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A clinical and laboratory follow-up study of high-risk tick-bitten individuals

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Original article

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ABSTRACT

Despite the presence of several microorganisms, other than *Borrelia burgdorferi* sensu lato (*Bbsl*) and TBE virus, in *Ixodes ricinus* ticks from the Nordic countries, data is lacking on their pathogenic potential in humans. In this study, we wanted to investigate the aetiology and clinical manifestations of tick-transmitted infections in individuals seeking medical care following a tick-bite.

The sampling frame was participants of a large-scale, prospective, multi-centre, follow-up study of tick-bitten volunteers recruited in Sweden, Finland and Norway in the years 2007–2015. Participants who sought medical care during the three-month follow-up period and from whom blood samples were collected during this healthcare visit (n = 92) were tested, using PCR, for exposure to spotted fever group (SFG) *Rickettsia* spp., *Anaplasma phagocytophilum* and *Babesia* spp. Moreover, 86 of these individuals had two serum samples, collected three months apart, tested serologically for six tick-borne microorganisms. The selected organisms – *Bbsl*, SFG rickettsiae, *Anaplasma phagocytophilum*, TBE virus, *Babesia microti* and *Bartonella henselae* – have all been detected in field-collected ticks from the Nordic countries. Medical records were reviewed and questionnaires were completed to determine clinical manifestations.

We found Lyme borreliosis to be the most common tick-transmitted infection as seen in 46 (54%) of the 86 participants with available medical records. Among the 86 participants with paired sera, serological or molecular evidence of recent exposure to other microorganisms than *Bbsl* could be demonstrated in eight (9%). Five participants (6%) exhibited serological evidence of recent concomitant exposure to more than one tick-borne microorganism. Clinical presentations were mild with one exception (TBE).

In conclusion, our data suggest a low risk of infection with tick-borne microorganisms, other than *Bbsl*, in immunocompetent tick-bitten persons from the examined regions, a low occurrence of co-infection and mostly mild or no overt clinical signs of infection in immunocompetent persons exposed to the studied agents.

1. Introduction

The castor bean tick (*Ixodes ricinus*) is widespread in the Nordic countries and is the tick species most frequently implicated in transmitting infectious agents to humans in northern Europe. It can carry and transmit potentially pathogenic microorganisms such as *Borrelia burgdorferi* sensu lato (*Bbsl*), spotted fever group (SFG) *Rickettsia* spp., *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, *Babesia* spp. as well as the European subtype of tick-borne encephalitis (TBE) virus (Andersson et al., 2013; Karlsson and Andersson, 2016; Wallménius et al., 2012). Moreover, studies have detected *Bartonella henselae* DNA in field-collected *I. ricinus* ticks but, thus far, no convincing clinical cases of cat-scratch disease, following a tick-bite, have been reported and the viability of ticks as vectors for this bacterium remains controversial (Telford and Wormser, 2010).

All of the aforementioned microorganisms have been associated with infections in humans (Fehr et al., 2010; Nilsson, 2009; Nilsson et al., 2011, 2010; Uhnoo et al., 1992; von Loewenich et al., 2010). Despite this, with the exception of *Bbsl* and TBE virus, little is known about their pathogenic potential in a northern European setting and the clinical features of patients seeking medical care due to these infections remain to be fully elucidated. Furthermore, because *I. ricinus* can simultaneously harbour multiple microorganisms, it can transmit more than one during a single tick-bite (Koetsveld et al., 2016; Lindblom et al., 2013; Nogueras et al., 2015; Swanson et al., 2006; Tijsse-Klasen et al., 2013). However, the risk of such an occurrence and its significance on the clinical presentation of the human host has received little attention in the Nordic countries.

In this study, we examined individuals seeking medical care in a three-month period following a tick-bite, to investigate 1) the proportion presenting with a tick-transmitted infection (TTI), 2) the microbial aetiology and clinical features of these infections, and 3) the proportion concomitantly exposed to more than one tick-borne microorganism.

2. Material and methods

2.1. The tick-borne diseases STING study

This study is based on data and material collected through the Tick-Borne Diseases (TBD) STING study, a prospective, multi-centre study examining tick-bitten persons from geographically different areas in southern, south-central, central and northern Sweden as well as the Åland Islands in southwestern Finland and Tromøy in southern Norway.

Men and women ≥ 18 years of age with a documented tick bite were enrolled in the TBD STING study between the years 2007 and 2015 (Wilhelmsson et al., 2010). Participants were recruited through an advertising campaign in media and public places, urging tick-bitten individuals to bring their detached tick(s) to one of the 67 primary healthcare (PHC) centres participating in the TBD STING study (Fig. 1a). Ticks delivered during this initial visit served as documentation of tick exposure. Following written consent and the fulfillment of a standardized questionnaire regarding participant health data, scheduled blood sampling was performed at the time of enrolment and at a final follow-up visit three months later. Further, all participants were informed to seek medical care and provide additional blood samples at a TBD STING study affiliated PHC centre in case they developed symptomatic disease during the three-month study period. These visits and corresponding blood samples will be referred to as "intermediate" in the following sections.

Any additional ticks detached in the three-month study period were also delivered to the PHC centres. Lastly, a self-completed questionnaire was delivered during the final three-month visit, reporting on symptoms experienced throughout the study period and inquiring on whether the participant has sought medical care due to these symptoms. Following collection, all blood samples were sent to Linköping University, Sweden, to be stored at – 70 °C until analysis.

2.2. Selection of study participants

In this study, we chose to focus on TBD STING study participants who developed symptomatic disease following a tick-bite and consequently sought medical care at some point during the three-month follow-up period. Of the 5080 participants initially enrolled in the TBD STING study, 4230 (83%) attended the final follow-up visit, as documented by the return of an at-least-partially filled out questionnaire, and/or had a blood sample delivered from an intermediate healthcare visit, thus enabling us to assess whether they had sought medical attention in the three-month study period (Fig. 2). Of these, a total of 432 (10%) participants were assessed to have sought medical care. Following the exclusion of those lacking intermediate blood samples (n = 323) and those who had sought medical care after the end of the three-month study period (n = 17), a total of 92 participants remained for inclusion in this sub-study. In addition to the tick(s) delivered at enrolment, a median of six additional ticks (range 0–53) were detached from these participants and delivered throughout the study period. One of the examined participants was being treated with methotrexate at the time of enrolment. None of the others were undergoing anti-neoplastic therapy or any other form of immunomodulating treatment that could potentially interfere with the host humoral response to tick-borne microorganisms. Paired sera, from the time of enrolment and the three-month follow-up, were available from 86 of these participants. Baseline characteristics of the study cohort are presented in Table 1.

2.3. Molecular analysis

Serum samples taken during the intermediate healthcare visits (n = 92) were analysed using real-time PCR for *Rickettsia* spp., *A. phagocytophilum* and *Babesia* spp. at the Division of Medical Microbiology at Linköping University in Sweden (Fig. 2). In addition, plasma samples from the same visits were prior to this study analysed for *N. mikurensis* at Sahlgrenska University Hospital (Grankvist et al., 2015).

An overview of the molecular assays is presented in Table 2a. Reverse transcribed total nucleic acid (cDNA) was prepared from 350 µl sera, as described elsewhere (Wilhelmsson et al., 2013). Two microliters of cDNA per reaction were used in separate real-time PCR assays to detect *Rickettsia* spp., *A. phagocytophilum* and *Babesia* spp., as previously described (Casati et al., 2006; Henningsson et al., 2015; Stenos et al., 2005).

2.4. Serological analysis

The 86 paired sera were examined at Statens Serum Institut in Copenhagen, Denmark, for IgG antibodies against *Bbsl*, SFG *Rickettsia* spp., *A. phagocytophilum*, *Ba. microti*, *B. henselae* and TBE virus (Fig. 2). Samples were analysed using current serological "gold standards" for diagnosing infections caused by these organisms with generally high reported sensitivities and specificities (Hansmann et al., 2019; Krause et al., 1994; Paris and Dumler, 2016; Zangwill et al., 1993).

An overview of the serological assays used is presented in Table 2b. Detection of IgG antibodies was done using commercially available IgG IFA (Focus Diagnostics, Inc., Cypress, CA, USA) for most microorganisms. For *Bbsl*, we used an in-house indirect IgG ELISA (Hansen and Asbrink, 1989) and for TBE virus we used a commercially available indirect IgG ELISA (Enzygnost, Siemens, Erlangen, Germany). For both ELISA assays, samples were run in duplicates. All IFA samples were titrated to end-point fluorescence in two-fold dilutions, as described elsewhere for SFG *Rickettsia* spp. (Kantsø et al., 2009). Fluorescent microscopy was performed according to the manufacturer's instructions by laboratory technicians with many years of experience in interpreting IFA results. Seroconversion was considered indicative of recent exposure for all assays. For the IFA assays, seroconversion was defined as
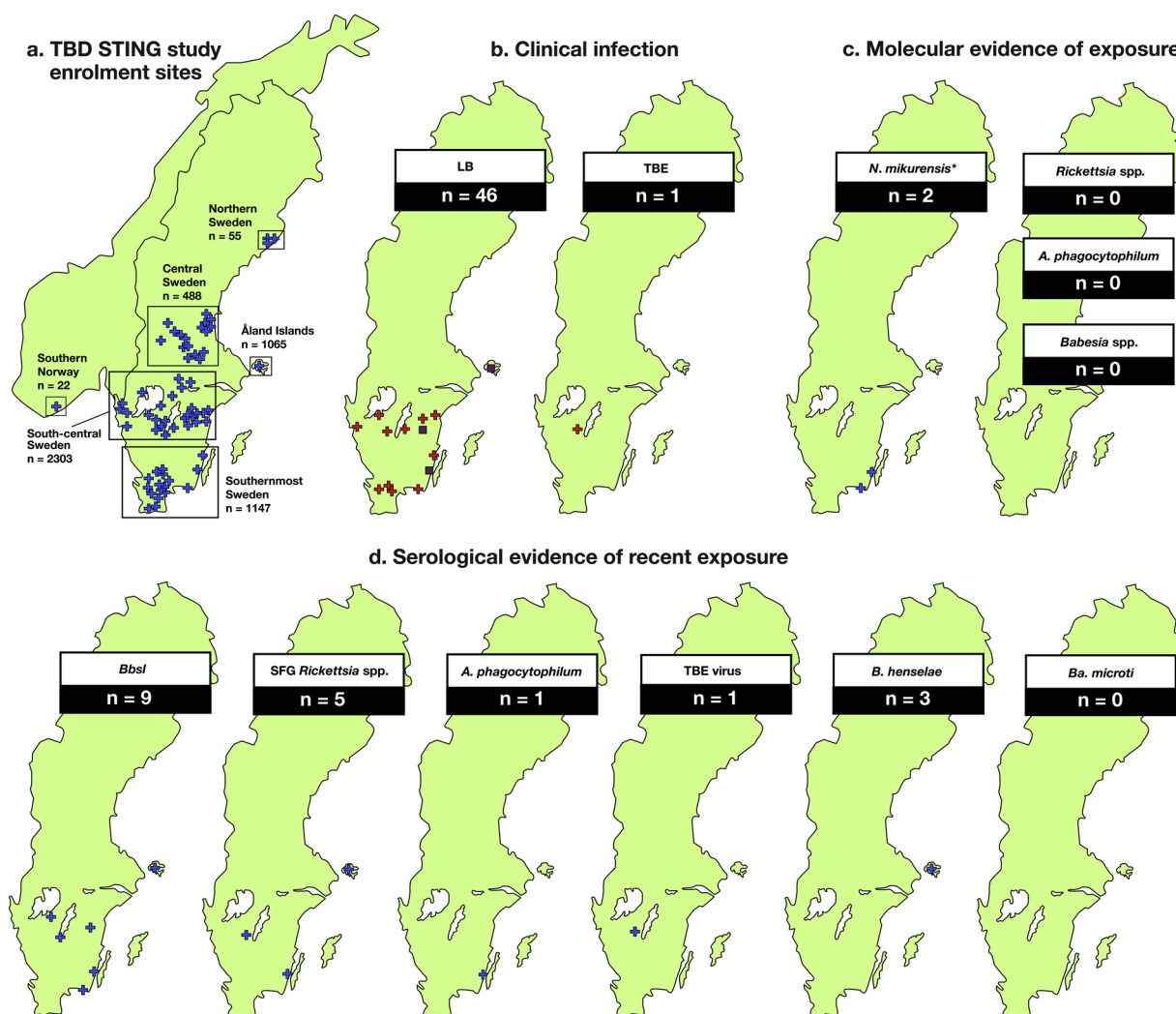
Fig. 1. Maps showing a) recruitment sites for the TBD STING study (2007–2015), b) sites reporting Lyme borreliosis and TBE with cases of confirmed Lyme neuroborreliosis marked as squares, c) sites with PCR findings, and d) sites associated with seroconversion between enrolment and the three-month follow-up visit. LB: Lyme borreliosis. TBE: Tick-borne encephalitis. SFG: Spotted-fever group. *Bbsl: Borrelia burgdorferi* sensu lato. *Published (Grankvist et al., 2015).

a fourfold rise in antibody titre or detectable IgG antibodies above cut-off level on the three-month visit in individuals who were seronegative at inclusion. For the ELISA assays, seroconversion was defined as an increase with > 3 arbitrary units, for *Bbsl*, and by a factor of ≥ 3, for TBE virus, or as detectable IgG antibodies on the three-month visit in individuals who were seronegative at inclusion (Hammers-Berggren et al., 1994).

2.5. Review of medical records

Physicians and co-authors JS, PF, MN and LFO reviewed the medical records of 86 participants (94%) for clinical signs of LB at the time of the intermediate healthcare visit (Fig. 2). The diagnosis of erythema migrans (EM) was based on the assessment of the attending physician and required a lesion diameter ≥ 5 cm in accordance with the European clinical case definitions for LB (Stanek et al., 2011). The diagnosis of Lyme neuroborreliosis (LNB) was based on the European Federation of Neurological Societies' criteria from 2010 (Mygland et al., 2010). Furthermore, medical records of participants with molecular or serological evidence of recent exposure to tick-borne organisms other than *Bbsl* were reviewed for clinical manifestations at the time of the healthcare visit.

2.6. Self-reported symptoms

Questionnaires were filled out at the three-month visit by 88 (96%) of the participants included in this study (Fig. 2). The questionnaires asked about symptoms experienced at any time during the three-month study period. Symptoms inquired about in the questionnaire were fever (≥ 38 °C), headache, myalgia/arthralgia, fatigue and nausea. These symptoms were selected as they have previously been reported for TTIs other than LB (Fournier et al., 2004; Henningsson et al., 2016; Lindblom et al., 2013; Uhnoo et al., 1992; von Loewenich et al., 2010).

2.7. Statistical analysis

Statistical significance for the categorical data obtained from the questionnaires was calculated with Fisher's exact test (two-tailed) using the statistical software R Studio (v1.0.153). $P < 0.05$ was considered significant.

3. Results

3.1. Molecular analysis

No participants had detectable DNA from *Rickettsia* spp., *A. phagocytophilum* or *Babesia* spp. (Fig. 1c) at the time of the intermediate
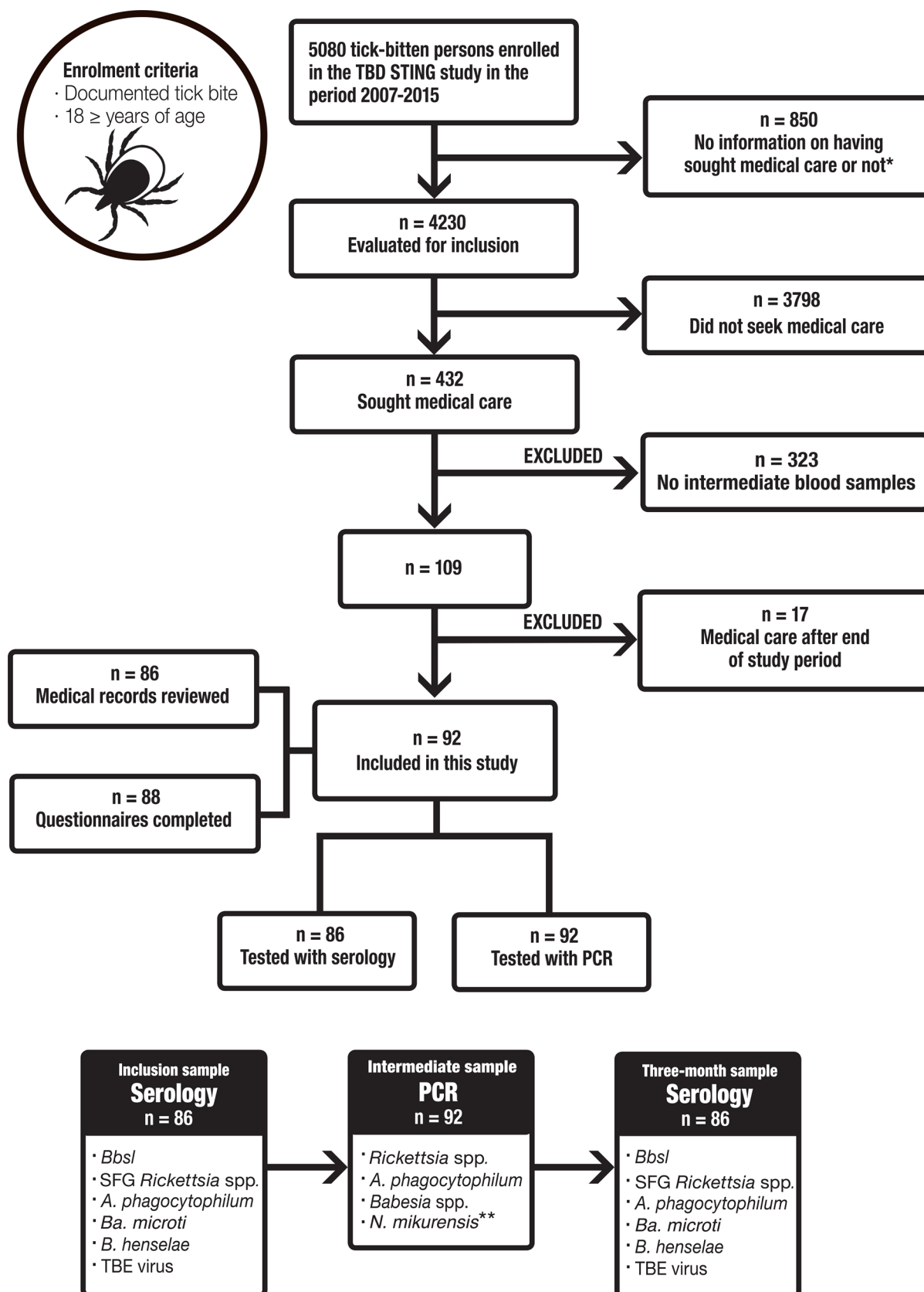
Fig. 2. Flow-chart showing inclusion and exclusion criteria as well as the participants analysed for this study. *Bbsl*: *Borrelia burgdorferi* sensu lato. SFG: Spotted-fever group. TBE: Tick- borne encephalitis. *No questionnaire on the final three-month follow-up visit and no blood samples collected from an intermediate healthcare visit. **Published (Grankvist et al., 2015).

Table 1

Overview of baseline characteristics, medical records and questionnaire data for the study participants. The diagnoses of possible/confirmed LNB and EM were based on the European Federation of Neurological Societies' criteria from 2010 and the European clinical case definitions, respectively. All questionnaire data were self-reported. IQR: Interquartile range. LB: Lyme borreliosis. EM: Erythema migrans. TBE: Tick-borne encephalitis. LNB: Lyme neuroborreliosis.

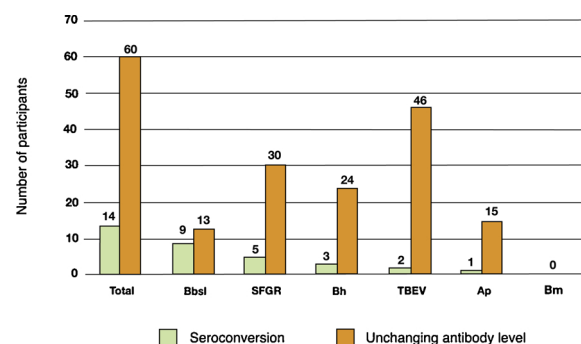| Baseline characteristics (n = 92)	
Age, y, median (IQR, range)	64 (56–69, 28–79)
Sex, male, %	26
Medical records (n = 86)	**%**
TBE	1
LB	54
- Physician-diagnosed EM	49
- Confirmed LNB	4
- Possible LNB	1
Questionnaire data (self-reported)	**% (no. of respondents)**
Co-morbidity at inclusion	
- Diabetes mellitus	5 (81)
- Neoplasia	3 (78)
- Asthma	13 (83)
Prior LB	57 (89)
Prior TBE	0 (88)
Immunised against TBE	55 (88)
Immunised against other flavivirus infections	
- Japanese encephalitis	0 (85)
- Yellow fever	7 (84)
Symptoms experienced during study period	
- Fever	15 (74)
- Myalgia/arthralgia	56 (81)
- Headache	39 (78)
- Fatigue	50 (82)
- Nausea	10 (73)

Fig. 3. Serological responses against a selection of tick-borne microorganisms observed for the 86 participants with paired sera from enrolment and the three-month follow-up visit. Of these, 60 (70%) had unchanging levels of IgG antibodies and 14 (16%) seroconverted against at least one of the investigated tick-borne microorganisms. Four participants seroconverted against two microorganisms, one participant seroconverted against three (see Table 3) and many participants had unchanging IgG levels above cut-off against multiple tick-borne microorganisms. No antibodies against *Babesia microti* were detected. *Bbsl*: *Borrelia burgdorferi* sensu lato. SFGR: Spotted fever group *Rickettsia*. TBEV: Tick-borne encephalitis virus. Bh: *Bartonella henselae*. Ap: *Anaplasma phagocytophilum*. Bm: *Babesia microti*.

healthcare visit. However, *N. mikurensis* DNA was detected in two of the participants, as described elsewhere (Grankvist et al., 2015).

3.2. Serological analysis

An overview of the serological results for the 86 participants with paired sera is presented in Figs. 3 and 1d. Most of the participants displaying seroconversion reacted against *Bbsl* (n = 9), followed by SFG *Rickettsia* spp. (n = 5), *B. henselae* (n = 3), TBE virus (n = 2), *A. phagocytophilum* (n = 1) and *Ba. microti* (n = 0). In total, 14 (16%) of the 86 participants seroconverted against at least one of the examined tick-borne microorganisms. Of note, IgG antibodies against *Bbsl* could be detected in both of the participants with *N. mikurensis* DNA (cases 6 and 7, Table 3) using our in-house assay (Hansen and Asbrink, 1989), with one of them (case 6) seroconverting between the two visits. Five participants (6%) seroconverted against more than one tick-borne microorganism (Table 3).

Table 2

Overview of the molecular (a) and serological (b) assays used to investigate the study samples. *Bbsl*: *Borrelia burgdorferi* sensu lato. ELISA: enzyme-linked immunosorbent assay. IFA: indirect immunofluorescence assay. SFG: Spotted fever group. TBE: Tick-borne encephalitis. * Values in arbitrary units, reflecting rise in optical density. ** Participants with follow-up samples showing a titre value of 1:128 had their initial inclusion samples re-examined at a dilution of 1:64 to determine if the rise in titre was at least fourfold between the two visits and thus indicative of recent exposure. *** Cut-off calculated as the mean absorbance value of the two negative controls + 0.200 (according to manufacturer's instructions).

a.

Microorganism	Primer/Probe	Nucleotide sequence (5'→3')	Target gene	Reference
Rickettsia spp.	CS-F	TCGCAAATGTTCACGGTACTTT	gltA	(Stenos et al., 2005)
	CS-R	TCGTGCATTTCTTTCCATTGTG		
	CS-P	FAM-TGCAATAGCAAGAACCGTAGG CTGGATG-BHQ1		
A. phagocytophilum	ApF	TTTTGGGCGCTGAATACGAT	gltA	(Henningsson et al., 2015)
	ApR	TCTCGAGGGAATGATCTAATAACGT		
	ApM	FAM-TGCCTGAACAAGTTATG-BHQ1		
N. mikurensis	Forward	CGGAAATAACAAAAGATGGA	groEL	(Grankvist et al., 2015)
	Reverse	ACCTCCTCGATTACTTTAG		
	Probe	6FAM-TTGGTGATGGAACTACA-MGB		
Babesia spp.	BJ1	GTCTTGTAATTGGAATGATGG	18S rRNA	(Casati et al., 2006)
	BN2	TAGTTTATGGTTAGGACTACG		

b.

Microorganism	Method	Manufacturer	Antigen	IgG cut-off
Bbsl	Indirect ELISA	In-house (Hansen and Asbrink, 1989)	Purified flagellum (*B. afzelii*)	*
SFG *Rickettsia* spp.	Indirect IFA	Focus Diagnostics	Inactivated *R. rickettsii*	1:128**
A. phagocytophilum	Indirect IFA	Focus Diagnostics	Infected HL60 cells	1:64
Ba. microti	Indirect IFA	Focus Diagnostics	Infected erythrocytes	1:64
B. henselae	Indirect IFA	Focus Diagnostics	Infected Vero cells	1:64
TBE virus	Indirect ELISA	Siemens	Inactivated TBE virus	***

3.3. Review of medical records

An overview of the 86 participants whose medical records were reviewed is presented in Table 1 and Fig. 1b. Forty-two (49%) presented with EM (Table 1). Three (4%) and one (1%) of the participants had confirmed and possible LNB, respectively. One participant (case 8, Table 3) presented with TBE and was hospitalized for 10 days (Henningsson et al., 2016). A total of 39 (45%) participants presented with complaints that were assessed to be unrelated to a TTI by the attending physician at the time of the intermediate healthcare visit.

The clinical manifestations of the eight participants with serological or molecular evidence of recent exposure to tick-borne microorganisms, other than Bbsl, are presented in Table 3. The two participants with N. mikurensis DNA (case 6 and 7, Table 3) presented clinically with isolated EM and are discussed in detail elsewhere (Grankvist et al., 2015).

3.4. Self-reported symptoms

The presence of self-reported symptoms at any time during the study period is summarised in Table 1. The proportion of participants reporting fever was significantly higher ($p = 0.02$) in those demonstrating seroconversion against SFG Rickettsia spp. compared with those lacking seroconversion between the two visits (Table 3). There was no statistically significant difference in the proportion of participants reporting myalgia/arthralgia ($p = 0.37$), headache ($p = 1.00$), fatigue ($p = 0.36$) or nausea ($p = 0.41$) between these groups.

4. Discussion

We found that, of the volunteers participating in the TBD STING study ($n = 4230$), 10% sought medical care during the study period, suggesting a generally low risk of developing symptoms prompting a healthcare visit following a tick-bite in the studied regions (Fig. 2). Among the examined 86 participants with paired sera, most ($n = 71$, 83%) had no molecular or serological evidence of recent exposure to a tick-borne microorganism. Of those with serological or molecular signs of recent exposure ($n = 15$), 14 seroconverted against at least one of the examined microorganisms and two exhibited microbial DNA (N. mikurensis; case 6 and 7, Table 3). Of those seeking medical care due to a TTI, we found a low proportion infected with other tick-borne microorganisms than Bbsl with only eight participants displaying serological or molecular evidence of recent exposure to other agents (Table 3). Apart from one participant who developed TBE, none were critically ill or in a clinical state necessitating swift hospital admittance. Furthermore, only 6% of the participants with paired sera displayed serological evidence of recent exposure to more than one tick-borne microorganism (Table 3), suggesting a low risk of infection with multiple tick-borne microorganisms. Interestingly, no participants had detectable IgG antibodies against Ba. microti, indicating low levels of exposure in the studied regions. This is in stark contrast to a recent Swedish study reporting IgG antibodies against Ba. microti in 9 of 86 individuals (11%) from a selected population of Bbsl seropositive persons (Svensson et al., 2019).

4.1. Bbsl and TBE virus

The high proportion of participants displaying IgG antibodies against Bbsl (Fig. 3) or presenting clinically with EM (Fig. 1b) is consistent with current data showing Bbsl to be the most common tick-borne microorganisms in the studied regions (Wilhelmsson et al., 2013). None of the three participants with EM who also displayed concomitant laboratory evidence of recent exposure to another tick-borne microorganism (cases 4, 6 and 7, Table 3) presented with any additional symptoms as noted in the medical records and self-reported questionnaires. One of them (case 7) recovered on penicillin and none of the remaining two returned for further follow-up, suggesting clinical

Table 3

Overview of the eight study participants with molecular or serological evidence of exposure to other tick-borne microorganisms than Borrelia burgdorferi sensu lato (Bbsl). One participant seroconverted against TBE virus due to immunisation and was omitted from this table. Serological results for Bbsl and TBE virus are provided in arbitrary units. F: Female. M: Male. EM: Erythema migrans. SFG: Spotted-fever group. Bbsl: Borrelia burgdorferi sensu lato. TBE: Tick-borne encephalitis. NA: Not applicable. * Based on medical records and questionnaire data. ** Reference value: CRP: < 10 mg/L.

	Co-morbidity*	Organism(s)	Serology 1 (inclusion)	Serology 2 (three months)	EM	Symptoms at time of healthcare visit (medical records)	Symptoms during study period (questionnaire)	Antimicrobial treatment	Follow-up
Case 1 71, M	None	SFG Rickettsia spp. Bbsl	<1:64 0	1:128 4	–	Isolated fever (38.5 °C) lasting for two days prior to visit. ↑ CRP 60**.	Fever, myalgia/arthralgia and fatigue	None	No fever at follow-up visit two days later. CRP 26 at two-week follow-up.
Case 2 71, F	–	SFG Rickettsia spp. B. henselae Bbsl	<1:128 <1:64 0	1:512 1:128 6	–	Multiple visits during study period. Mainly neuralgia/hyperesthesia.	Fever and fatigue	IV ceftriaxone on multiple occasions	Many follow-up visits. Persistent symptoms. No clear diagnosis.
Case 3 61, F	Asthma, hypertension	SFG Rickettsia spp.	<1:128	1:256	–	Pain in upper right abdominal quadrant. Gall stones suspected by attending physician.	Fever, headache and fatigue	None	–
Case 4 64, F	–	B. henselae SFG Rickettsia spp.	<1:64 <1:128	1:512 1:256	+	Isolated EM	None	Amoxicillin 500 mg × 3 for 14 days	–
Case 5 66, F	Diabetes	SFG Rickettsia spp. B. henselae	1:128 <1:64	1:1024 1:256	–	Joint pain in foot for months. Diagnosed with gout and osteoarthritis.	Myalgia/arthralgia and fatigue	None	–
Case 6 57, F	None	A. phagocytophilum Bbsl N. mikurensis	<1:64 0 NA	1:128 2 NA	+	Isolated EM (Grankvist et al., 2015)	None	Phenoxymethylpenicillin 1 g × 3 for 10 days	–
Case 7 68, F	None	N. mikurensis	NA	NA	+	Isolated EM (Grankvist et al., 2015)	None	Phenoxymethylpenicillin 1 g × 3 for 10 days	Rash disappears following treatment.
Case 8 67, M	Arrhythmia, hypertension	TBE virus	NEG	POS	–	TBE (Henningsson et al., 2016)	Fever, headache, myalgia/ arthralgia and fatigue	Doxycycline 200 mg × 1 for 10 days	Full recovery at follow-up six weeks post-discharge

Table 4

Overview of self-reported symptoms during the three-month study period in those with and without seroconversion against SFG *Rickettsia* spp. between enrolment and the three-month visit, among the 86 study participants tested serologically. Participants who did not report on the presence or absence of a specific symptom were left out of the calculations for that symptom (missing data; fever, n = 14; myalgia/arthralgia, n = 7, headache, n = 10, fatigue, n = 6, nausea, n = 15). SFG: Spotted fever group.

	Seroconversion against SFG *Rickettsia* spp.	No seroconversion against SFG *Rickettsia* spp.	P-value
Fever (%)	3/5 (60)	8/67 (12)	**0.02**
Myalgia/arthralgia (%)	4/5 (80)	39/74 (53)	0.37
Headache (%)	2/5 (40)	28/71 (39)	1.00
Fatigue (%)	4/5 (80)	36/75 (48)	0.36
Nausea (%)	1/5 (20)	6/66 (9)	0.41

recovery.

Of note, approximately half of all included participants had received immunisation against TBE virus prior to enrolment, as reflected in the high seroprevalence of anti-TBE virus IgG antibodies in the examined subjects (Table 1 and Fig. 3). Two participants seroconverted against TBE virus between the two visits. However, as one of them was immunised around the time of enrolment, the seroconversion was most likely in response to the immunisation. The other participant was never immunised and presented with TBE as previously described (Henningsson et al., 2016).

4.2. N. mikurensis

Neoehrlichia mikurensis DNA was detected in two participants, both presenting clinically with EM, as described elsewhere (Grankvist et al., 2015). One of these participants was reported to have had persisting but unchanging anti-*Bbsl* IgG antibodies above cut-off and the other participant lacked anti-*Bbsl* antibodies on both visits (Grankvist et al., 2015). However, on repeat testing using an in-house assay (Hansen and Asbrink, 1989), we detected anti-*Bbsl* IgG in both participants (cases 6, 7, Table 3) with one of them (case 6) seroconverting between the two visits, indicating recent *Bbsl* exposure and possibly suggesting an incidental finding of *N. mikurensis* DNA in a patient with EM of borrelial aetiology. Despite previous reports of *N. mikurensis* DNA detected in immunocompetent patients with EM (Jahfari et al., 2016; Quarsten et al., 2017), it has also been reported in asymptomatic persons (Welc-Faleciak et al., 2014) and no causality has been established between EM-like skin lesions and infection with *N. mikurensis*.

4.3. SFG Rickettsia spp

High bacterial loads are uncommon in the blood of patients with rickettsiosis resulting in a reduced clinical sensitivity of PCR assays which could explain the observed discrepancy between our serological and molecular results (Paris and Dumler, 2016). Only one (case 1, Table 3) of the five participants who seroconverted against SFG *Rickettsia* spp. exhibited clinical features suggestive of rickettsiosis during the intermediate healthcare visit, indicating a substantial proportion of asymptomatic or mild infections. This participant presented with isolated fever lasting for a period of two days prior to the intermediate healthcare visit and had slightly elevated CRP at the time of the consultation. No treatment was initiated and clinical recovery was observed after two days with a spontaneously waning CRP over the next two weeks. The remaining four participants had either no apparent clinical signs of infection (cases 3 and 5), displayed clinical signs of EM (case 4) or presented with clinical manifestations unlikely to be associated with rickettsiosis (case 2). Of note, two participants (cases 2 and 3) reported fever in the self-completed questionnaire despite it not being mentioned in the medical records, suggesting that fever had been present during the study period but did not lead to a healthcare visit. Moreover, the proportion of participants reporting fever in the self-completed questionnaires was significantly higher in persons demonstrating seroconversion against SFG *Rickettsia* spp. compared with those

lacking seroconversion (p = 0.02, Table 4). These findings are consistent with most previous studies reporting mild febrile disease following exposure to *R. helvetica* (Fournier et al., 2004, 2000; Phongmany et al., 2006), the most common SFG *Rickettsia* sp. in field-collected ticks from Sweden (Wallménius et al., 2012). With this in mind, it is equally important to note that all the symptoms inquired upon in the self-completed questionnaires are non-specific and no causal association between symptom and infectious agent can be inferred from the current study. No eschars were noted in any of the participants and, despite previous reports linking *R. helvetica* to neuroinfection (Nilsson et al., 2011, 2010), no such cases were encountered in the current study.

Of note, three participants (cases 2, 4 and 5) exhibited both anti-*Rickettsia* antibodies and antibodies against *B. henselae*. Given the low occurrence of *B. henselae* in Scandinavian ticks and the as yet unestablished viability of ticks as vectors for this bacterial species (Telford and Wormser, 2010), serological cross-reactivity between *B. henselae* and SFG *Rickettsia* spp. is a possibility and has been reported elsewhere (Takeda et al., 2007).

4.4. Strengths and limitations

This is the first study examining the aetiology of TTIs in high-risk individuals seeking medical care following a tick-bite in the Nordic countries with all available blood samples having been tested for some of the most common tick-borne microorganisms present in these geographical regions. By selecting TBD STING study participants who had sought medical care during the three-month study period, we included those who were ill to such a degree that they chose to make a healthcare visit. Thus, the participants with TTI included in this study are the ones with the most severe manifestations of their infections. Given that participants not seeking medical care in this period of time could still have experienced symptoms of TTI, those were most likely of a milder and self-limiting nature.

It is important to note that selection bias is present and the study participants do not constitute a random population sample. This is reflected by a predominance of females (74%) and a high median age of 64 years. Furthermore, the high number of ticks detached in the three-month study period (median 6) as well as the high proportion of participants with a self-reported history of LB or prior immunisation against TBE (Table 1), suggests a population at particular risk of tick-bites and exposure to tick-borne microorganisms. Previous studies have observed increased participation rates in epidemiologic studies among women and persons more likely to view the issue being studied as particularly important to their way of life and this is likely the case in the current study (Galea and Tracy, 2007). With this in mind, one must exercise caution when extrapolating these results to the general population. However, assuming that the study population is at a particularly high risk of tick-bites and thus of contracting a TTI, it is likely that the proportion of people seeking medical care following a tick bite in the general population is lower than what we report in this study.

The high prevalence of persisting but unchanging anti-*Rickettsia* antibody levels above cut-off (Fig. 3) could be indicative of prior tick-mediated exposure. However, the prevalence is unexpectedly high,

even surpassing the seroprevalence of antibodies against *Bbsl*, the most frequently detected microorganisms in field-collected ticks from the studied regions (Wilhelmsson et al., 2013). This could be suggestive of non-specific background reactivity or other routes of exposure and underscores the importance of local cut-off levels based on regional seroprevalence studies and the importance of testing paired sequential samples for diagnostic purposes. Of particular interest, high background seroprevalence levels of anti-*Rickettsia* IgG have previously been reported among healthy blood donors in neighbouring Denmark (Kantsø et al., 2009). It is likewise important to note that *R. rickettsii* was used as antigen which could have reduced the sensitivity of the assay for other species of SFG *Rickettsia*. However, due to the considerable homology among the SFG *Rickettsia*, we expect the assay to be capable of detecting the relevant tick-borne *Rickettsia* spp. present in the Nordic countries, as has been previously suggested (Nilsson et al., 1999). Since *R. helvetica* is the most common *Rickettsia* sp. in field-collected ticks from this region, the detected anti-*Rickettsia* antibodies most likely reflect exposure to this particular species (Wallménius et al., 2012). However, it is important to keep in mind that the serological assay used is not species-specific and could reflect exposure to other species of SFG *Rickettsia*, including *R. sibirica*, which has previously been detected in field-collected *I. ricinus* ticks from Sweden (Wallménius et al., 2012).

5. Conclusions

Our study indicates that, following a tick-bite in the studied regions, the proportion of people developing symptoms leading to a healthcare visit is generally low. Among the examined high-risk individuals who sought medical care within three months following a tick-bite, many presented with complaints unrelated to TTIs. Of those presenting with TTIs, a large proportion displayed clinical signs of EM. Laboratory evidence of recent exposure to tick-transmitted microorganisms other than *Bbsl* was infrequent in this population of mostly immunocompetent individuals, with those exposed to these agents presenting with no or only mild overt symptoms of infection. Finally, concomitant exposure to more than one tick-borne microorganism was uncommon and of no demonstrable clinical implication in the current study.

Ethics statement

The study was approved by the Regional Ethics Committee in Linköping, Sweden (M132-06), and by the local Ethics Committee of the Åland Health Care, Mariehamn, Finland (2008-05-23). Written consent was obtained from all participants in the TBD STING study.

Declaration of Competing Interest

The authors have no competing interests to declare.

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