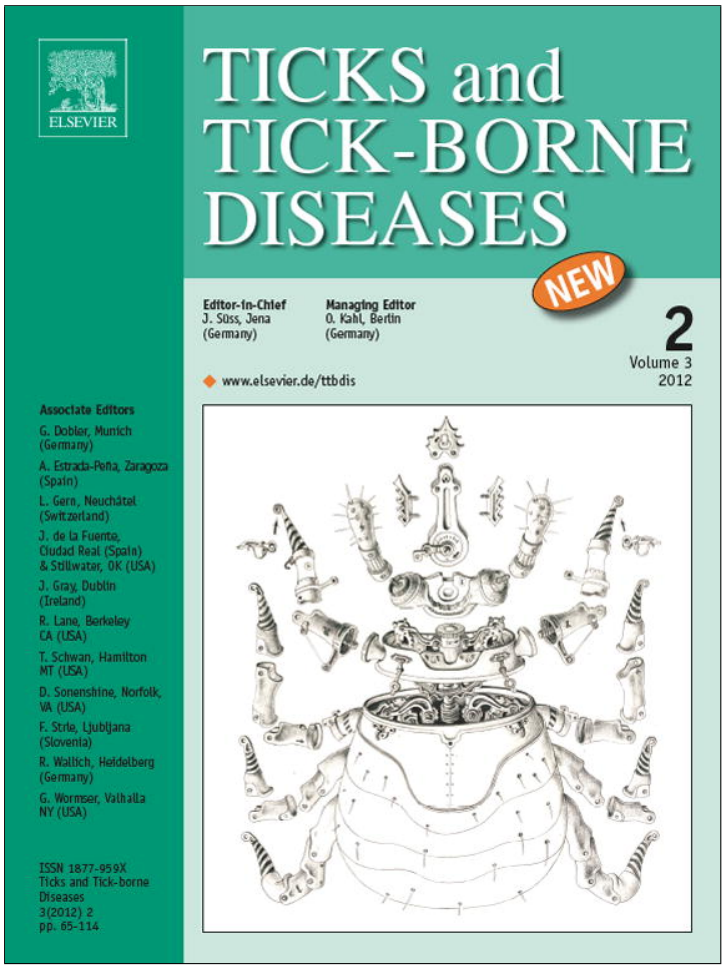

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Original article

Prevalence of *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Coxiella burnetii* in adult *Ixodes ricinus* ticks from 29 study areas in central and southern Sweden

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ABSTRACT

A total of 887 adult *Ixodes ricinus* ticks (469 females and 418 males) from 29 different localities in Sweden were screened for *Rickettsia*, *Anaplasma*, and *Coxiella* DNA using PCR and then subjected to gene sequencing. Rickettsial DNA was detected in 9.5–9.6% of the ticks. Most of the positive ticks were infected with *Rickettsia helvetica*. One tick harbored another spotted fever rickettsia, closely related to or identical with *R. sibirica* not previously found in *I. ricinus* nor in Sweden. Six of the ticks (0.7%) were infected with an *Anaplasma* sp., presumably *A. phagocytophilum*. *Coxiella burnetii* DNA was not detected in any of the ticks. The detection of *R. helvetica* and *A. phagocytophilum* in several of the localities sampled suggests that these potentially human-pathogenic agents are common in Sweden.

Introduction

Rickettsia spp., *Anaplasma* spp., and *Coxiella burnetii* are all obligate intracellular Gram-negative bacteria that cause emerging infections worldwide. The *Rickettsia* and *Anaplasma* species are all vector-borne, primarily tick-borne, while *C. burnetii* is transmitted mainly by inhalation or by contact with infectious tissues, although tick-transmission of this bacterium has also been reported (Rolain et al., 2005). *Rickettsia helvetica*, the only documented tick-borne *Rickettsia* species in Sweden, was first isolated from *Ixodes ricinus* ticks in Switzerland in 1979 (Burgdorfer et al., 1979). *I. ricinus* is the tick species that most commonly bite humans in most areas of Sweden and in Europe (Jaenson et al., 1994; Piesman and Gern, 2004) and the main European vector of the agents of Lyme borreliosis, *Borrelia burgdorferi* sensu lato, and of the tick-borne encephalitis (TBE) virus (Parola and Raoult, 2001). It is also the presumed main vector of *R. helvetica* (Burgdorfer et al., 1979; Parola et al., 1998) and *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis (HGA) (Dumler et al., 2001; Hildebrandt et al., 2011a), and one of several putative vectors of *C. burnetii* (Franke et al., 2010; Hildebrandt et al., 2011).

R. helvetica belongs to the spotted fever group (SFG) of rickettsiae, which includes 25 validated species, at least 16 of which are recognized pathogens in humans (Fournier and Raoult, 2009). A patient with disease symptoms due to SFG rickettsial infection typically present flu-like symptoms but more severe conditions such as meningitis occurs (Fournier et al., 2004; Parola and Raoult, 2001; Nilsson et al., 2010).

Previous, geographically more limited studies showed a prevalence of *Rickettsia* spp. ranging from 1.7 to 36.8% in Swedish ticks, some of which were analyzed as pooled samples (Nilsson et al., 1997, 1999; Severinsson et al., 2010). In Denmark and Poland, *R. helvetica* prevalences of 1.1–13.0% (Svendsen et al., 2009, and references therein) and 5.5–10.6% (Stanczak et al., 2008), respectively, have been recorded in *I. ricinus*.

Von Stedingk et al. (1997) found a prevalence of 6.6–9.2% of *A. phagocytophilum* in *I. ricinus* along coastal areas in Sweden, and a recent study of pooled *I. ricinus* specimens, collected in coastal and inland areas, showed a prevalence ranging between 1.3 and 15.0% (Severinsson et al., 2010).

Human infections with *C. burnetii* are usually contracted by aerosol transmission during contact with infected animal faeces or bird residues (Tissot-Dupont et al., 2004), but ticks may sometimes transmit *C. burnetii* (Rolain et al., 2005). Wild and domestic mammals serve as reservoirs and are part of the bacterium's life cycle,

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while humans are accidental hosts that may develop Q fever, a self-limiting or chronic disease. The aim of the present study was to obtain, using PCR techniques and Sanger sequencing, more reliable data on the distribution and prevalence of *Rickettsia* spp., *Anaplasma* spp., and *C. burnetii* in adult *I. ricinus* ticks collected at different locations in Sweden.

Materials and methods

Collection of ticks

Questing (host-seeking) adult *I. ricinus* ticks were collected during May 20th–September 23rd, 2008, from 29 different localities in southern and central Sweden. The localities were chosen based on previous experience of vegetation types harboring relatively abundant tick populations (Lindström and Jaenson, 2003) within reasonable distance from the research center (Fig. 1). At each of the 29 localities, with mixed coniferous/deciduous or deciduous woodland vegetation, ticks were sampled by dragging a 1-m^2 white woollen flannel cloth over the ground vegetation. Any adult female and male ticks present were put individually into separate Eppendorf tubes. However, 4 male and 6 female ticks collected at Gotska Sandön and Särö Västerskog were analyzed in 5 pooled samples.

Five male and 7 female *I. ricinus* ticks, 4 of which were blood-fed, collected from a domestic dog [*Canis lupus familiaris* (Canidae)] at Norbo Finnmark, were also included (Table 1). When the collection was completed, species identification was performed within 2 weeks on all 887 ticks (469 females and 418 males), during which time the ticks were kept in a refrigerator room at +4 °C and thereafter moved to a freezer at −70 °C. The nucleic acid (DNA) isolation was done in a later stage after which those samples were kept at −70 °C from the time of extraction.

DNA extraction and screening using real-time PCR

The ticks were disinfected and homogenized in sodium phosphate-buffered (100 mM; pH 7.2) isotonic (0.9% NaCl, w/v) saline, after which RNA and DNA were extracted from the homogenate from each tick using 800 μL TriPure reagent (Roche Diagnostics) and 2 μL (20 mg/mL) glycogen (Invitrogen, Carlsbad, CA), and applying a protocol with chloroform, as previously described (Severinsson et al., 2010). All extracted DNA specimens were screened using real-time PCR, targeting the *gltA* gene of SFG *Rickettsia* spp. (Stenos et al., 2005), the 16S rRNA gene of *Anaplasma* spp. (Jäderlund et al., 2009), and a modified and single PCR targeting the *icd* gene (Klee et al., 2006) of *C. burnetii* in the above order (Table 2). The real-time PCR assays were performed in a Rotor-Gene 3000 (Corbett Research, Sydney, Australia) using LightCycler® TaqMan® Master (Roche, Mannheim, Germany) for the *Rickettsia* PCR and TaqMan® Universal PCR Master Mix, No AmpErase® UNG and TaqMan® Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA) for the *Anaplasma* and *Coxiella* PCRs, respectively. Plasmid standard controls were used in real-time PCR of *Rickettsia* and for the *Anaplasma* 16S rRNA gene (Severinsson et al., 2010; Jäderlund et al., 2009) and for *C. burnetii*, purified chromosomal DNA of the agent was used as a positive control (Vircell, Granada, Spain). Two negative controls of distilled water were also included in each PCR run. After all other PCR tubes had been sealed, 2 positive controls were added in each PCR run, as a final stage, to avoid contamination. In the *Rickettsia* and *Coxiella* PCR assays, uracil-DNA glycosylase (UNG) was used to avoid contamination by carry-over amplified products. For *Anaplasma*, the amplified real-time PCR product is long enough for successful sequencing, which is why no UNG was added.

PCR amplification

For *Rickettsia* spp., the real-time PCR-positive samples were further analyzed using conventional and nested PCR assays targeting the *ompB* and 17-kDa genes, with the expected fragment lengths of 475 bp (or nested 267 bp) for *ompB* (Choi et al., 2005); 371 bp and nested 214 bp (Leitner et al., 2002), and 434 bp (Carl et al., 1990) for 17 kDa, respectively, together with a semi-nested PCR targeting the *gltA* gene yielding a 832-bp long fragment (Table 2).

A 5-μL portion of DNA template was amplified in a reaction mixture containing 500 nM of both primers, 0.625 units *Taq* polymerase, 200 μM of each dNTP, PCR buffer, and Q solution from *Taq* Core kit (Qiagen, Hilden, Germany), yielding a total reaction volume of 25 μL. Amplification was carried out under the following conditions: an initial 3-min denaturation step at 94 °C followed by 35 cycles consisting of 1 min each at 94 °C, 60 °C, and 72 °C, and a final extension at 72 °C for 10 min. A negative control of distilled water was included in each PCR run, and, as a positive control, extracted DNA from *R. helvetica* isolated from an *I. ricinus* tick (Nilsson et al., 1999) was added after all other PCR tubes had been sealed to avoid contamination. Nested and conventional PCR assays were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). Amplified PCR products were separated by electrophoresis on a 1% agarose gel stained with ethidium bromide, illuminated by UV light and compared with the DNA molecular weight marker GeneRuler™ Express DNA ladder (Fermentas GmbH, St. Leon-Rot, Germany).

The *Anaplasma* sp. samples that were positive in real-time PCR were further analyzed using a nested PCR assay that amplifies partial sequences of the 16S rRNA gene (1433 and 928 bp), as previously reported (Barlough et al., 1996) (Table 2). As a positive control in the conventional PCR, we used extracted DNA from *A. phagocytophilum* (received from the Zoonotic Ecology and Epidemiology Section, Kalmar University, Kalmar, Sweden). In a few samples, the volume of extracted DNA was sufficient only for real-time PCR screening and conventional PCR for identification of *Rickettsia* species. Therefore, some specimens could not be screened for *Anaplasma* spp. or *C. burnetii*; for this reason, no data on coinfections were obtained.

Sequencing and identification of Rickettsia and Anaplasma spp.

Positive PCR products were purified with Exonuclease I and FastAP™ Thermosensitive Alkaline Phosphatase according to manufacturer's instructions (Fermentas GmbH) and thereafter sequenced using BigDye® Terminator v 3.1 Cycle Sequencing Kit performed in an ABI 3130 instrument (Applied Biosystems) or at Uppsala Genome Center (Science for Life Laboratory, Dept. of Immunology, Genetics and Pathology, Uppsala University, Rudbeck Laboratory, Sweden). When sequencing the *gltA* gene, an additional primer, CS535d, was used (Roux et al., 1997). All sequences were analyzed for species identification and pairwise similarities using DNA Baser version 2.80.0 (Heracle Software, Lilienthal, Germany) and compared to deposited sequences in GenBank (http://blast.ncbi.nim.nih.gov/Blast.cgi) using the Basic Local Alignment Search Tool (BLAST). Sequence alignments were conducted using Clustal W in BioEdit Sequence Alignment Editor version 7.0.5.3 (Ibis Therapeutics, Carlsbad, CA).

Statistical analysis

Odds ratio procedures (OR), Fisher's exact test and χ^2-test were used to compare the proportions and a p value <0.05 was considered statistically significant. Statistical analyses were conducted using Predictive Analytics Software (PASW®) Statistics 20.

Table 1
Study areas (GPS coordinates in order from north to south) and results, based on PCR, for *Rickettsia* spp. and *Anaplasma* spp.

Study area (no.)	Locality	*I. ricinus*: No. PCR-positive for *R. helvetica*/No. examined/(% pos)			*I. ricinus*: No. PCR-positive for *Anaplasma* sp./No. examined/(% pos)			GPS coordinates	
		Males	Females	Total	Males	Females	Total	LAT	LON
1	Hudiksvall	0/6 (0)	0/5 (0)	0/11 (0)	0/6 (0)	0/5 (0)	0/11 (0)	61.6372963	17.4464491
2	Stenö/Källskär	16/90 (18)	25/92 (27)	41/182 (22.5)	1/77 (1)	2/73 (3)	3/150 (2)	61.2535660	17.1885400
3	Gävle	0/1 (0)	–	0/1 (0)	0/1 (0)	–	0/1 (0)	60.8506019	17.1951389
4	Trödje	0/2 (0)	1/2 (50)	1/4 (25)	0/2 (0)	0/2 (0)	0/4 (0)	60.8259167	17.2384444
5	Skutskär	0/11(0)	1/15 (7)	1/26 (4)	0/11 (0)	0/14 (0)	0/25 (0)	60.6225000	17.4671019
6	Älvkarleby	0/6 (0)	0/13 (0)	0/19 (0)	0/6 (0)	0/13 (0)	0/19 (0)	60.5335000	17.4389676
7	Borlänge	0/4 (0)	1/4 (25)	1/8 (13)	0/4 (0)	0/3 (0)	0/7 (0)	60.4677778	15.5894444
8	Vikmanshyttan	0/2 (0)	0/4 (0)	0/6 (0)	0/2 (0)	0/4 (0)	0/6 (0)	60.3223565	15.8886204
9	Östhammar	0/10 (0)	0/15 (0)	0/25 (0)	0/10 (0)	0/15 (0)	0/25 (0)	60.2984769	18.4109306
10	Norbo Finnmark	1/11 (9)	2[a]/12 (17)[b]	3[a]/23 (13)[b]	0/10 (0)	0/12 (0)[b]	0/22 (0)[b]	60.2811111	15.4547222
11	Väddö	0/10 (0)	1/7 (14)	1/17 (6)	0/10 (0)	0/6 (0)	0/16 (0)	59.9595509	18.8499583
12	Skebobruk	0/13 (0)	0/19 (0)	0/32 (0)	0/13 (0)	1/19 (5)	1/32 (0)	59.9447546	18.6482130
13	Morga	1/31 (3)	8/55 (15)	9/86 (10)	0/30 (0)	0/47 (0)	0/77 (0)	59.7577778	17.6480093
14	Rimbo	0/2 (0)	1/4v(25)	1/6 (17)	0/2 (0)	0/3 (0)	0/5 (0)	59.7402870	18.2275370
15	Kapellskär	2/59 (3)	1/70 (1)	3/129 (2)	0/56 (0)	1/69 (1)	1/125 (0.8)	59.7202824	18.9147917
16	Kolarvik	8/57 (14)	6/49 (12)	14/106 (13.2)	0/49 (0)	0/43 (0)	0/92 (0)	59.5755556	17.0950000
17	Västerås	1/22 (5)	0/13 (0)	1/35 (3)	0/22 (0)	0/13 (0)	0/35 (0)	59.5380556	16.5386111
18	Strängnäs	1/27 (4)	0/22 (0)	1/49 (2)	0/26 (0)	1/22 (5)	1/48 (2)	59.4026806	17.0283750
19	Eskilstuna	0/4 (0)	3/6 (50)	3/10 (30)	0/4 (0)	0/3 (0)	0/7 (0)	59.3611296	16.4171620
20	Karlstad	0/5 (0)	1/3 (33)	1/8 (13)	0/5 (0)	0/2 (0)	0/7 (0)	59.3589259	13.4355694
21	Värmdö	0/3 (0)	1/6 (17)	1/9 (11)	0/3 (0)	0/5 (0)	0/8 (0)	59.2958333	18.5817546
22	Askersund	–	0/5 (0)	0/5 (0)	–	0/5 (0)	0/5 (0)	58.8813519	14.9522315
23	Herrhamra	0/32 (0)	1/35 (3)	1/67 (1)	0/30 (0)	0/35 (0)	0/65 (0)	58.8070231	17.8255556
24	Kapellängen, GS (P)	0/2 (0)	0/2 (0)	0/4 (0)	0/2 (0)	0/2 (0)	0/4 (0)	58.3872685	19.2005093
25	Gamla gården, GS (P)	0/2 (0)	1–2/2 (50–100)	1–2/4 (25–50)	0/2 (0)	0/2 (0)	0/4 (0)	58.3500741	19.2168472
26	Jönköping	0/2 (0)	0/1 (0)	0/3 (0)	0/2 (0)	0/1 (0)	0/3 (0)	57.8301481	14.3044444
27	Västervik	0/4 (0)	0/5 (0)	0/9 (0)	0/4 (0)	0/5 (0)	0/9 (0)	57.7876528	16.5827593
28	Vg. Änggårdsbergen	–	0/1 (0)	0/1 (0)	–	0/1 (0)	0/1 (0)	57.6774074	11.9501296
29	Hall. Särö Västerskog (P)	–	0/2 (0)	0/2 (0)	–	0/2 (0)	0/2 (0)	57.5138889	11.9282407
Total		30/418 (7.2)	54–55/469 (11.5–11.7)	84–85/887 (9.4–9.5)	1/389 (0.3)	5/426 (1.2)	6/815 (0.7)		
CI		(4.7–9.7)	(8.6–14.4)	(7.5–11.4)	(−0.2–0.8)	(0.2–2.2)	(0.1–1.3)		

GS, Gotska Sandön; (P), pooled samples; CI, confidence interval.

[a] One tick was fully fed.

[b] Four ticks were fully fed.

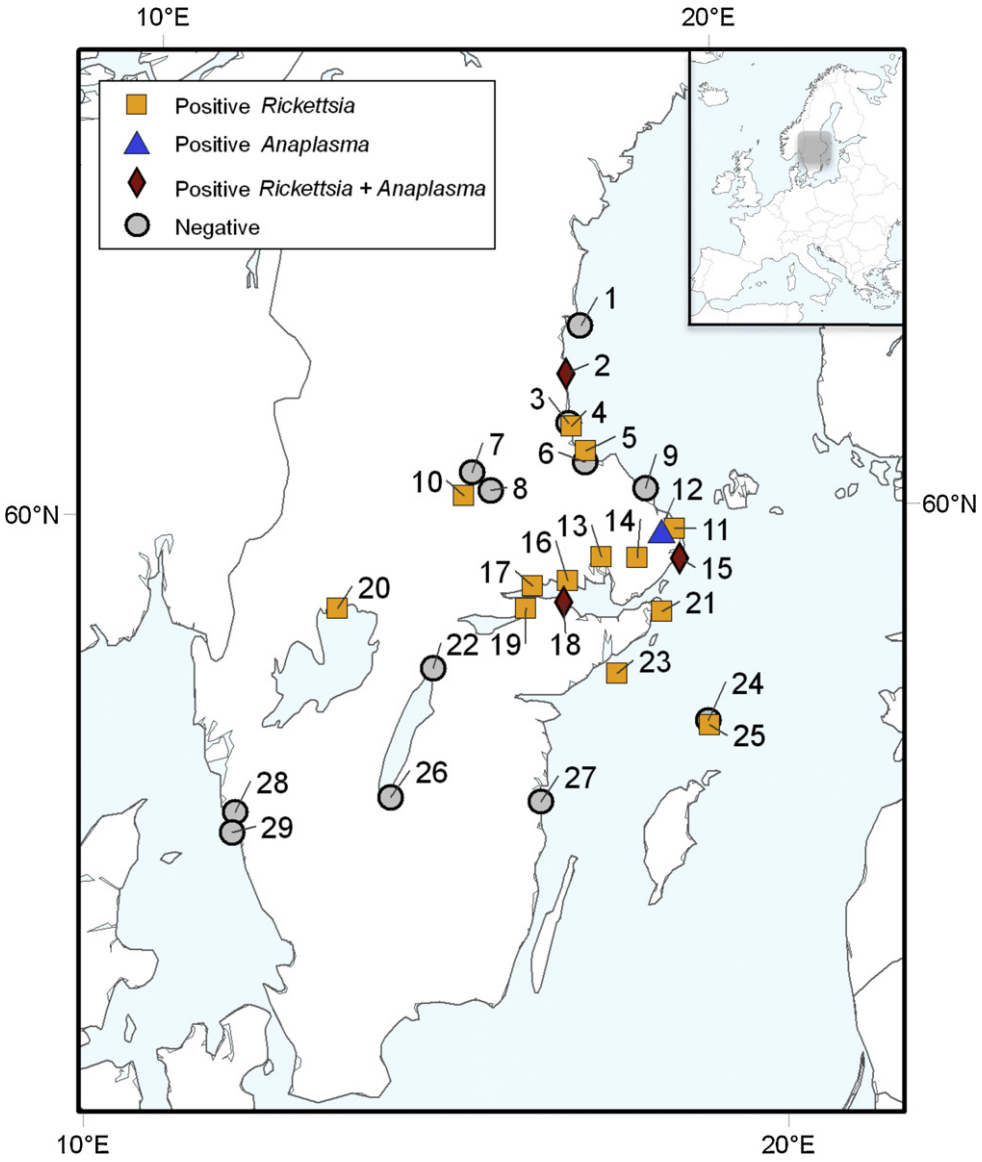

Fig. 1. Tick sampling localities in Sweden in order from north to south. Names of the localities are listed in Table 1.

Results

Prevalence of Rickettsia spp.

Of the 887 adult tick samples, 84–85 (9.54–9.6%) were positive for SFG *Rickettsia* according to real-time PCR; of the positive ticks, 54–55 were females and 30 were males. The prevalence of *Rickettsia* spp. between habitats was not statistically different with regard to coastal or inland localities ($p > 0.05$, Fisher's exact test) (Table 1).

For 45 of these samples there was enough material for further analysis by sequencing, and the amplified *ompB* gene products were successfully sequenced for 39 of these ticks, and all but one were identified as *R. helvetica*, showing 100% similarity with the corresponding gene sequences deposited in GenBank (accession number EF219461).

In addition, the partial 17-kDa gene was amplified and sequenced for 21 of 39 *ompB*-positive and 6 other real-time PCR-positive ticks, all identified as *R. helvetica* (accession number GU827073). The semi-nested *gltA* PCR runs, in 3 of the *ompB*- and 17-kDa-positive samples, resulted in 791-bp-long fragments (excluding primer regions) of which 2 of the sequences were identified as *R. helvetica*, presenting 100% resemblance to the accession number U59723.

One adult male tick [No. 977, collected from a dog in Norbo Finnmark (Table 1)] showed 98.6–99% sequence identity (*ompB*) with the spotted fever subgroup of *Rickettsia* spp., including among others, *R. parkeri* (accession number FJ644549), *R. conorii* (accession number AE006941), *R. slovaca* (accession number AF123723), and *R. sibirica* (accession number AF123722). The difference was 2–3 bp within the cluster and 6 bp for *R. helvetica*. The *gltA* 791-bp long fragment sample was 96.8% (766/791 bp) identical with *R. helvetica*, 99.5% (787/791 bp) identical with *R. slovaca* (accession number U59725), 99.6% identical (788/791 bp) with *Rickettsia* sp. 'Atlantic rainforest' (accession number GQ855235) and *R. mongolotimonae* isolate URRMTMFEe65 (accession number DQ097081), *Rickettsia* sp. BJ-90 (AF178035), and 100% (791/791 bp) identical with *R. sibirica* 246 (accession number RSU59734). These results therefore indicate that tick No. 977, collected from a domestic dog, was infected with a spotted fever group rickettsia closely related to or identical with *R. sibirica*.

Table 2
Primers and probes used in the real-time PCR assays and primers for PCR amplification and sequencing of *Rickettsia* spp. and *Anaplasma* spp.

Primer	Species	Gene	Nucleotide sequence (5′–3′)	Product size (bp)
CS-F	*Rickettsia*	*gltA*	TCG CAA ATG TTC ACG GTA CTTT	
CS-R			TCG TGC ATT TCT TTC CAT TGT G	74
CS-P			6FAM-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-BBQ	
GER3	*Anaplasma*	16S rRNA	TAG ATC CTT CTT AAC GGA AGG GCG	
GER4			AAG TGC CCG GCT TAA CCC GCT GGC	151
AP16S-P			6FAM-CTG TCG TCA GCT CGT GTC GTG AGA TGT TG-BBQ	
icd-439F	*C. burnetii*	*Icd*	CGT TAT TTT ACG GGT GTG CCA	
icd-514R			CAG AAT TTT CGC GGA AAA TCA	76
icd-464P			6FAM-CAT ATT CAC CTT TTC AGG CGT TTT GAC CGT-BBQ	
RH314	*Rickettsia*	*gltA*	AAA CAG GTT GCT CAT CAT TC	837
CSF-R			AAG TAC CGT GAA CAT TTG CGA	
CS-Ric-R			CAG TGA ACA TTT GCG ACG GTA	832
CS535d			GCA ATG TCT TAT AAA TAT TC	Sequencing primer
ompB-YF		*ompB*	GTC AGC GTT ACT TCT TCG ATG C	
ompB-YR			CCG TAC TCC ATC TTA GCA TCA G	475
ompB-IF			CCA ATG GCA GGA CTT AGC TAC T	
ompB-IR			AGG CTG GCT GAT ACA CGG AGT AA	267
Ric17-YF		17 kDa	GGA ACA CTT CTT GGC GGT G	
Ric17-YR			CAT TGT CCG TCA GGT TGG CG	371
Ric17-IF			GCA TTA CTT GGT TCT CAA TTC GG	
Ric17-IR			AAC CGT AAT TGC CGT TAT CCG G	214
Rr17kDa.61p			GCT CTT GCA ACT TCT ATG TT	
Rr17kDa.492n			CAT TGT TCG TCA GGT TGG CG	432
EE-1	*Anaplasma*	16s rRNA	TCC TGG CTC AGA ACG AAC GCT GGC GGC	
EE-2			AGT CAC TGA CCC AAC CTT AAA TGG CTG	1433
EE-3			GTC GAA CGG ATT ATT CTT TAT AGC TTG C	
EE-4			CCC TTC CGT TAA GAA GGA TCT AAT CTC C	928

Prevalence of Anaplasma spp.

DNA from 815 tick samples was screened for *Anaplasma* spp. using real-time PCR, resulting in 6 positive ticks (0.7%), of which 5 were female and one male. Of the 6 PCR products, 4 were successfully sequenced and were 100% identical with *A. phagocytophilum* (accession number AB604783). However, the length of the amplified sequence region was only 102 bp, which is not optimal for discrimination between different species of *Anaplasma* spp., and, unfortunately, the nested PCR assays resulted in no analysable products. Of the 6 positive ticks, 3 were collected at Stenö (Table 1).

Prevalence of C. burnetii

Because the tick samples had been used for previous analyses, extracted DNA from 786 of the ticks were available for PCR analyses of *C. burnetii*, which could not be detected in DNA samples from any of these ticks.

Discussion

The present findings are the first to show the prevalence and distribution of spotted fever rickettsiae and *Anaplasma* sp. closely related to or identical with *A. phagocytophilum* in individual adult *I. ricinus* ticks collected from a number of field localities in Sweden. The prevalence of 9.5–9.6% for *Rickettsia* spp. is in accordance with results from previous European investigations, e.g., in Poland where a prevalence of 2.8–8.5% was recorded, and Spain where 16.7% of ticks were positive for *R. helvetica* (Parola and Raoult, 2001). Our results are also in line with a previous study in central Sweden, in which Severinsson et al. (2010) recorded an infection rate of 2.6–10% in pooled samples of adult *I. ricinus* ticks. No statistical differences in prevalence were found with regard to coastal or inland localities. However, the scattered occurrence supports the view that this tick-borne rickettsiosis is endemic to Sweden.

When aligned, all but one of the sequenced products showed 100% homology with *R. helvetica*. The product of tick No. 977 differed from the *R. helvetica* sequence, but was the same as that of *R. sibirica*. This species has previously been detected in various species of *Haemaphysalis* and *Dermacentor*, but, to our knowledge, it has not previously been found in any *Ixodes* species (Piesman and Gage, 2004). *R. sibirica* is the aetiologic agent of Siberian tick typhus (North Asian tick typhus). Migratory passerine birds may have contributed to the geographic dissemination of this spotted fever *Rickettsia* (Elfving et al., 2008). *R. monacensis* and *R. slovaca* have been found in *I. ricinus* (Sekeyova et al., 2000; Simser et al., 2003), but none of these rickettsiae have been found in Sweden. *R. felis* has previously been recorded in Sweden in 2 human patients with subacute meningitis; the vector(s) in these cases is/are unknown (Lindblom et al., 2010).

R. helvetica is the only species previously found in free-living ticks in Sweden. However, data on the potential presence and modes of maintenance and transmission of *R. helvetica* in a vertebrate reservoir have not been studied sufficiently.

While bacteria of the genus *Rickettsia* are generally characterized by their transovarial (vertical) mode of transmission in the vector population, this route of transfer seems not to occur in *A. phagocytophilum*. This *Anaplasma* would therefore need one or more vertebrate reservoirs for maintenance of its life cycle. Accordingly, small mammals (*Apodemus* spp., *Myodes glareolus*, *Microtus agrestis*, *Mi. oeconomus*), medium sized mammals such as the hedgehog (*Erinaceus europaeus*), and large mammals such as sheep (*Ovis aries*), cattle (*Bos taurus*), and roe deer (*Capreolus capreolus*) have been identified as or suspected to be reservoir hosts for *A. phagocytophilum* in Europe (Alberdi et al., 2000; Stuen, 2007; Rosef et al., 2009; Skuballa et al., 2010; Rar et al., 2011).

In a previous study in Sweden, *A. phagocytophilum* was detected in nymphs at a prevalence of 1.7–19.4%, but not in any larval or adult ticks (Severinsson et al., 2010). Our present result, i.e., 6 positive adult ticks out of 815 adults (0.7%) is at the lower end of the reported prevalence range, i.e., 0.4–66.7% in European countries (Blanco and Oteo, 2002; Bown et al., 2003). In a study in Denmark, Skarphédinsson et al. (2007) detected a greater prevalence in adult ticks (40.5%) than in nymphs (14.5%). Rosef et al. (2009) found a high infection prevalence in *I. ricinus* nymphs and adults at sites where deer were abundant. These authors concluded that in Norway cervids are important reservoirs of *A. phagocytophilum*.

Several genetic variants of *A. phagocytophilum* have been recorded from mammals and ticks in Europe (Stuen, 2007). Biological and ecological differences exist between these variants which behave differently in the mammalian hosts (Stuen, 2007). The large differences in infection prevalence among different investigations may be explained by the presence of different variants of *A. phagocytophilum* so that one particular variant in one locality has a particular association with a limited number of species of related vertebrates, while another variant is associated with another guild of reservoir species (Rar et al., 2011). Another explanation for a low prevalence, such as in this study, or a complete absence of *A. phagocytophilum* in adult ticks (Severinsson et al., 2010) may be that the transstadial transmission of the variant(s) of *A. phagocytophilum*, present in the biotopes sampled, is not efficient. Kurtenbach et al. (1998) described the presence of borreliacidal activity of deer sera which explains the incompetence of cervids to act as efficient reservoirs for *Borrelia burgdorferi* s.l. The low prevalence of *A. phagocytophilum* in adult ticks of the present study and that of Severinsson et al. (2010) may be hypothesized to depend on similarly acting factors in the nymphal host's serum. Thus, if larval *I. ricinus* became infected while feeding on *Anaplasma*-infective small mammals (rodents) and then ingested their second blood meal as nymphs from larger mammals (cervids), factors present in the second host's blood would eliminate the bacteria before the ticks were captured as host-seeking adult ticks. Further studies are needed to show which one(s) of these hypotheses is/are valid.

The primers GER 3 and GER 4 (Table 2) correspond to bases 950–973 and 1077–1101, respectively, and amplify a 151-bp fragment from species of the *Ehrlichia phagocytophila* group (e.g., *E. equi* and the agent causing human granulocytic ehrlichiosis), but not from monocytic ehrlichia, including the closely related *E. canis* (Jäderlund et al., 2009). After a previous revision of the family Anaplasmataceae, *Ehrlichia equi*, *E. phagocytophila*, and the human granulocytic ehrlichiosis (HGE) agent are represented by the single species *A. phagocytophilum* (Dumler et al., 2001), which is also the only probable species detected by the PCR.

Q fever is a common infection in Sweden, based on the fact that *C. burnetii* has been isolated from placentas of sheep, which have been collected at farms with humans seropositive to the organism, and that seropositivity in humans is found throughout the country (Åkesson et al., 1991). A serosurvey was conducted in Sweden in 1993 where antibodies to the agent were detected in 10 of 784 cattle (1.3%).

In 2008/2009, a screening for antibodies against Q fever in bulk milk from 1537 randomly selected cattle holdings showed a presence of antibody-positive herds in about 8% (National Veterinary Institute, Sweden; official report).

C. burnetii has been reported in less than 2% of *I. ricinus* ticks in Europe (Astobiza et al., 2011). The absence of *C. burnetii* in ticks may have no relation to the prevalence of the pathogen in the study area. This is because *C. burnetii* may circulate among wild and domestic ungulates without any involvement of ticks in its transmission.

Conclusions

The data obtained suggest that in areas of Sweden where *I. ricinus* occurs *R. helvetica* and *Anaplasma* sp. are prevalent. It appears that another spotted fever *Rickettsia*, possibly *R. sibirica*, is present in Sweden. The results can be used as a basis for further studies on the distribution of the agents in nature and the potential risk of their transmission to humans. Based on the prevalence in the study areas, tick-borne SFG rickettsioses and HGA should always be considered in patients presenting disease symptoms after tick bites.

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