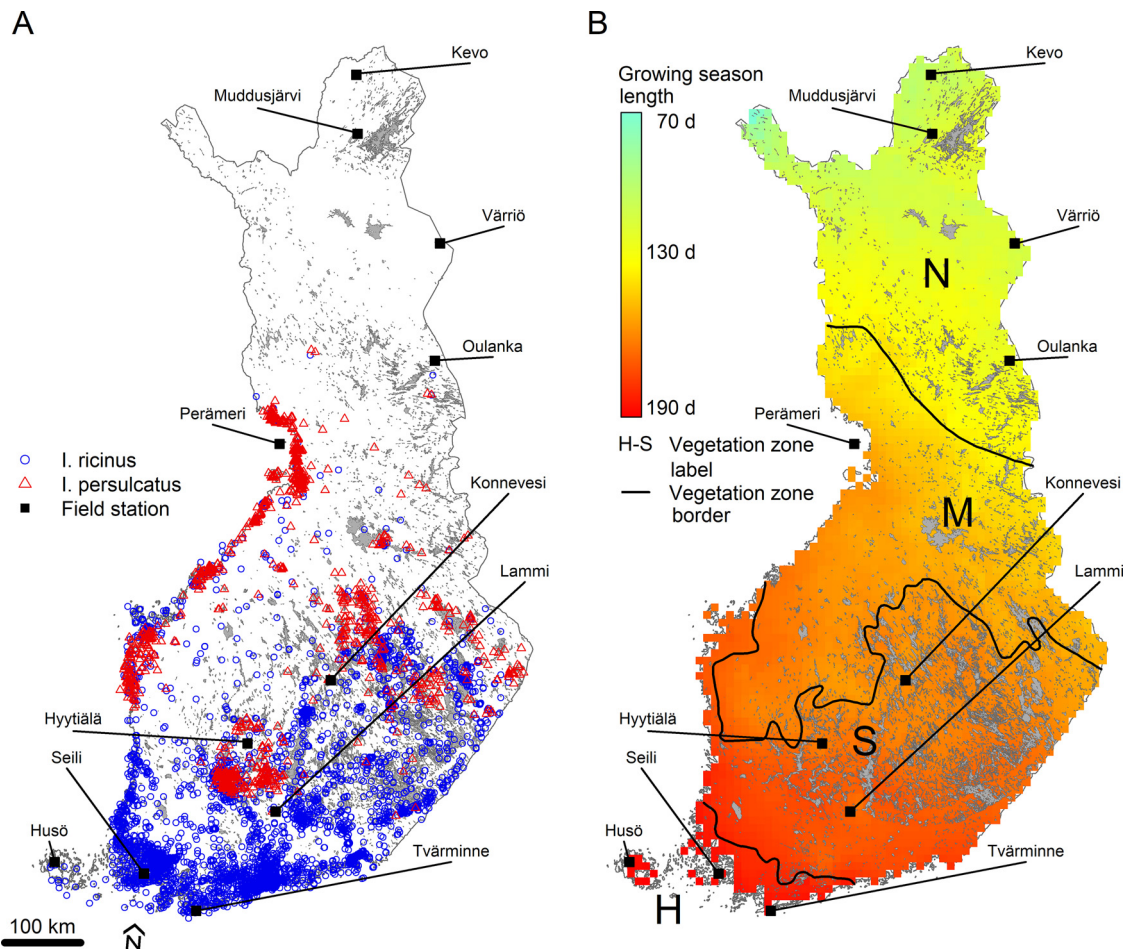


Fig. 1. Locations of research stations participating in the collaboration. In panel A, the background map depicts the distributions of *Ixodes ricinus* (blue circles) and *I. persulcatus* (red triangles) in Finland, based on a citizen science survey conducted in 2015 (Laaksonen et al., 2017). Vegetation zone labels: H = hemiboreal zone, S = southern boreal zone, M = middle boreal zone, N = northern boreal zone (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

sample) due to low expected prevalence. Individual samples from a pool found positive were subsequently re-analyzed separately. The primers used for each pathogen are reported in the Technical Appendix.

Real-time quantitative PCR (henceforth abbreviated qPCR) assays were carried out using SensiFAST™ Probe Lo-ROX Kit (for DNA) and SensiFAST™ Probe Lo-ROX One-Step Kit (for RNA) (Bioline, Germany). All DNA/RNA samples were analyzed in two replicate reactions carried out on 96 or 384-well plates. At least two blank water samples were used as negative controls in each assay. Samples were considered positive when successful amplification was detected in both replicate reactions or in two consecutive assays. Assay protocols have been reported previously for all screened pathogens (Laaksonen et al., 2018; Sormunen et al., 2018), apart from BBSL genospecies (for which see Technical Appendix). Also see the Technical Appendix for mastermix contents.

Samples found positive for *Rickettsia* and *Babesia* with qPCR were subsequently amplified by conventional PCR and Sanger-sequenced in order to determine species (Table A1). Likewise, BBSL-positive samples that could not be identified to the genospecies level by qPCR were Sanger-sequenced to determine species (Table A1). Assay protocols and mastermix contents for PCR amplification of these were as reported previously (Sormunen et al., 2018; Sormunen et al., 2016a,2016b), with the following modifications regarding *Borrelia*: reaction volume was increased to 15 μ L, with 3 μ L DNA sample, and thermal cycling profile was run for 50 cycles at 54 $^{\circ}$ C annealing temperature.

Pathogen analyses for samples collected from Seili have recently

been published independently and are thus not discussed in any part of this manuscript (Sormunen et al., 2018).

2.4. Statistical analysis

We refrained from formal analyses of differences in tick abundance among the research stations, because the null hypothesis on equal abundance is redundant to start with; there are no plausible arguments to expect same amounts of ticks in different geographical regions or different vegetation types and biotopes.

Research station or tick species-specific differences in tick questing activity among study years and months were modeled by generalized linear mixed models (GLMM) with Poisson distribution and log link function. In models of species-specific monthly activity, differences between study areas and years were controlled for as a random effect (Area nested within Year). In models of research station-specific monthly activity, differences between study years were controlled for as a random effect (Year). Regarding larvae, analysis only included data from Seili Island, from where sufficient data was available. No statistical analyses regarding TBPs were attempted, as the numbers of positive samples were too low to allow for any meaningful analysis. All the GLMMs were run with the GLIMMIX procedure of SAS v. 9.4. using restricted maximum likelihood estimation. The method by Kenward and Roger (2009) (Kenward and Roger, 2009) was chosen to adjust standard errors and denominator degrees-of-freedom for tests of the fixed factors.

Table 1
Annual tick densities and distances dragged at research stations.

Research station ^a	Study year	Distance dragged (m)	Species	Adults	Nymphs	Larvae	Adults/100 m ²	Nymphs/100 m ²	Larvae/100 m ²
Seili	2012	11 250	<i>I. ricinus</i>	44	540	1356	0.4	4.8	12.1
	2013	6 000	<i>I. ricinus</i>	24	311	476	0.4	5.2	7.9
	2014	5 250	<i>I. ricinus</i>	20	352	1030	0.4	6.7	19.6
	2015	5 250	<i>I. ricinus</i>	38	268	559	0.7	5.1	10.6
	2016	5 250	<i>I. ricinus</i>	44	626	1006	0.8	11.9	19.2
	2017	6 000	<i>I. ricinus</i>	117	1438	1941	2	24	32.4
	2018	7 450	<i>I. ricinus</i>	140	877	2924	1.9	11.8	39.2
Husö	2015	350	<i>I. ricinus</i>	6	50	37	1.7	14.3	10.6
	2016	360	<i>I. ricinus</i>	8	46	229	2.2	12.8	63.6
	2017	350	<i>I. ricinus</i>	12	86	77	3.4	24.6	22
	2018	350	<i>I. ricinus</i>	7	91	14	2	26	4
Tvärminne	2015	940	<i>I. ricinus</i>	37	38	3	3.9	4	0.3
	2016	700	<i>I. ricinus</i>	11	25	1	1.6	3.6	0.1
	2017	500	<i>I. ricinus</i>	3	17	52	0.6	3.4	10.4
	2018	700	<i>I. ricinus</i>	25	60	0	3.6	8.6	–
Perämeri	2015	1 050	<i>I. persulcatus</i>	6	0	0	0.6	–	–
	2016	600	<i>I. persulcatus</i>	6	0	0	1	–	–
	2017	600	<i>I. persulcatus</i>	3	0	0	0.5	–	–
	2018	600	<i>I. persulcatus</i>	6	0	0	1	–	–
Konnevesi	2015	400	N/a	0	0	0	–	–	–
	2016	700	<i>I. persulcatus</i>	1	0	0	0.1	–	–
	2017	700	<i>I. persulcatus</i>	0	1	0	–	0.1	–
	2018	700	<i>I. persulcatus</i>	23	0	0	3.3	–	–
Lammi	2016	710	<i>I. ricinus</i>	0	1	0	–	0.1	–
	20,151,718	1 820	N/a	0	0	0	–	–	–
Hyytiälä	2015–2018	2 860	N/a	0	0	0	–	–	–
Värriö	2015–2018	2 000	N/a	0	0	0	–	–	–
Oulanka	2015–2018	1 900	N/a	0	0	0	–	–	–
Kevo	2015–2018	2 150	N/a	0	0	0	–	–	–
Muddusjärvi	2015–2016	850	N/a	0	0	0	–	–	–

^a Research stations: The Archipelago Research Institute, University of Turku (Seili); Husö Biological Station, Åbo Akademi University (Husö); Tvärminne Zoological Station, University of Helsinki (Tvärminne); Bothnian Bay Research Station, University of Oulu (Perämeri); Konnevesi Research Station, University of Jyväskylä (Konnevesi); Lammi Biological Station, University of Helsinki (Lammi), Hyytiälä Forestry Field Station, University of Helsinki (Hyytiälä); Värriö Subarctic Research Station, University of Helsinki (Värriö); Oulanka Research Station, University of Oulu (Oulanka); Kevo Subarctic Research Station, University of Turku (Kevo); Muddusjärvi Research Station, University of Helsinki (Muddusjärvi).

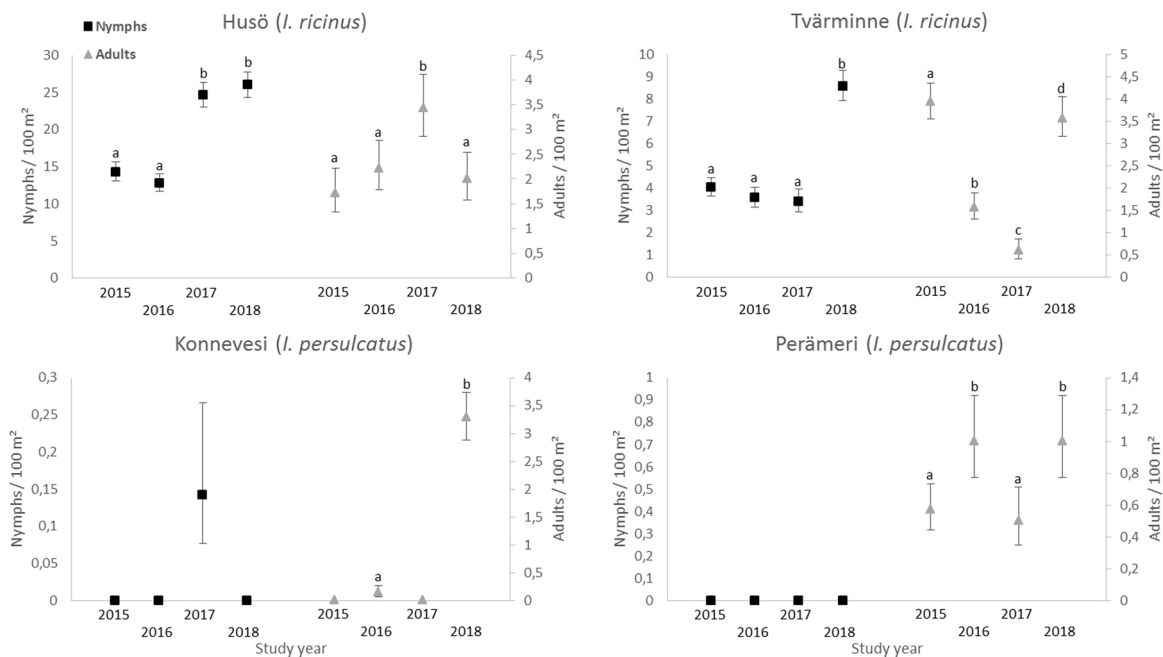


Fig. 2. Annual densities (with 95% CL) of nymphs and adults at research stations where studies have been conducted since 2015. Mismatching letters denote statistically significant differences between years with different letters ($p < 0.05$). Note the different scales in y-axis.

Multiple, *a posteriori*, pairwise comparisons for differences of the estimated marginal means (i.e., ls-means in SAS) were adjusted by the Tukey–Kramer method. These results are visually depicted (Figs. 2–5), using $\alpha = 0.05$ as a threshold for significant difference. Detailed

statistical values of the conducted analyses are provided in the Technical Appendix.

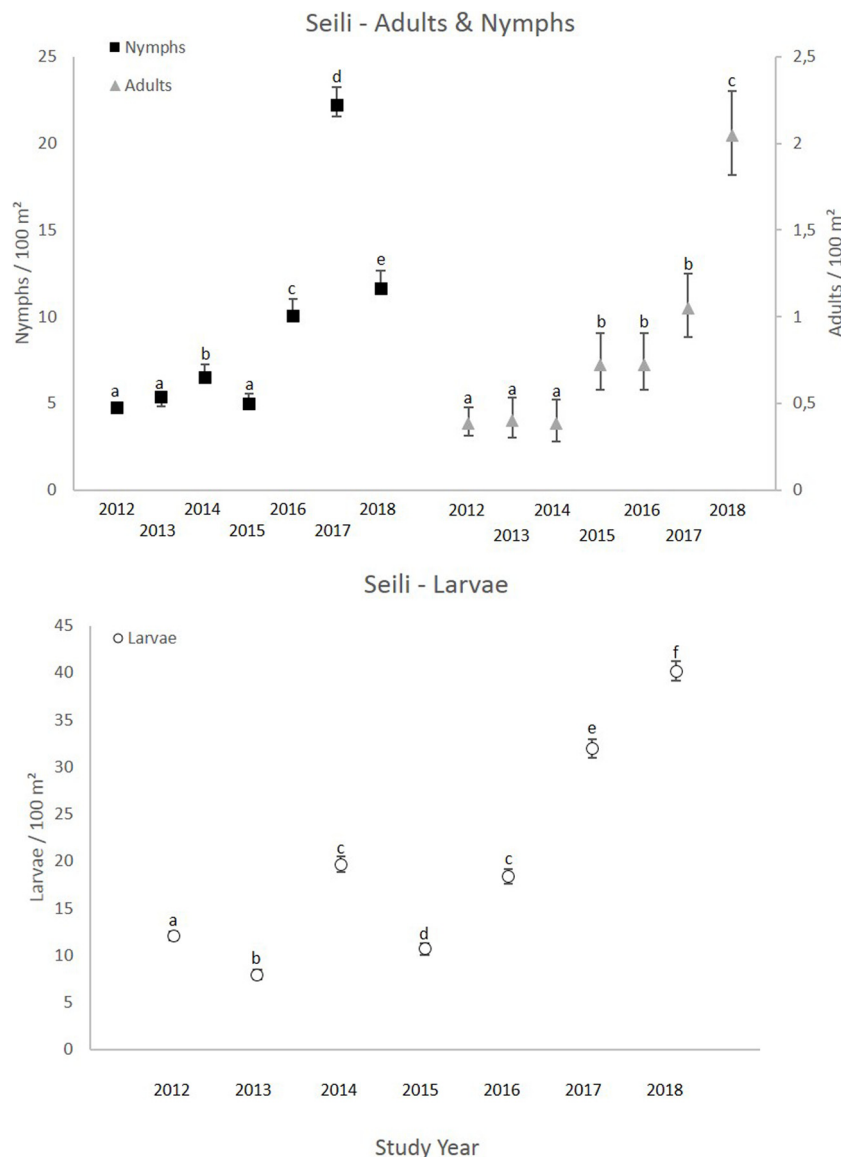


Fig. 3. Annual densities (with 95% CL) of *I. ricinus* adults, nymphs and larvae on Seili Island. Mismatching letters denote statistically significant differences between years with different letters ($p < 0.05$). Note the different scales in y-axis.

3. Results

3.1. Tick distribution, activity and abundance across Finland

A total of 936 *I. ricinus* (109 adults, 414 nymphs and 413 larvae) and 46 *I. persulcatus* (45 adults and one nymph) were collected during 21.9 km of cloth dragging (covering an area of 21,900 m²) in 2015–2018 at ten participating research stations (Table 1). In addition, a total of 13 534 *I. ricinus* (364 adults, 4163 nymphs and 9007 larvae) were collected during 45.7 km of cloth dragging (45,700 m²) in Seili during 2012–2018. During the study period, ticks were consistently detected from five locations: Seili, Husö and Tvärminne (*I. ricinus*), and Konnevesi and Perämeri (*I. persulcatus*) (Table 1). Only either *I. ricinus* or *I. persulcatus* was detected from each research station. From Lammi, only one *I. ricinus* nymph was found in 2016, and from Hyttiälä, Värriö, Oulanka, Kevo and Muddusjärvi, no ticks were detected.

The study areas exhibited varying patterns of seasonal tick activity and inter-annual tick densities (Fig. 2–5). Regarding adult and nymph (and larvae in Seili) abundances across the study years, while the exact patterns were not exactly the same, 2017 or 2018 were peak years at all locations that consistently yielded ticks (Fig. 2,3). Konnevesi displayed

the most interesting annual patterns, as *I. persulcatus* was not detected there at all in 2015, but was then annually detected since 2016, with peak abundance in 2018 (Fig. 2). As for seasonal activity patterns, differences among study areas appear mostly to be determined by the tick species present (research station -specific figures are presented in Technical Appendix) (Figs. 4,5). For *I. ricinus*, seasonal patterns included pronounced August–September peaks for nymphs and July–August peaks for adults, whereas for *I. persulcatus* adults, activity stopped after July, with peak activity in May–June. For *I. ricinus* nymphs and larvae in Seili, seasonal patterns varied depending on year, but commonly displayed late season peak densities (Fig. 5).

3.2. Tick-borne pathogen diversity and prevalence

Altogether nine different pathogens were detected from studied ticks: *Borrelia afzelii*, *B. garinii*, *B. burgdorferi* s.s., *B. valaisiana*, *B. miyamotoi*, *Babesia venatorum*, *R. helvetica*, *A. phagocytophilum* and ca. *N. mikurensis* (Table 2). Bacteria from the BBSL-group were the most commonly detected pathogens, with $48.9 \pm 8.4\%$ of adult ($54.3 \pm 10.2\%$ for *I. ricinus* and $37.8 \pm 14.2\%$ for *I. persulcatus*) and $25.3 \pm 4.4\%$ of nymph samples carrying at least one genospecies

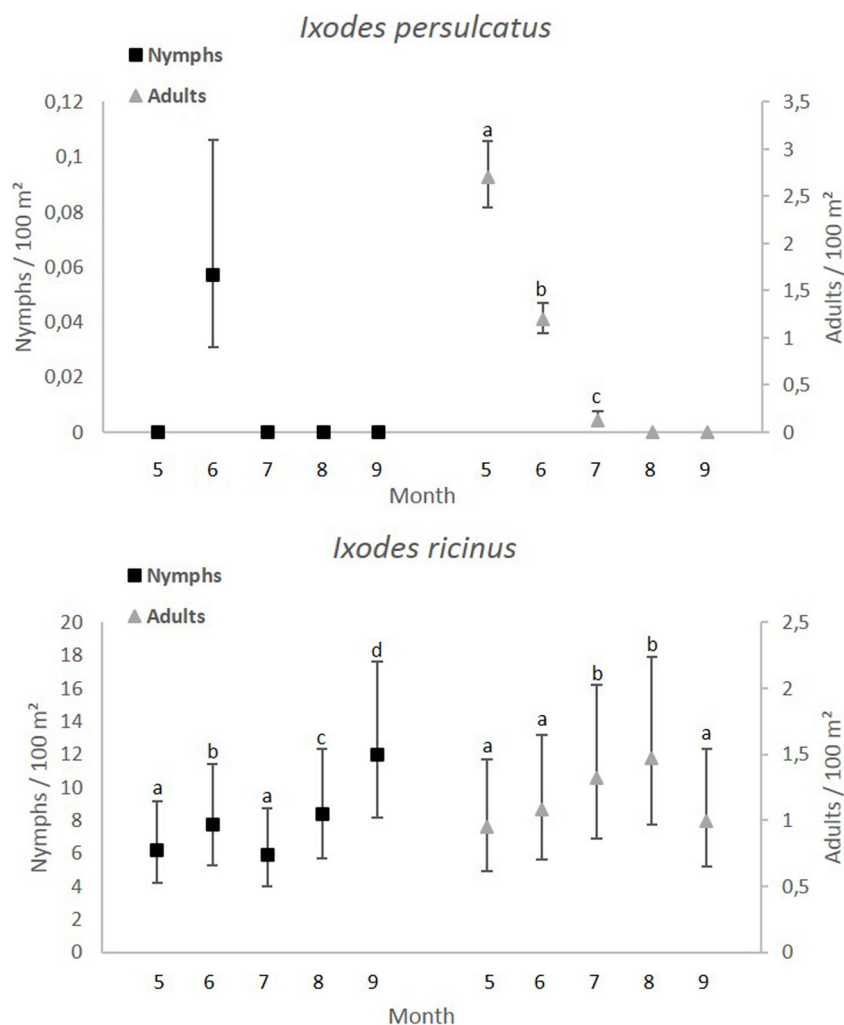


Fig. 4. Seasonal activity of *I. ricinus* and *I. persulcatus* nymphs and adults (with 95% CL) based on data from all research stations. Mismatching letters denote statistically significant differences between months with different letters ($p < 0.05$). Note the different scales in y-axes.

(prevalence percentage and binomial 95% confidence interval given). Within the BBSL group, *B. garinii* was the most common genospecies (42.5% and 42.4% of positive samples for adults and nymphs, respectively), followed by *B. afzelii* (39.7% and 36.4%), *B. valaisiana* (4.1% and 7.1%) and *B. burgdorferi* s.s. (5.5% and 2%). For 8.2% of positive adults and 12.1% of positive nymphs, BBSL genospecies could not be determined.

Prevalence rates for pathogens other than BBSL were modest or low (Table 2). No samples positive for TBEV, *Bartonella* spp. or *F. tularensis* were detected. The only pathogen detected from all tick-yielding research areas was *B. garinii*, whereas *B. afzelii*, *B. valaisiana* and *R. helvetica* were each detected from all but one area (Table 2). The overall proportion of ticks infected with at least one pathogen was $54.7 \pm 8.3\%$ (95% CI) for adults and $32.5 \pm 4.7\%$ for nymphs. Finally, a total of 19 adults (13.9% of adult samples) and 9 nymphs (2.3% of nymph samples) were found to carry more than one pathogen (Table A5). Interestingly, despite their lower BBSL prevalence compared to *I. ricinus*, adult *I. persulcatus* from Perämeri were commonly co-infected with more than one BBSL genospecies (28.3% of all ticks co-infected; $n = 21$). Corresponding values were 0% for Konnevesi ($n = 25$), 0% for Husö ($n = 33$) and 1.8% for Tvärminne ($n = 57$). Overall, the most commonly observed coinfection was *B. afzelii* and *ca. N. mikurensis*, which comprised 31.6% (6) of adult and 66.7% (6) of nymph coinfections. Coinfection with *B. afzelii* and *ca. N. mikurensis* was more common than expected by random co-occurrence (6.2 expected, 12 observed; $\chi^2 = 8.1$, $p = 0.006$, $df = 1$).

4. Discussion

The Finnish research station collaboration presented here was designed as an effective and non-laborious sampling scheme, to ensure that annual data collection is possible by the personnel working at the stations. While not yet being suitable for comprehensive analyses regarding, for example, the occurrence of rare pathogens or the exact environmental factors driving the observed seasonal activity patterns, the data generated can be applied to study several other tick-related subjects, such as nationwide trends in tick abundance and activity, as well as the spreading of ticks and tick-borne pathogens to novel areas. The environments surrounding the research stations form a gradient of different statuses of tick occurrence, from the established tick areas in southern Finland, through the lower-density and sporadic areas of occurrence in central Finland, and all the way up north to the insofar uninhabitable areas in the Finnish Lapland. This allows for the assessment of different tick related phenomena between areas where one might expect differences to be manifested. Furthermore, as the monitoring project carries on to the future, it presents a good opportunity to observe the spread of ticks and/or tick-borne pathogens to new areas. Consequently, with this data, assessing the factors promoting the observed changes becomes feasible.

4.1. Tick distribution, activity and abundance across Finland

Expectedly, the highest tick densities were consistently observed at

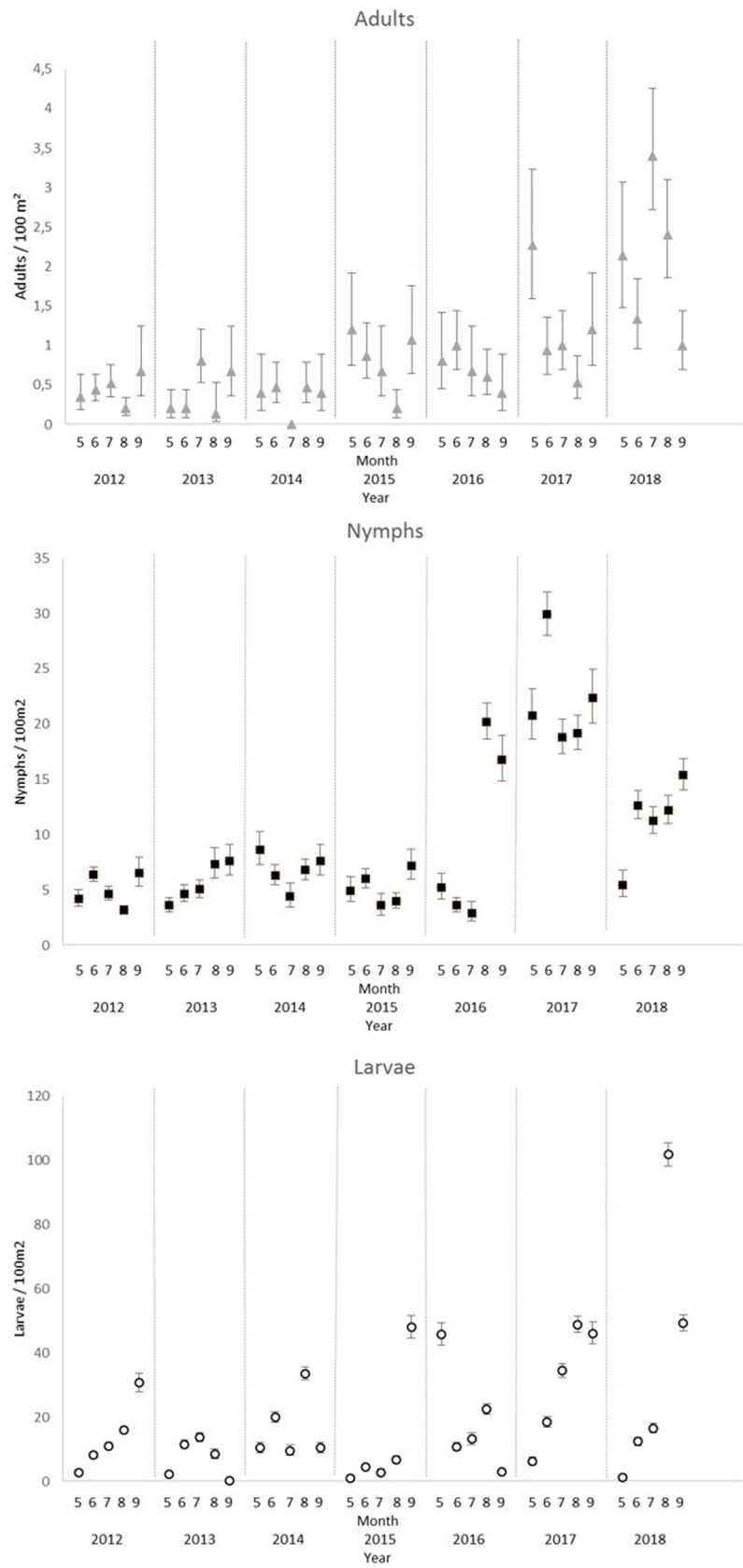


Fig. 5. Seasonal patterns of adult, nymph and larva *I. ricinus* activity on Seili Island (with 95% CL). Information on statistically significant differences between specific months and years are available from the authors on request.

Table 2
Prevalence of tick-borne pathogens detected from nymphs and adults in the current study.

Pathogen:	Adults (n = 137)			<i>I. ricinus</i> nymphs (n = 378)			Detected from	
	Positive samples	Prevalence + 95% CI		Positive samples	Prevalence + 95% CI		Study site ^b	Tick species ^c
<i>Borrelia burgdorferi</i> s.l.	67(73) ^a	48.9 ± 8.4		96(99) ^a	25.4 ± 4.4		All	Ir, Ip
<i>B. afzelii</i>	29	21.2 ± 6.8		36	9.5 ± 3		Tv, Hu, Pe	Ir, Ip
<i>B. garinii</i>	31	22.6 ± 7.0		42	11.1 ± 3.2		All	Ir, Ip
<i>B. valaisiana</i>	3	2.2 ± 2.5		7	1.9 ± 1.4		Tv, Hu, Ko	Ir, Ip
<i>B. burgdorferi</i> s.s.	4	2.9 ± 2.8		2	0.5 ± 0.7		Tv, Hu, Pe	Ir, Ip
Unidentified <i>Borrelia</i>	6	5.1 ± 3.7		12	3.2 ± 1.8			Ir, Ip
<i>Ca. Neoehrlichia mikurensis</i>	8	5.8 ± 3.9		12	3.2 ± 1.8		Tv, Hu	Ir
<i>Anaplasma phagocytophilum</i>	3	2.2 ± 2.5		5	1.3 ± 1.2		Tv, Hu	Ir
<i>Rickettsia helvetica</i>	6	4.4 ± 3.4		14	3.7 ± 1.9		Tv, Hu, Pe	Ir, Ip
<i>Babesia venatorum</i>	2	1.5 ± 2		4	1.1 ± 1		Tv, Hu	Ir
<i>Borrelia miyamotoi</i>	1	0.7 ± 1.4		1	0.3 ± 0.5		Tv, Hu	Ir
Any pathogen	75	54.7 ± 8.3		124	32.5 ± 4.7			

^a Number in brackets is total number of BBSL detections – some samples were co-infected with two genospecies.

^b Abbreviations: Tv = Tvärminne; Hu = Husö; Pe = Perämeri; Ko = Konnevesi.

^c Abbreviations: Ir = *Ixodes ricinus*; Ip = *Ixodes persulcatus*.

the southernmost research stations at Seili, Husö and Tvärminne. All these research stations are situated on the shoreline of the Baltic Sea, where high tick densities and borreliosis incidence have been reported in recent years (Klemola et al., 2019; Sajanti et al., 2017; Sormunen et al., 2016a,2016b). In contrast, the situation at the central research stations (Lammi, Konnevesi and Hyytiälä) was unexpected, as they all are situated in areas where tick occurrence has been recorded, but few or no ticks were caught in the field collections (Laaksonen et al., 2017). In Konnevesi, no ticks were detected in 2015, but from 2016 onward *I. persulcatus* has been reported annually, with the highest densities in 2018. From Lammi, only one *I. ricinus* nymph was reported in 2016 and from Hyytiälä, no ticks have been found. As the environments surrounding these central research stations are known to host tick populations (Fig. 1), the observed absence/rarity of ticks is likely an effect of smaller scale, local environmental conditions, which cannot be pinpointed with the available data. Regarding annual tick abundances, tick densities were generally higher in the latter years of the study, although whether densities were highest in 2017 or 2018 varied depending on research station. These observations are in line with other studies from Fennoscandia, which have reported increasing tick densities in the 21st century (Bugmyrin et al., 2019; Jaenson et al., 2012; Jore et al., 2011; Sormunen et al., 2016a,2016b).

The seasonal activity patterns exhibited by adults and nymphs of *I. ricinus* and *I. persulcatus* in the current study fit well within the guidelines provided by literature (Gray, 1991, 2008; Korenberg, 2000; Sirotkin and Korenberg, 2018; Uspensky, 2016). For *I. persulcatus*, activity was observed to be unimodal, with the highest activity period located in May–June and with non-existent activity from July onwards, as observed commonly also in studies conducted in Russia (Uspensky, 2016). For *I. ricinus*, nymph activity was bimodal, with the late season peak in August–September more pronounced, and adult activity unimodal, with peak activity in August. These species-specific differences between the peak activity periods of adults and nymphs may have implications for public health, especially since many Finns partake in berry and mushroom picking and hunting particularly from August to October. Consequently, the risk involved in these activities may be vastly different between *I. ricinus* or *I. persulcatus* dominated areas, highlighting the importance of identifying the locally present species for risk assessments.

Concerning *I. ricinus* larvae, the annual activity patterns observed in Seili were fluctuating, but commonly included high activity peaks in August or September. Nymph activity peaks in Seili were likewise more common in the late than in the early season. These observations may have implications regarding the transmission of at least the European subtype of TBEV, for which the transmission of viruses from nymphs to

larvae during proximal co-feeding is an important part of the sylvatic cycle (Randolph, 2011). Co-feeding has often been linked particularly to spring temperatures in the northern parts of Europe, with warmer or more quickly warming springs allowing for earlier activation of wintering larvae and, consequently, their simultaneous activity with nymphs (Randolph, 2011; Randolph et al., 2000). However, the tick activity data from Seili presented here would suggest late season co-feeding potentially being more common at Seili Island, located in the southwestern archipelago of Finland, an area considered endemic for TBEV (Tonteri et al., 2015). Indeed, TBEV has been detected also from Seili Island since 2016 (Sormunen et al., 2018). In the future, trappings of rodents are planned for spring and autumn seasons in Seili to assess potential differences in the numbers of co-feeding juvenile ticks found on hosts.

Another difference between the tick species was observed regarding the proportions of adults and nymphs being caught. In the current study, the life stage distribution of caught *I. persulcatus* was different from that of *I. ricinus*, with noticeably more adults than nymphs being caught for *I. persulcatus* (45 adults vs. 1 nymph) and vice versa for *I. ricinus* (109 adults vs. 414 nymphs; 364 vs. 4163 in Seili). Furthermore, whereas 413 (9007 in Seili) *I. ricinus* larvae were collected, no *I. persulcatus* larvae were found. These observations coincide with study results from Russia, where it has been reported that mostly *I. persulcatus* adults can be collected via cloth dragging, with nymphs and larvae being rare finds (Uspensky, 2016). While the reasons for this have not been thoroughly explored, resource partitioning in space to minimize inter-stage competition has been suggested as the cause. More precisely, it has been proposed that, due to them being active at the same, limited period of time, the different life stages of *I. persulcatus* have diversified or focused their use of hosts to prevent competition, for example by adopting different questing behavior (partitioning in space). In contrast, for *I. ricinus*, competition is minimized by the activity periods of life stages being more spread out (partitioning in time) (Uspensky, 2016).

4.2. Tick-borne pathogen diversity and prevalence

Bacteria from the BBSL-group were expectedly the most common pathogens detected. Previously reported results from Finland (Laaksonen et al., 2018, 2017; Sormunen et al., 2016a,2016b), as well as the whole of Europe (Gustafson et al., 1995; Strnad et al., 2017), have suggested that members of the group are present in most of the areas where *I. ricinus* or *I. persulcatus* are found. Likewise, the prevalence rates for nymphs and adults reported here coincide with the values reported more widely from Europe (Strnad et al., 2017).

Apart from BBSL, other tick-borne pathogens were detected in modest numbers. Whereas the prevalence rates reported for these pathogens mostly coincide with previously reported values from other wilderness areas in Finland (Aleksiev et al., 2007; Laaksonen et al., 2018, 2017; Sormunen et al., 2016a, 2016b), it is worth noting that a recent investigation in Turku in southwestern Finland revealed much higher *R. helvetica* prevalence in urban and suburban areas of the city (Klemola et al., 2019). Furthermore, surveys in other parts of Europe have occasionally revealed much higher prevalence for several of the screened pathogens, suggesting that they can achieve higher rates when conditions are favorable (Christova et al., 2003; Cotté et al., 2009; Derdakova et al., 2014; Kantsø et al., 2010; Portillo et al., 2018; Silaghi et al., 2016).

It is also worth noting that for *ca. N. mikurensis*, co-infection with *B. afzelii* was observed to be more common than expected by random co-occurrence. This observation is in line with similar reports from e.g. Finland, Austria and Norway (Andersson et al., 2013; Glatz et al., 2014; Kjelland et al., 2018; Klemola et al., 2019; Laaksonen et al., 2018; Sormunen et al., 2018), and the detection of several nymphs co-infected with these pathogens offers support to the previously suggested notion of a common rodent reservoir animal (Andersson and Råberg, 2011; Andersson et al., 2014). While not documented regarding this pathogen combination, co-infections of multiple pathogens have on occasion been demonstrated to be able to cause more severe diseases in humans (Krause et al., 1996; Swanson et al., 2006). Therefore, it is important to continue assessing the range and frequency of co-infections occurring naturally in ticks.

4.3. Conclusions and future prospects of the collaboration

Data from the first four years of the nationwide research station collaboration have already revealed interesting patterns and differences in the occurrence of ticks and tick-borne pathogens across Finland, showcased in the current study. In the future, longer cloth dragging sessions are planned particularly for research stations in central Finland, where, despite low or non-existent findings by cloth dragging, ticks are consistently found attached to dogs by research station personnel. Indeed, cloth dragging is known not to be a particularly sensitive method for catching ticks in lower density areas, so longer dragging lengths might be required to detect any ticks in such areas. In order to prioritize the successful accomplishment of monthly and annual collections, the amount of cloth dragging per station was limited during these first years of the research project. However, now that the practices of tick collection have become established, more extensive sampling can also be pursued.

Although several changes in tick populations linked to climate change have already been observed in Fennoscandia, it is likely that tick and TBP populations will undergo further changes in the future – possibly even more significant ones than those recorded thus far. This is particularly conceivable here in the North, where *I. ricinus* and *I. persulcatus* live at the latitudinal extremes of their distributional ranges. Due to their role as vectors for pathogens causing serious diseases, the tracking of these changes is important not only for scientific purposes, but potentially also for public health. By setting up a sampling scheme that can be successfully completed in the midst of all the other activities the research station personnel undertake, we have ensured long-term tick data collection to the foreseeable future. These data will in turn provide a solid backbone for future studies of Finnish tick populations and the changes occurring therein.

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Appendix A. Supplementary data

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