Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Department of Biomedical Sciences and Veterinary Public Health

Species identification and pathogen detection by FLUIDIGM, a high-throughput PCR technique in ticks collected from northern Sweden

Phimphanit Choklikitumnuey

Master's degree Project in Biology, 30.0 credits. Spring 2019
Department: Biomedical Sciences and Veterinary Public Health (BVF), Section for Parasitology, Swedish University of Agricultural Sciences (SLU)
Supervisor: Giulio Grandi

Species identification and pathogen detection by FLUIDIGM, a high-throughput PCR technique in ticks collected from northern Sweden

Phimphanit Choklikitumnuey

Supervisor: Giulio Grandi, SLU, Department of Biomedical Sciences and Veterinary Public Health

Examiner: Eva Tyden, SLU, Department of Biomedical Sciences and Veterinary Public Health

Credits: 30 credits

Level: Advanced, A2E

Course title: Independent project in Biology

Course code: EX0871

Programme/education: Biology

Course coordinating department: Department of Animal Breeding and Genetics

Place of publication: Uppsala Year of publication: 2020

Online publication: https://stud.epsilon.slu.se

Keywords: ticks, tick-borne diseases, fluidigm

Swedish University of Agricultural Sciences Faculty of Veterinary Medicine and Animal Science
Department of Clinical Sciences

Abstract

Due to the environmental climate changes, northern Sweden is potentially a geographical area where new tick species could become established and introduce along with them new tick-borne pathogens. High-throughput real-time PCR, FLUIDIGM, was selected to perform the pathogen detection in this study. In this study ticks (n=1,421) from northern Sweden in 2018 (between June and October), either collected from different host species (dog, cat, horse, rabbit, cattle, mice, rabbit and human) or questing ticks collected from the environment, were identified at species level using morphological keys; *Ixodes ricinus*/*I. persulcatus* identification was confirmed by a PCR assay included within FLUIDIGM analyses. The ticks were identified belonging to the species as follow: 1,381 *Ixodes ricinus* ticks (adult females; n=1167, adult males; n=176 and nymphs; n=38), 27 *Ixodes persulcatus* ticks (adult females; n=21, adult males; n=5 and nymphs; n=1), 4 most likely hybrid species of *Ixodes ricinus* and *Ixodes persulcatus* (adult females; n=4), 12 *Ixodes trianguliceps* (adult females; n=11 and nymphs; n=1) and 1 adult female *Hyalomma marginatum.* Ticks were positive for the following tick-borne pathogens: 25.26% *Borrelia* spp., 4.08% *Babesia venatorum*, 0.28% *B. microti*,*B. divergens/B. capreoli*., 9.15% *Anaplasma* spp., 9.22% *Anaplasma phagocytophilum*, 6.19% *Neoehrlichia mikurensis*, 25.62% *Rickettsia helvetica*, 0.07%, *Rickettsia aeschlimannii*, 0.49%, tickborne encephalitis virus (TBEV), 2.25% Uukuniemi virus (UUKV), 4.08% *Babesia venatorum*, 0.28% *Babesia microti*, and 0.49% *Babesia divergens/B. capreoli*. This is the first time that this form of study on ticks and tick-borne pathogens has been performed in this northern area of Sweden. The results confirm that *I. ricinus* is the dominant tick species in this area and the increased spreading of *I. persulcatus* compared to previous reports should be observed. These results confirm that even ticks in this northern are can also be vectors of important medical and veterinary pathogens. Moreover, this is the first report of *H. marginatum* in this northern area.

Table of Contents

Introduction

Tick species in Sweden focusing on northern Sweden

Ticks are blood-feeding ectoparasites of most vertebrates which are distributed around the world. Ticks are considered as some of the most relevant arthropod vectors of several human and animal infectious diseases, since they are able to transmit wide range of pathogens including bacteria, viruses, parasites and protozoa (de la Fuente et al., 2017; Gulia-Nuss et al., 2016; Parola et al., 2013; Parola and Raoult, 2001). According to Barker and Murrell (2004), around 900 tick species are currently identified and classified worldwide. The majority of tick in northern Europe belong to the hard tick or *Ixodidae* family, and the minority belongs to soft tick or Argasidae family. Since 1952, several studies have investigated the Swedish tick fauna (i.e., Nilsson, 1988; Brinck et al., 1967; Arthur, 1952) and one study in 1994 of Jaenson et al. (1994) has recorded around 14 tick species that have been found in Sweden considering their geographic distribution and host relationships. Several tick species mentioned as permanently present in Sweden were classified according to their host species. Firstly, the tick species that feed on birds are *Ixodes* (*I.*) *arboricola*, *I. caledonicus*, *I. lividus*, *I. unicavatus* and *I uriae*. Secondly, the tick species that feed on mammals are *Carios vespertilionis*, *I. canisuga*, *I. hexagonus* and *I. trianguliceps*. Lastly, the tick species feeding on both birds and mammals are *Haemaphysalis punctata* and *I. ricinus*. Other tick species are introduced (but still not established in the country) either by imported dogs – as *Rhipicephalus sanguineus* or by migratory birds, like *Hyalomma marginatum*. Recently *I. persulcatus*, probably introduced by birds, seems to be established in northern Sweden (Jaenson et al., 2016). Moreover, imported exotic zoo animals and pets can transport several tick species non-endemic in Sweden (Jaenson et al., 1994).

Throughout these last three decades, *Ixodes ricinus* as the most common tick species present in Sweden has become more abundant in central and southern Sweden and has gradually spread its range of territory expansion northwards on account of varied factors. One of the probable factors is climate change. Due to the warmer climate and milder winters effect, the vegetation period was extended which increase the possibility of the tick's maintenance hosts ubiquity, distribution and existence which support the increasing of tick and its expansion (Jaenson et al., 2012).

In northern Sweden considering all municipalities laying north of the river Dalälven, around six tick species have been recorded; *Ixodes ricinus* has been recorded from an extensive range of host animals that resembles to cover nearly all mammals and more than fifty bird species inhabiting in the area. *I. trianguliceps* has been found on eleven species of rodents and shrews. *I. persulcatus* has been reported from a variety of mammal and bird species while small mammals and birds served as hosts of immature ticks, medium-sized to large mammals served as hosts of adult ticks. *I. uriae* has been recorded from more than 48 bird species of colony-nesting marine birds while *I. lividus* has been recorded from only one bird species, the sand martin (*Riparia riparia*) or could be found on other bird species utilizing the nests of the sand martin. Lastly, *Rhipicephalus sanguineus* was found on imported dogs from southern latitudes (Hillyard, 1996; Jaenson et al., 1994).

Tick-borne pathogens

In Europe, ticks are some of the most important arthropod vectors that transmit several pathogens of medical and veterinary importance (Heyman et al., 2010). Humans served as accidental hosts of these diseases which are regularly sustained in constant natural cycles engaging ticks, wildlife and/or domestic animals (de la Fuente et al., 2017; Colwell et al., 2011; De et al., 2008).

Ixodesricinus is a tick species widespread in Europe; it can transmit several diseases as Lyme borreliosis caused by spirochete bacteria from the *Borrelia* genus (*Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii,* and *Borrelia spielmanii* in Europe), and the relapsing fever spirochete which also caused by other spirochete bacteria from the *Borrelia* genus (*Borrelia miyamotoi*), which has been reported in Sweden (Henningsson et al., 2019; Hovius et al., 2013). Except for *Borrelia* transmission, *I. ricinus* can transmit several different pathogens, including other bacteria as *Anaplasma* spp. (*Anaplasma phagocytophilum* being the most commonly reported in Europe). *A. phagocytophilum* bacteria dwell inside host white blood cells causing anaplasmosis. *I. ricinus* can also transmit bacteria causing various medical infectious diseases such as an obligate intracellular Gram-negative bacteria *Rickettsia* spp. from the spotted fever group, an obligate intracellular bacteria Candidatus *Neoehrlichia mikurensis*, *Ehrlichia* spp., *Francisella tularensis* and *Bartonella* spp. (Portillo et al., 2018; Parola et al., 2013; Parola and Raoult, 2001; Cotté et al., 2008).

Additionally, ticks can transmit intracellular parasites as protozoa from *Babesia* genus: *Babesia divergens, Babesia* sp. EU1 and *Babesia microti* causing piroplasmosis*,* and *Theileria* spp. (Bonnet et al., 2007; Bishop et al., 2004). Moreover, ticks can also transmit viruses which called tick-borne viruses (TBVs) including tick borne encephalitis virus (TBEV) which is a single-stranded RNA virus considered as one of the most important medical pathogens, since it is causing tick-borne encephalitis (TBE) in humans (Belova et al., 2012). Three subtypes of TBEV considered European (EU), Eastern, and Far-Eastern subtype are discriminated by different tick vector species, different geographic distribution and different degrees of pathogenicity (Dobler et al., 2018). EU subtype of TBEV is the one found until now in *I. ricinus* ticks in Sweden (Golovljova et al., 2004). Another virus of significant medical concern is a single-stranded RNA virus as Uukuniemi virus (UUKV) which is also transmitted by *I. ricinus* (Papa et al., 2018; Mazelier et al., 2016). Other than TBEV and UUKV, ticks can also transmit louping ill virus (LIV), Kemerovo virus and Eyach virus (EYAV) (Rizzoli et al., 2014; De et al., 2008)

For as much as ticks withhold a diversity of pathogens, some of them are obligate intracellular organisms, which are difficult to isolate and grow *in vitro*. Therefore, molecular methods are commonly used for TBP identification. A High-throughput real-time PCR, microfluidic system (BioMark™ dynamic array system, FLUIDIGM), is a method that is able to perform many real-time PCRs at the same time/on the same chip using low-volume of sample. This method has been tested to be effectively used to perform enhanced monitoring of public and animal health emerging diseases (Michelet et al., 2014).

Several terrestrial ecosystems have already been affected by the increasing world surface temperature due to the climate change, which in turn affects the distribution of animal and plant species (Dantas-Torres, 2015). Northern Sweden is considered to be a geographical area where due to climate change new tick species could become established and introduce along with them new tick-borne pathogens. This study aims to provide updated information i) on the tick species found on animals and humans in northern Sweden and ii) on the tick-borne

pathogens they might carry by using a high-throughput real-time PCRs (FLUIDIGM) to screen relevant pathogens potentially present or introduced by ticks in Northern Sweden.

Materials and methods

Tick collection

One thousand-four-hundred-twenty-one ticks were selected from the tick collection of Climate change effects on the epidemiology of infectious diseases and the impacts on Northern societies (CLINF) project (https://clinf.org/) in 2018 (totally, more than 3,000 ticks were collected). CLINF is a project aimed at investigating the potential expansion of infectious diseases in the nordic countries. The ticks were collected and sent by people to the National Veterinary Institute (SVA, Uppsala) between June and October 2018; the area of interest was defined as north of the river Dalälven.

The selected ticks were divided in 3 groups based on the geographical origin: 1. "N group": ticks collected from all municipalities in Norrbotten, Västerbotten (except Umeå municipality) and Jämtland counties 2. "M group": ticks collected from other municipalities located north of the river Dalälven 3. "S group": ticks collected from the municipalities of Gävle, Sundsvall and Umeå. Since the northernmost area of collection (i.e. "group N") is considered the most interesting for the purpose of assessing changes in tick populations and in tickborne pathogens distribution study, all the ticks collected from this area in 2018 were analysed. The "S group" was created since the amount of ticks originating from these municipalities were far higher than the amounts from all the other areas of collection (i.e. "N group" and "M group") and they would have been overrepresented in the present study if we had been selecting samples based on the sole randomization.

Tick morphological identification

After arrival by mail at SVA, all ticks were transferred individually in 2 ml tube and stored at -80°C until morphological identification. Each specimen was then washed in 1ml of 70 percent concentration of ethanol by gently mixing for 1 minute and then ethanol was poured off; this procedure was carried out to remove potential environmental bacterial contamination. After that, 1 ml of MilliQ water was added and gently mixed with the tick for one minute before it was poured off. After being washed, the ticks were let dry individually on a single clean paper sheet while waiting for species identification. A stereomicroscope (Leica MZ16, Leica Microsystems, Stockholm, Sweden) with up to x200 of magnifications was used for species identification together with morphological taxonomic keys and illustrations (Author: Estrada-Peña et al., 2018; Manilla, 1998; Hillyard, 1996; Filippova, 1977; Arthur, 1963) which provided morphological descriptions and characteristics of all the species potentially occurring in the study area. Tick identification was performed by the student and was confirmed by the supervisor afterwards. In the case of *I. ricinus* and *I. persulcatus*, due to their rather similar appearance, the species identification was confirmed by two species-specific PCR reactions (one for each species) included on the same chip used for tick-borne pathogen detection (see "High-throughput real-time PCR").

Tick homogenization

The homogenization of ticks was performed right after species identification. Each tick was then put individually in a clean 2 ml screw-cap micro tube (Sarstedt AG, Nümbrecht, Germany). Each tube had been prepared in advance by adding four hundred-fifty microliters of mixed lysis buffer solution [made up of 441μl of RNeasy Lysis Buffer (Qiagen, Hilden, Germany) plus 9 μl of 2M Dithiothreitol (DTT)] and a five-millimeter diameter sterile stainless-steel bead (Qiagen, Hilden, Germany). Tubes containing the tick, the buffer and the bead were shaken in batches for two rounds of 1 minute each (frequency: 30 times per second) with a 'TissueLyser' machine (Qiagen). Each batch contained 48 samples including one negative control (buffer and bead only).

Tick Nucleic acid (NA) extraction

The tubes containing homogenized ticks were centrifuged for 3 minutes at 20,000 ×g to be prepared for the extraction. Ninety microliter of tick supernatant were transferred by pipetting manually and individually to the 96 wells extraction plate; the extraction plate had been prepared by adding 10 μl of Proteinase K (from *Tritirachium*: buffer aqueous glycerol solution; Sigma life science, Germany) to each well. Total nucleic acids (NA) extraction was performed by an extraction robot (Magnatrix 8000+) using a commercial extraction kit (Vet Viral NA kit, NorDiag, Sweden). NA were extracted from 94 samples (92 tick samples, 1 negative control, 1 positive control) at the same time. The positive control comprised 5 μl of inactivated TBEV strain K23 (Encepur®, Chiron Vaccines, Marburg, Germany) and 5 μl of *Borrelia burgdorferi* sensu stricto B31 ATCC 35210 (10^8 cells/ml).

cDNA Synthesis

The RNAs extracted from each specimen were used to synthesize their complementary DNA (cDNA) by reverse-transcription, using Illustra Ready-To-Go RT-PCR Beads (GE Healthcare, Amersham Place, UK). Twenty microliters of each extracted sample were manually pipetted to 96 wells PCR plate, 10 μl of pd(N)6: random hexamer primers (0.25 μg/μl) were added to each sample and the plate was incubated for 5 minutes at 97°C using a PTC-100 thermal cycler (MJ Research, MA, USA). After that, 20 μl of the RT-PCR mixture (obtained by dissolving a RT-PCR bead in 20 μl RNAse-free water) were added to each sample and the plate was incubated for 30 minutes at 42°C, followed by 5 minutes at 97°C. The final volume of product was 50 μl of cDNA for each sample.

Primers and probe design for High-throughput real-time PCR

Pathogens, targeted genes and primers/probe sets are listed in *Table1.* and *Table 2*. For each pathogen or tick, primers and probes were designed following Gondard et al. (2018) and Michelet et al. (2014). Each primer or probe set validation had been performed in the abovementioned publications by real-time TaqMan PCR sona Light Cycler® 480(LC480) (Roche Applied Science, Germany) and on dilution range of several positive controls (*Table2.* and *3.*). These four pathogens: *Borrelia valaisiana*, *Francisella tularensis*, *Coxiella burnetii*,

and *Theileria annulata* were targeted by two different real-time PCR reactions (using 2 different target regions) in order to enhance detection.

Table 1. List of pathogens, targets, primers/probe sets, and positive controls (in each name F means forward primer, R means reverse primer and P means Probe)

Species	Target	Name	Sequence	Length (bp)	Positive control
Borrelia burgdorferi sensu stricto	*rpoB*	Bo_bu_rpoB_F	GCTTACTCACAAAAGGCGTCTT	83	Culture of B31 strain
		Bo_bu_rpoB_R	GCACATCTCTTACTTCAAATCCT		
		Bo_bu_rpoB_P	AATGCTCTTGGACCAGGAGGACTTTCA		
Borrelia garinii	*rpoB*	Bo_ga_rpoB_F	TGGCCGAACTTACCCACAAAA	88	Culture of NE11 strain
		Bo_ga_rpoB_R	ACATCTCTTACTTCAAATCCTGC		
		Bo_ga_rpoB_P	TCTATCTCTTGAAAGTCCCCCTGGTCC		
Borrelia afzelii	*Fla*	Bo_af_fla_F	GGAGCAAATCAAGATGAAGCAAT	116	Culture of VS641 strain
		Bo_af_fla_R	TGAGCACCCTCTTGAACAGG		
		Bo_af_fla_P	TGCAGCCTGAGCAGCTTGAGCTCC		
Borrelia valaisiana	*ospE*	Bo_val_ospE_F	GAAACTTAGGGAGTATCTTATGAAT	143	Culture of VS116 strain
		Bo_val_ospE_R	CTTGCCCCCTTAAACTAATATCT		
		Bo_val_ospE_P	TGCTCACTCAACCTGCCTTGCTCGC		
	ospA	Bo_va_ospA_F	ACTCACAAATGACAGATGCTGAA	135	
		Bo_va_ospA_R	GCTTGCTTAAAGTAACAGTACCT		
		Bo_va_ospA_P	TCCGCCTACAAGATTTCCTGGAAGCTT		
Borrelia miyamotoi	*glpQ*	B_miya_glpQ_F	CACGACCCAGAAATTGACACA	94	Plasmid[a]
		B_miya_glpQ_R	GTGTGAAGTCAGTGGCGTAAT		
		B_miya_glpQ_P	TCGTCCGTTTTCTCTAGCTCGATTGGG		
Borrelia spielmanii	*fla*	Bo_spi_fla_F	ATCTATTTTCTGGTGAGGGAGC	71	Plasmid[a]
		Bo_spi_fla_R	TCCTTCTTGTTGAGCACCTTC		
		Bo_spi_fla_P	TTGAACAGGCGCAGTCTGAGCAGCTT		
Borrelia lusitaniae	*rpoB*	Bo_lus_rpoB_F	CGAACTTACTCATAAAAGGCGTC	87	Culture of Poti-B1 strain
		Bo_lus_rpoB_R	TGGACGTCTCTTACTTCAAATCC		
		Bo_lus_rpoB_P	TTAATGCTCTCGGGCCTGGGGGACT		
Borrelia bissettii	*rpoB*	Bo_bi_rpoB_F	GCAACCAGTCAGCTTTCACAG	118	Plasmid[a]
		Bo_bi_rpoB_R	CAAATCCTGCCCTATCCCTTG		
		Bo_bi_rpoB_P	AAAGTCCTCCCGGCCCAAGAGCATTAA		

Borrelia spp.	23S rRNA	Bo_bu_sl_23S_F	GAGTCTTAAAAGGGCGATTTAGT	73	
		Bo_bu_sl_23S_R	CTTCAGCCTGGCCATAAATAG		
		Bo_bu_sl_23S_P	AGATGTGGTAGACCCGAAGCCGAGT		
Anaplasma marginale	*msp1b*	An_ma_msp1_F	CAGGCTTCAAGCGTACAGTG	85	Experimentally infected cow
		An_ma_msp1_R	GATATCTGTGCCTGGCCTTC		
		An_ma_msp1_P	ATGAAAGCCTGGAGATGTTAGACCGAG		
Anaplasma platys	*groEL*	An_pla_groEL_F	TTCTGCCGATCCTTGAAAACG	75	Infected dog blood
		An_pla_groEL_R	CTTCTCCTTCTACATCCTCAG		
		An_pla_groEL_P	TTGCTAGATCCGGCAGGCCTCTGC		
Anaplasma ovis	*msp4*	An_ov_msp4_F	TCATTCGACATGCGTGAGTCA	92	Plasmid[a]
		An_ov_msp4_R	TTTGCTGGCGCACTCACATC		
		An_ov_msp4_P	AGCAGAGAGACCTCGTATGTTAGAGGC		
Anaplasma centrale	*groEL*	An_cen_groEL_F	AGCTGCCCTGCTATACACG	79	Plasmid[a]
		An_cen_groEL_R	GATGTTGATGCCCAATTGCTC		
		An_cen_groEL_P	CTTGCATCTCTAGACGAGGTAAAGGGG		
Anaplasma phagocytophilum	*msp2*	An_ph_msp2_F	GCTATGGAAGGCAGTGTTGG	77	Infected embrionary cells of *Ixodes scapularis*
		An_ph_msp2_R	GTCTTGAAGCGCTCGTAACC		
		An_ph_msp2_P	AATCTCAAGCTCAACCCTGGCACCAC		
Ehrlichia ruminantium	*dsb*	Eh_ru_dsb_F	CTCAGAGGGTAATAGATTTACTC	107	Culture of Gardel strain
		Eh_ru_dsb_R	GTATGCAATATCTTCAAGCTCAG		
		Eh_ru_dsb_P	ACTACAGGCCAAGCACAAGCAGAAAGA		
Ehrlichia canis	*dsb*	Eh_ca_dsb_F	AATACTTGGTGAGTCTTCACTCA	110	Plasmid[a]
		Eh_ca_dsb_R	GTTGCTTGTAATGTAGTGCTGC		
		Eh_ca_dsb_P	AAGTTGCCCAAGCAGCACTAGCTGTAC		
Ehrlichia chaffeensis	*dsb*	Eh_ch_dsb_F	TATTGCTAATTACCCTCAAAAAGTC	117	Infected wild *Amblyomma*
		Eh_ch_dsb_R	GAGCTATCCTCAAGTTCAGATTT		*Americanum*
		Eh_ch_dsb_P	ATTGACCTCCTAACTAGAGGGCAAGCA		
Candidatus Neoehrlichia mikurensis	*groEL*	Neo_mik_groEL_F	AGAGACATCATTCGCATTTTGGA	96	Infected tick
		Neo_mik_groEL_R	TTCCGGTGTACCATAAGGCTT		
		Neo_mik_groEL_P	AGATGCTGTTGGATGTACTGCTGGACC		
Rickettsia conorii	23S-5S ITS	Ri_co_ITS_F	CTCACAAAGTTATCAGGTTAAATAG	118	Culture
		Ri_co_ITS_R	CGATACTCAGCAAAATAATTCTCG		

		Ri_co_ITS_P	CTGGATATCGTGGCAGGGCTACAGTAT		
Rickettsia slovaca	23S-5S ITS	Ri_slo_ITS_F	GTATCTACTCACAAAGTTATCAGG	138	Culture
		Ri_slo_ITS_R	CTTAACTTTTACTACAATACTCAGC		
		Ri_slo_ITS_P	TAATTTTCGCTGGATATCGTGGCAGGG		
Rickettsia massiliae	23S-5S ITS	Ri_ma_ITS_F	GTTATTGCATCACTAATGTTATACTG	128	Culture
		Ri_ma_ITS_R	GTTAATGTTGTTGCACGACTCAA		
		Ri_ma_ITS_P	TAGCCCCGCCACGATATCTAGCAAAAA		
Rickettsia helvetica	23S-5S ITS	Ri_he_ITS_F	AGAACCGTAGCGTACACTTAG	79	Culture
		Ri_he_ITS_R	GAAAACCCTACTTCTAGGGGT		
		Ri_he_ITS_P	TACGTGAGGATTTGAGTACCGGATCGA		
Spotted fever group	*gltA*	SFG_gltA_F	CCTTTTGTAGCTCTTCTCATCC	145	
		SFG_gltA_R	GCGATGGTAGGTATCTTAGCAA		
		SFG_gltA_P	TGGCTATTATGCTTGCGGCTGTCGGT		
Bartonella henselae	*pap31*	Bar_he_pap31_F	CCGCTGATCGCATTATGCCT	107	Culture of Berlin 1 strain
		Bar_he_pap31_R	AGCGATTTCTGCATCATCTGCT		
		Bar_he_pap31_P	ATGTTGCTGGTGGTGTTTCCTATGCAC		
Bartonella quintana	*bqtR*	Bar_qu_bqt_F	TCCATCACAAGATCTCCGCG	80	Culture
		Bar_qu_bqt_R	CGTGCCAATGCTCGTAACCA		
		Bar_qu_bqt_P	TTTAAGAGAGGAGGTAGAAGAGGCTCC		
Francisella tularensis	*tul4*	Fr_tu_tul4_F	ACCCACAAGGAAGTGTAAGATTA	76	Culture of CIP 5612T strain
		Fr_tu_tul4_R	GTAATTGGGAAGCTTGTATCATG		
		Fr_tu_tul4_P	AATGGCAGGCTCCAGAAGGTTCTAAGT		
	fopA	Fr_tu_fopA_F	GGCAAATCTAGCAGGTCAAGC	91	
		Fr_tu_fopA_R	CAACACTTGCTTGAACATTTCTAG		
		Fr_tu_fopA_P	AACAGGTGCTTGGGATGTGGGTGGTG		
Coxiella burnetii	*idc*	Co_bu_icd_F	AGGCCCGTCCGTTATTTTACG	74	Culture
		Co_bu_icd_R	CGGAAAATCACCATATTCACCTT		
		Co_bu_icd_P	TTCAGGCGTTTTGACCGGGCTTGGC		
	IS*1111*	Co_bu_IS111_F	TGGAGGAGCGAACCATTGGT	86	

		Co_bu_IS111_R	CATACGGTTTGACGTGCTGC		
		Co_bu_IS111_P	ATCGGACGTTTATGGGGATGGGTATCC		
Babesia divergens	*hsp70*	Bab_di_hsp70_F	CTCATTGGTGACGCCGCTA	83	Culture of RFS strain
		Bab_di_hsp70_R	CTCCTCCCGATAAGCCTCTT		
		Bab_di_hsp70_P	AGAACCAGGAGGCCCGTAACCCAGA		
Babesia caballi	*Rap1*	Ba_cab_rap1_F	GTTGTTCGGCTGGGGCATC	94	Plasmid[a]
		Ba_cab_rap1_R	CAGGCGACTGACGCTGTGT		
		Ba_cab_rap1_P	TCTGTCCCGATGTCAAGGGGCAGGT		
Babesia canis	18S rRNA	Ba_ca_RNA18S_F	TGGCCGTTCTTAGTTGGTGG	104	Infected dog blood
		Ba_ca_RNA18S_R	AGAAGCAACCGGAAACTCAAATA		
		Ba_ca_RNA18S_P	ACCGGCACTAGTTAGCAGGTTAAGGTC		
Babesia vogeli	*hsp70*	Ba_vo_hsp70_F	TCACTGTGCCTGCGTACTTC	87	Infected dog blood
		Ba_vo_hsp70_R	TGATACGCATGACGTTGAGAC		
		Ba_vo_hsp70_P	AACGACTCCCAGCGCCAGGCCAC		
Babesia microti	*CCTeta*	Bab_mi_CCTeta_F	ACAATGGATTTTCCCCAGCAAAA	145	Culture of R1 strain
		Bab_mi_CCTeta_R	GCGACATTTCGGCAACTTATATA		
		Bab_mi_CCTeta_P	TACTCTGGTGCAATGAGCGTATGGGTA		
Babesia bovis	*CCTeta*	Ba_bo_CCTeta_F	GCCAAGTAGTGGTAGACTGTA	100	Culture of MO7 strain
		Ba_bo_CCTeta_R	GCTCCGTCATTGGTTATGGTA		
		Ba_bo_CCTeta_P	TAAAGACAACACTGGGTCCGCGTGG		
Babesia bigemina	18S rRNA	Ba_big_RNA18S_F	ATTCCGTTAACGAACGAGACC	99	Plasmid[a]
		Ba_big_RNA18S_R	TTCCCCCACGCTTGAAGCA		
		Ba_big_RNA18S_P	CAGGAGTCCCTCTAAGAAGCAAACGAG		
Babesia major	*CCTeta*	Ba_maj_CCTeta_F	CACTGGTGCGCTGATCCAA	75	Plasmid[a]
		Ba_maj_CCTeta_R	TCCTCGAAGCATCCACATGTT		
		Ba_maj_CCTeta_P	AACACTGTCAACGGCATAAGCACCGAT		
Babesia ovis	18S rRNA	Ba_ov_RNA18S_F	TCTGTGATGCCCTTAGATGTC	92	Plasmid[a]
		Ba_ov_RNA18S_R	GCTGGTTACCCGCGCCTT		
		Ba_ov_RNA18S_P	TCGGAGCGGGGTCAACTCGATGCAT		
Theileria equi	*ema1*	Th_eq_ema1_F	GGCTCCGGCAAGAAGCACA	66	Plasmid[a]
		Th_eq_ema1_R	CTTGCCATCGACGACCTTGA		

		Th_eq_ema1_P	CTTCAAGGCTCCAGGCAAGCGCGT		
Theileria annulata	18S rRNA	Th_an_18S_F	GCGGTAATTCCAGCTCCAATA	126	Culture of D7 strain
		Th_an_18S_R	AAACTCCGTCCGAAAAAAGCC		
		Th_an_18S_P	ACATGCACAGACCCCAGAGGGACAC		
	Tams1	Th_an_Tams1_F	CGATTACAAACCAGTTGTCGAC	82	
		Th_an_Tams1_R	GTAAAGGACTGATGAGAAGACG		
		Th_an_Tams1_P	TGAGTACTGAGGCGAAGACTGCAAGG		
Escherichia coli	*eae*	eae-F2	CATTGATCAGGATTTTTCTGGTGATA	102	Culture of EDL933 strain
		eae-R	CTCATGCGGAAATAGCCGTTA		
		eae-P	ATAGTCTCGCCAGTATTCGCCACCAA TACC		

[a]*Plasmids are recombinant pBluescript IISK+ containing the target gene. (Michelet et al., 2014)*

Table 2. List of pathogens, tick species, targets, primers/probe sets, and positive controls

Species	**Target**	**Name**	**Sequence**	**Length (bp)**	**Positive control**
African swine fever virus	vp72	AFSV_vp72_F	CGATGATGATTACCTTTGCTTTG	84	Culture of ASFV
		AFSV_vp72_R	AAAATTCTCTTGCTCTGGATACG		Georgia strain
		AFSV_vp72_P	AAGCCACGGGAGGAATACCAACCCAG		
Thogoto virus	M	Thogoto_seg6_F	GGTCCTCAAGAACGTCAGCA	113	Plasmid[a]
		Thogoto_seg6_R	CATGTAAGTACCAAGACTCATCG		
		Thogoto_seg6_P	AAAGTCGCCCTTCTCCGGGAAAGCAT		
Dhori virus	PB1	Dhori_seg2_F	CAAGCTCTGGTGTGCCTGT	81	Plasmid[a]
		Dhori_seg2_R	CAGTTACTTCTGAGACAGCCT		
		Dhori_seg2_P	AGGAGGGGAAGAGAAGTTGGCCAAG		
Kemerovo virus	Vp3	Kemerovo_seg2_F	GTCAGACGGATTTTCGACCTC	71	Plasmid[a]
		Kemerovo_seg2_ R	GCGAGCCAGATCCCGATGT		
		Kemerovo_seg2_P	ACGGGCCAACACTCGTTCATCACAG		
Colorado tick fever virus	Vp2	Colorado_vp2_F	TTCTTGCTTCTTCCCGGATCA	80	Culture of Florio
		Colorado_vp2_R	CGATTCGGTTTCCGGTAACAT		VR-1233 strain
		Colorado_vp2_P	CATGACCATATCCACGGGAAGCTATCA		
Eyach virus	Vp2	Eyach_vp2_F	TGGCTGACAACATGACGGATA	98	Eyach virus grown in
		Eyach_vp2_R	GGCCTCACGATACTTTCGATT		suckling mice brains
		Eyach_vp2_P	ACGGGCTCGGTACTTCGGTTGAGAT		
Crimean-Congo Hemorrhagic fever virus	N	CCHF_segS_F	CC-For CAAGGGGTACCAAGAAAATGAAGAAGG C	181	Plasmid[a]
		CCHF_segS_R	CC-Rev GCCACAGGGATTGTTCCAAAGCAGAC		
		CCHF_segS_P1	CC-PrSE01 TGTCAACACAGCAGGGTGCATGTAGAT		
		CCHF_segS_P2	CC-PrSE03 TGTAAGCACGGCAGGGTGCATGTAAAT		
		CCHF_segS_P3	CC-PrSE0A ACTCCAATGAAGTGGGGGAAGAAGCT		
Dugbe virus	N	Dugbe_segS_F	GCACAAGGAGCACAAATAGAC	134	Culture of Dugbe
		Dugbe_segS_R	TTTTTGCCTCCTCTAGCACTC		virus

		Dugbe_segS_P	TGGCCCATCTCAAAGAGGAATTGAGAC		
Nairobi sheep disease virus (NSDV)	G1	NSDV_G1_F	TCTAAGTGCTAGCCCTGATGT	112	Culture of NSDV
		NSDV_G1_R	GCCAACTGAGTGTTCTTCTTC		
		NSDV_G1_P	TTCTACAGGCCGTCCGTCAAGGAAGA		
	G1	NSDV_G1Bis_F	ACTAAGTGCAAGCTCAGAAGC	112	Culture of NSDV
		NSDV_G1Bis_R	ACCCACAGAATGTTCATCCTC		
		NSDV_G1Bis_P	TCCTACTGTGTGTCCTTCAGGGGTTG		
Uukuniemi virus	RNA-dependent, RNA polymerase	Uukuniemi_segL_F	GTGGCAGCTTTTCTCTGGTTT	82	Culture of TC259 strain
		Uukuniemi_segL_R	GGGGAAACTGTCATGCCTAAT		
		Uukuniemi_segL_P	CCTTTTGCCAGTTTGGTCAGTTGCTCC		
Schmallenberg virus	N	SBV_segS_F	CGTTGGATTGCTGATACATGC	102	Culture of SBV 1568 V3 strain
		SBV_segS_R	GGCCCAGGTGCATCCCTT		
		SBV_segS_P	AACCTCAGCAAGGGGCATGACAATCTG		
Tick borne encephalitis virus	E	TBEV_euro_geneE_F	TCCTTGAGCTTGACAAGACAG	91	Culture of Absettarov, Hypr, Neudoerfl, Salem strains
		TBEV_euro_geneE_R	TGTTTCCATGGCAGAGCCAG		
		TBEV_euro_geneE_P	TGGAACACCTTCCAACGGCTTGGCA		
	E	TBEV_fareast_geneE_F	TCAGAACACCTACCGACGG	121	Plasmid[a]
		TBEV_fareast_geneE_R	CTCCAAACTCAACCAGCCGT		
		TBEV_fareast_geneE_P	CTGGCAGGTCCACCGGGACTGGT		
	E	TBEV_sibe_geneE_F	TTGTTGTGCAGAGTCGCCAG	82	Plasmid[a]
		TBEV_sibe_geneE_R	TCGGAAGGTGTTCCAGAGTC		
		TBEV_sibe_geneE_P	TGGCGTTGACTTGGCTCAGACTGTCA		
Louping ill virus	E	Louping_geneE_F	GCTGTCAAGATGGATGTGTACAA	113	Culture of 369T2 strain
		Louping_geneE_R	CCACTCTTCAGGTGATACTTGT		
		Louping_geneE_P	CTTGGAGATCAGACTGGAGTGCTGCT		
Powassan virus	C	Powassan_poly_F	TGGGGATTCTTTGGCACGC	75	Culture of LB, 64-7062 strains
		Powassan_poly_R	GTGGTACCGTTTTCCAGAACA		
		Powassan_poly_P	TTTTCAGCACTGGGGGTCTGGCCGT		
Deer tick virus	5'NCR/C	Deertick_poly_F	GACAGCTTAGGAGAACAAGAG	94	Culture of CT390, FDRSP-08, JHSP-08 strains
		Deertick_poly_R	CGGTCACTTTCAGCTTTCGC		
		Deertick_poly_P	CTGGGAGTGGTCATGGTGACTACTTC		
Omsk hemorrhagic fever virus	NS5	Omsk_NS_F	AATGGGAGCATTCAGCTGGC	87	Plasmid[a]
		Omsk_NS_R	GTCCGTCCTTCATCACCAAC		
		Omsk_NS_P	TCATGGAAATGGTGCGAGCAGAAGGG		
Kyasanur forest disease virus	M	Kyasanur_poly_F	ACACGATGCACACACCTGC	72	Plasmid[a]
		Kyasanur_poly_R	CACCAATGAAACTCTAGTCGTC		
		Kyasanur_poly_P	AGAACCGGGACTTTGTCTCAGGGAC		
Langat virus	E	Langat_geneE_F	ATACCATAAAGGTGGAGCCAC	84	Culture of TP21 strain
		Langat_geneE_R	CTGTGAACGAGGCTGACTTC		
		Langat_geneE_P	ACACTGGAGAGTTTGTGGCAGCCAATG		
West Nile virus	E	WNV_poly_F	CAGCGATCTCTCCACCAAAG	69	Culture of IS98, Kunjin, MP22 strains
		WNV_poly_R	GGGTCAGCACGTTTGTCATTG		
		WNV_poly_P	TGGCTTCTCCCATGGTCGGGCAC		

Meaban Virus	NS5	Meaban_NS5_F	TGAGAAGAGCGGTGGAGGA	87	Culture of Meaban virus
		Meaban_NS5_R	TTTCCTCCCTCAAGCTCGG		
		Meaban_NS5_P	CCAAGTCTTTCACGAGCCATCCGAG		
Ixodes ricinus	CO1	Ix_ri_CO1_F	TGGGGCAGGAACTGGATGAA	180	Tick
		Ix_ri_CO1_R	CGTTCTAAAGATAGTCCTGGTG		
		Ix_ri_CO1_P	CAGTATACCCCCCACTTTCAGCAAATATTTCT		
Ixodes persulcatus	CO1	Ix_per_CO1_F	CAGGGACAGGATGAACTGTTTA	166	Tick
		Ix_per_CO1_R	GATATTCCAGGGGAACGTATG		
		Ix_per_CO1_P	TCCTCCTCTATCATCTAACATCTCCCATTCA		

[a]*Plasmids are recombinant pBluescript IISK+ containing the target gene. (Gondard et al., 2018)*

DNA pre-amplification for High-throughput real-time PCR

The Perfecta PreAmp SuperMix (Quanta Biosciences, Beverly, USA) was used for DNA pre-amplification according to the manufacturer's instructions. All primers, except target tick DNA primers, were pooled to combining equal final volume 200 nM each. The final volume to perform the reaction was 5 µl which containing 1 µl of Perfecta PreAmp SuperMix, 1.25 µl pooled primers, 1.5 µl H_2O and 1.25 µl cDNA. The 400 reactions were performed with 1 cycle at 95◦C for 2 minutes, 14 cycles at 95◦C for 10 second and 3 minutes at 60◦C. At the end of the cycling program, the samples were prepared for the FLUIDIGM Biomark dynamic array with a dilution 1:10 by adding 45µl of H_2O per well or with a dilution 1:5 for virus by adding 20 µl of H_2O per well. The final volume was 50 µl then the pre-amplified cDNAs were conserved at −20◦C until needed.

High-throughput real-time PCR

For high-throughput microfluidic real-time PCR the BioMark^{TM} real-time PCR system (FLUIDIGM, USA) with 48.48 dynamic arrays (FLUIDIGM, USA) was used. Forty-eight samples and 48 PCR mixes were dispensed into individual wells by these chips**,** after which on-chip microfluidics assemble PCR reactions in individual chambers prior to thermal cycling resulting in 2304 individual reactions per chip.

6-carboxy-fluorescein (FAM)- and black hole quencher (BHQ1)-labeled TaqMan probes (Applied Biosystems, France) combined with TaqMan Gene Expression Master Mix were used to perform Real-time PCRs. The real-time PCR reactions were performed under the following thermal cycling conditions: 2 minutes at 50◦C after that 10 minutes at 95◦C, followed by 40 cycles of 2-step amplification of 15 seconds at 95◦C, and 1 minute at 60◦C. Result data were achieved on the BioMarkTM real-time PCR system then the FLUIDIGM real-time PCR Analysis software was used to analyse the data to reach crossing point (CP) values. For more detail see Michelet et al. (2014). A sample was considered positive if CP>30.

To evaluate microfluidic tool, specificity against RNA reference materials of primers and probes were evaluated in duplicate screen field samples. On each chip one negative water controls were added. *Ixodes ricinus* RNA was used as an RNA extraction control and also to confirm the tick species reaction. The DNA of *Escherichia coli* strain was added to each chip to ascertain if any factor present in the batch/chip could inhibit the PCR. Specific primers and probe for the *Escherichia coli* eae gene (Nielsen and Andersen, 2003) were used for this internal inhibition detection.

Tick-borne Pathogens detection in *Hyalomma marginatum*

Homogenization, nucleic acid extraction as well as cDNA synthesis of the single specimen of *Hyalomma marginatum* collected were performed as described in previous sections, with the exception that exception that the tick was incubated in ethanol 70% for 24 hours to inactivate potentially present tick-borne pathogens that could represent an hazard for laboratory staff during tick processing. *H. marginatum* was tested with individual PCR reactions for Crimean-Congo Haemorrhagic fever virus (CCHFV) using a real-time RT-PCR. *Rickettsia* spp. DNA (amplification of the gltA gene part) was amplified using a pan-*Rickettsia* real-time PCR, then the *Rickettsia* species were identified by analysing sequences of a 23S-5S intergenic spacer region. *Babesia* spp. and *Theileria* spp. (amplification of the 18S rRNA gene part) detection was done by using a conventional PCR. All the PCR methods used are reported in Chitimia-Dobler et al. (2019).

Results

Tick collection

Of the selected ticks (n=1,421), 640 ticks were collected from dogs, 626 ticks were collected from cats, 42 ticks were collected from other animal species (cattle, horses, rodents and rabbits), 100 ticks were collected from humans, 9 ticks were collected from the environment and in 4 cases the collector did not specify the host species from which they were collected (*Figure 1*.).

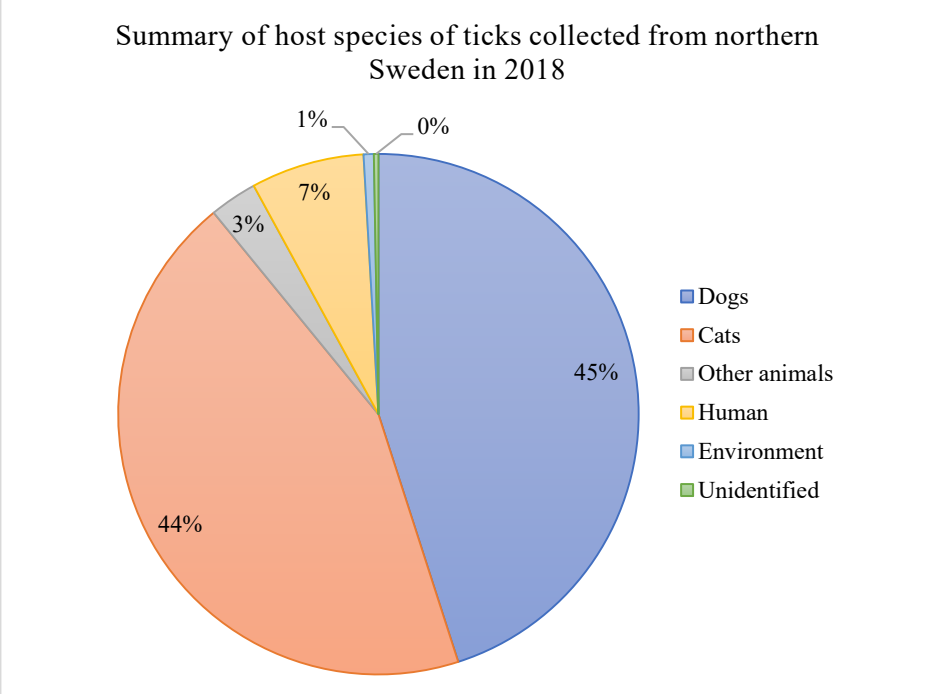

Figure 1. Host species of ticks collected from Northern Sweden in 2018

The geographical representation of the three areas of collection (i.e. “groups”) is described in *Figure 2* and the summary of life stage of ticks collected from northern Sweden in each collection area (i.e. “group”) in 2018 is described in *Table 3*.

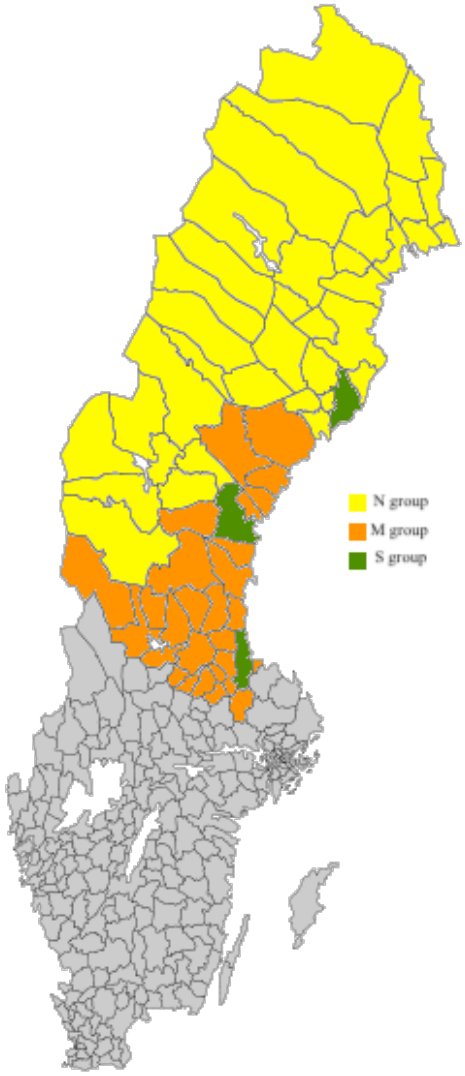

Figure 2. Map of the areas used to build up the 3 groups of collection

Table 3. Summary of life stage of ticks collected from northern Sweden based on the collection area group in 2018

Collecting area group	Females (n=1,199)	Males (n=183)	Nymphs (n=39)	Total
N	233	12	3	248
M	682	110	24	816
S	284	61	12	357

Tick species identification

One thousand-three-hundred-eighty-one ticks found on different host species (dogs, cats, cattle, horses, mouse, rabbits and humans) as well as collected from the environment were identified as *Ixodes ricinus*: 1167 females, 176 males and 38 nymphs; 14 females were primarily morphologically identified as *Ixodes* spp. due to the tick damaged appearance, then the species identification was confirmed as *I. ricinus* by FLUIDIGM. Twenty-seven ticks were identified as *Ixodes persulcatus* by FLUIDIGM: 21 females, 5 males and 1 nymph (while 32 ticks - 25 females and 7 males – had been primarily morphologically identified as *Ixodes persulcatus*). Seven adult female ticks from 3 domestic cats (*Felis catus*) and 4 dogs (*Canis lupus*) resulted to be positive both for *Ixodes ricinus* and *Ixodes persulcatus* species-specific PCR reactions, which could be either the result of cross contamination or due to their potential nature of hybrid species. Twelve ticks were identified as *Ixodes trianguliceps*: 11 females and 1 nymph. Lastly, one adult female tick from a horse was identified belonging to the species *Hyalomma marginatum* (*Table 4*.).

Table 4. Summary of species identification of ticks collected from Northern Sweden in 2018

Species	*Ixodes ricinus**			*Ixodes persulcatus**			*Ixodes trianguliceps*			*Hyalomma marginatum*
Sex and life stage	N	M	F	N	M	F	N	M	F	FF
	38	176	1167	1	5	21	1	0	11	1
Total	1381			27			12			1

**species identification confirmed by FLUIDIGM*
***N = nymph, M = adult male and F = adult female*

Tick-borne pathogens detection

Tick-borne pathogens were detected by FLUIDIGM-PCR as follows: 25.26% (359 ticks) were positive for *Borrelia* spp., 9.15% (130 ticks) were positive for *Anaplasma* spp., 6.19% (88 ticks) were positive for *Neoehrlichia mikurensis*, 25.62% (364 ticks) were positive for *Rickettsia helvetica*, 0.07% (1 tick, *Hyalomma marginatum*) of the ticks detected positive PCR reactions to *Rickettsia aeschlimannii*, 0.49% (7 ticks) were positive for TBEV, 2.25% (32 ticks) were positive for UUKV, 4.08% (58 ticks) were positive for *Babesia venatorum*, 0.28% (4 ticks) for *Babesia microti* and 0.49% (7 ticks) for *Babesia divergens/B. capreoli*.

The positive PCR reactions of tick-borne pathogens detection can be categorized in 3 area groups as follow:

1. M group (n=816 ticks), 19.36% (158 ticks) were positive for *Borrelia* spp., 6.74% (55 ticks) were positive for *Anaplasma* spp., 3.68% (30 ticks) were positive for *Anaplasma phagocytophilum*, 2.21% (18 ticks) were positive for *Neoehrlichia mikurensis*, 17.65% (144 ticks) were positive for *Rickettsia helvetica*, 0.12% (1 tick) were positive for *Rickettsia aeschlimannii*, 0.86% (7 ticks) were positive for UUKV, 1.84% (15 ticks) were positive for *Babesia venatorum*, 0.49% (4 ticks) were positive for *Babesia microti*, and 0.25% (2 ticks) were positive for *Babesia divergens/B. capreoli*.

2. N group (n=248 ticks), 25.00% (62 ticks) were positive for *Borrelia* spp., 17.34% (43 ticks) were positive for *Anaplasma* spp., 15.73% (39 ticks) were positive for *Anaplasma phagocytophilum*, 16.13% (40 ticks) were positive for *Neoehrlichia mikurensis*, 29.44% (73

ticks) were positive for *Rickettsia helvetica*, 0.40% (1 tick) were positive for TBEV, 2.02% (5 ticks) were positive for UUKV, 9.27% (23 ticks) were positive for *Babesia venatorum* and 0.40% (1 tick) were positive for *Babesia divergens/B. capreoli*.

3. S group (n=357 ticks), 38.94% (139 ticks) were positive for *Borrelia* spp., 8.96% (32 ticks) were positive for *Anaplasma* spp., 17.37% (62 ticks) were positive for *Anaplasma phagocytophilum*, 8.40% (88 ticks) were positive for *Neoehrlichia mikurensis*, 41.18% (147 ticks) were positive for *Rickettsia helvetica*, 1.68% (6 ticks) were positive for TBEV, 5.60% (20 ticks) were positive for UUKV, 5.60% (20 ticks) were positive for *Babesia venatorum*, and 1.12% (4 ticks) were positive for *Babesia divergens/B. capreoli* as described in *Figure 3*.

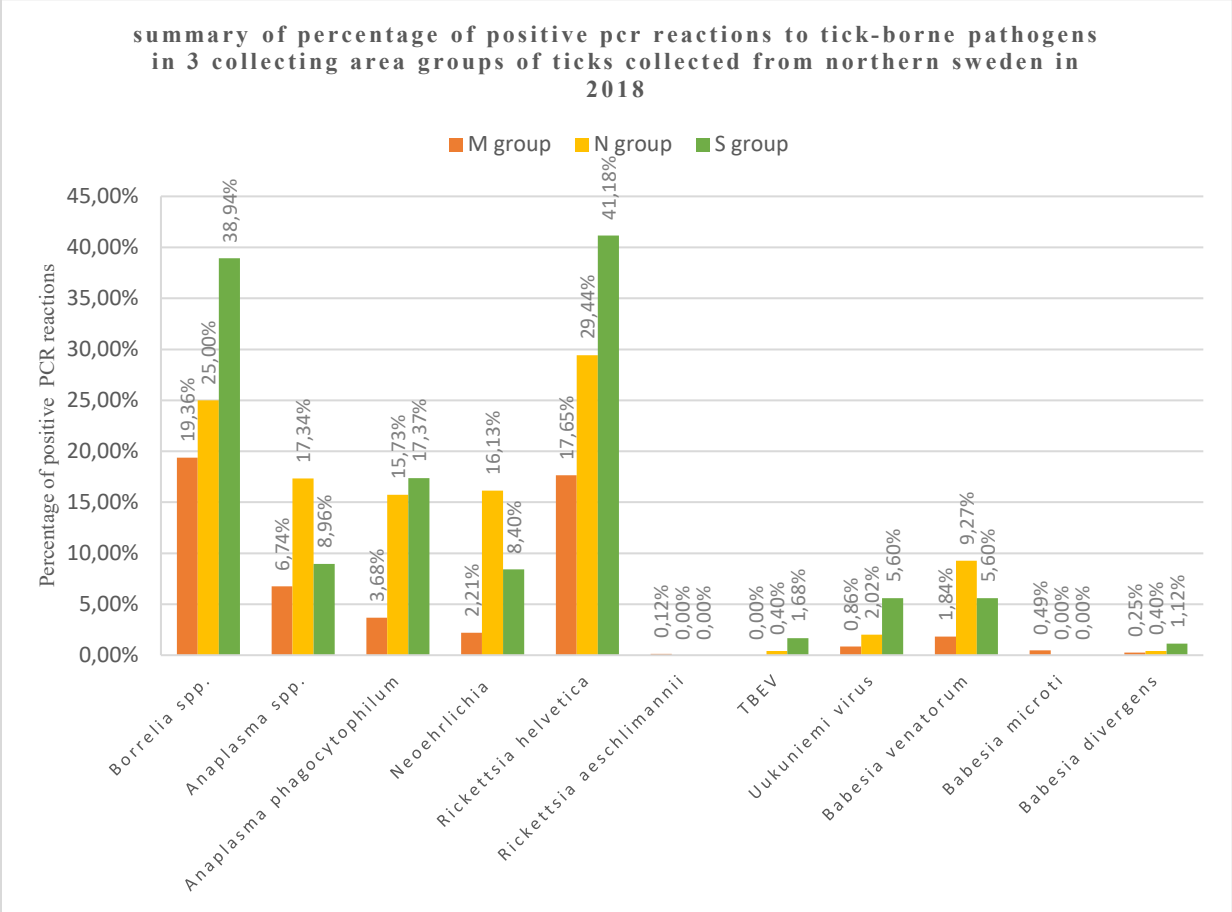

Figure 3. Positive PCR reactions to tick-borne pathogens divided by collecting area group

From 1,421 ticks, there were positive PCR reactions to tick-borne pathogens detected categorized in 4 host specie groups as follow:

1. Dogs (n=640 ticks), 23.44% (150 ticks) were positive for *Borrelia* spp., 9.06% (58 ticks) were positive for *Anaplasma* spp., 8,91% (57 ticks) were positive for *Anaplasma phagocytophilum*, 6.09% (39 ticks) were positive for *Neoehrlichia mikurensis*, 24.53% (157 ticks) were positive for *Rickettsia helvetica*, 0.31% (2 ticks were positive for TBEV, 2.03% (13 ticks) were positive for UUKV, 3.44% (22 ticks) were positive for *Babesia venatorum*, 0.16% (1 tick) were positive for *Babesia microti*, and 0.16% (1 tick were positive for *Babesia divergens/B. capreoli*.

2. Cats (n=626 ticks), 26.36% (165 ticks) o were positive for *Borrelia* spp., 9.27% (58 ticks) were positive for *Anaplasma* spp., 9.27% (58 ticks) were positive for *Anaplasma*

phagocytophilum, 5.91% (39 ticks) were positive for *Neoehrlichia mikurensis*, 28.27% (177 ticks) were positive for *Rickettsia helvetica*, 0.48% (3 ticks) were positive for TBEV, 2.40% (15 ticks) were positive for UUKV, 4.31% (27 ticks) were positive for *Babesia venatorum*, 0.16% (1 tick) were positive for *Babesia microti* and 0.96% (6 ticks) were positive for *Babesia divergens/B. capreoli*.

3. Human (n=100 ticks), 34.00% (34 ticks) were positive for *Borrelia* spp., 4.00% (4 ticks) were positive for *Anaplasma* spp., 5.00% (5 ticks) were positive for *Anaplasma phagocytophilum*, 6.00% (6 ticks) were positive for *Neoehrlichia mikurensis*, 21.00% (21 ticks) were positive for to *Rickettsia helvetica*, 1.00% (1 tick) were positive for TBEV, 3.00% (3 ticks) were positive for UUKV and 9.00% (9 ticks) were positive for *Babesia venatorum*.

4. Others (n=55 ticks), 18.18% (10 ticks) were positive for *Borrelia* spp., 18.18% (10 ticks) were positive for *Anaplasma* spp., 20.00% (11 ticks) were positive for *Anaplasma phagocytophilum*, 10.91% (6 ticks were positive for *Neoehrlichia mikurensis*, 16.36% (21 ticks) were positive for *Rickettsia helvetica*, 1.82% (1 tick) were positive for *Rickettsia aeschlimannii*, 1.82% (1 tick) were positive for TBEV, 1.82% (1 tick) were positive for UUKV, 3.64% (2 ticks) were positive for *Babesia microti*., as described in *Figure 4*.

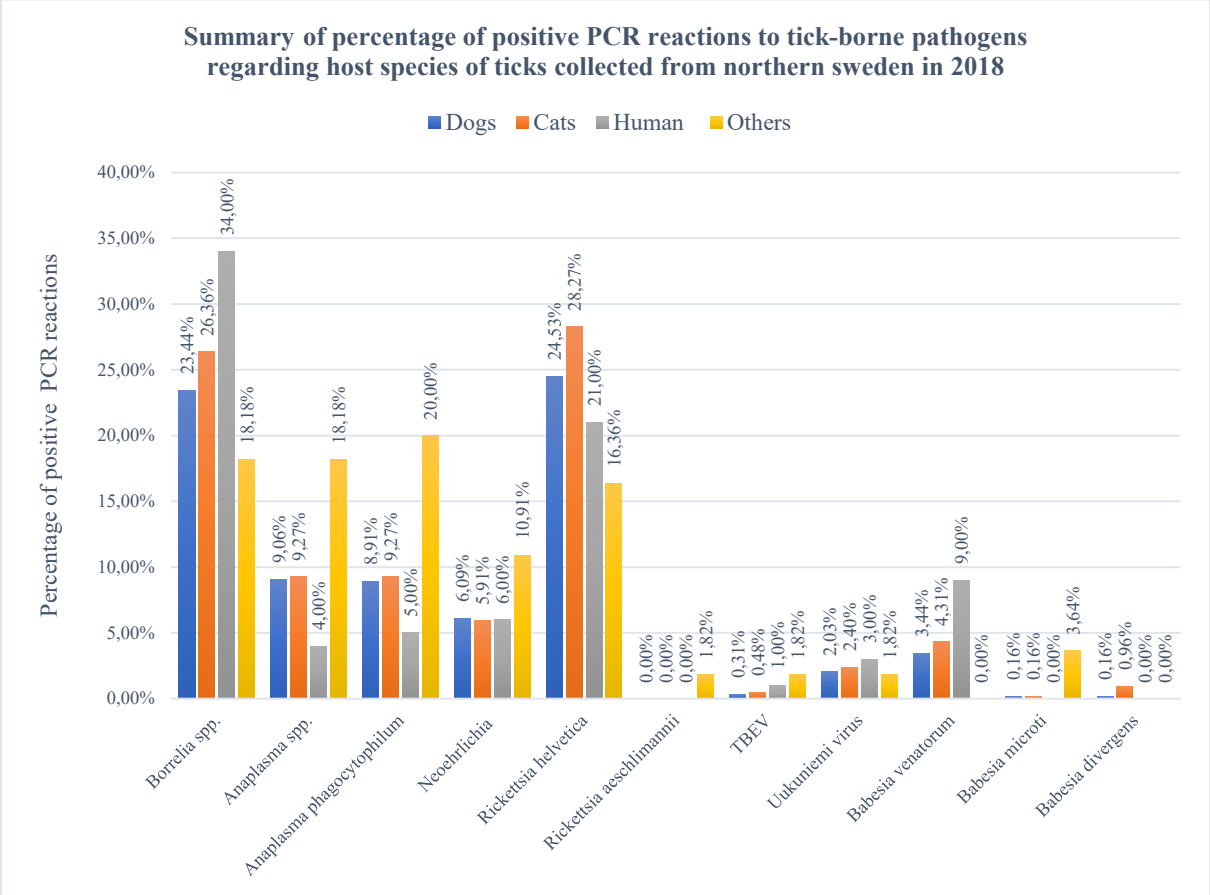

Figure 4. Positive PCR reactions to tick-borne pathogens divided by host species

The detection of positive PCR reactions to tick-borne pathogens were summarized regarding to pathogen group and tick species as follow:

Bacteria

There were 25.26% (359 ticks) of ticks collected from Northern Sweden (n=1,421) detected positive PCR reactions to *Borrelia* spp. which could divide in tick species groups as follow: 355 ticks belonged to *Ixodes ricinus*, 3 ticks belonging to *Ixodes persulcatus* and 1 tick belonged to *Ixodes trianguliceps*. There were 9.15% (130 ticks) of the ticks detected positive PCR reactions to *Anaplasma* spp. which could divide in tick species groups as follow: 119 ticks belonged to *Ixodes ricinus*, 6 ticks belonging to *Ixodes persulcatus* and 5 ticks belonged to *Ixodes trianguliceps*. There were 9.22% (131 ticks) of the ticks detected positive PCR reactions to for *Anaplasma phagocytophilum* which could divide in tick species groups as follow: 123 ticks belonged to *Ixodes ricinus*, 4 ticks belonging to *Ixodes persulcatus* and 4 ticks belonged to *Ixodes trianguliceps*. While 5.21% (88 ticks) of the ticks were detected positive PCR reactions to *Neoehrlichia mikurensis* which could divide in tick species groups as follow: 82 ticks belonged to *Ixodes ricinus*, 5 ticks belonging to *Ixodes persulcatus* and 1 tick belonged to *Ixodes trianguliceps*. Moreover, there were 25.62% (364 ticks) of the ticks were detected positive PCR reactions to *Rickettsia helvetica* which could divide in tick species groups as follow: 357 ticks belonged to *Ixodes ricinus*, 6 ticks belonging to *Ixodes persulcatus* and 1 tick belonged to *Ixodes trianguliceps*. Lastly, 0.07% (1 tick) turned to be positive at PCR reaction to *Rickettsia aeschlimannii* belonged to *Hyalomma marginatum* as shown in *Table 5*.

Table 5. Summary of positive PCR reactions to bacteria pathogens divided by tick species of ticks collected from northern Sweden in 2018

Species	*Ixodes ricinus*	*Ixodes persulcatus*	*Ixodes trianguliceps*	*Hyalomma marginatum*	Total
Borrelia spp.	355	3	1	0	359
Anaplasma spp.	119	6	5	0	130
Anaplasma phagocytophilum	123	4	4	0	131
Neoehrlichia mikurensis	82	5	1	0	88
Rickettsia helvetica	357	6	1	0	364
Rickettsia aeschlimannii	0	0	0	1	1

Virus

There were 0.49 % (7 ticks) of ticks collected from northern Sweden (n=1,421) found to be positive at PCR reactions for TBEV; all 7 ticks were *Ixodes ricinus*. While 2.25% (32 ticks) of the ticks detected positive PCR reactions to UUKV which all 32 ticks belonged to *Ixodes ricinus* as shown in *Table 6*..

Table 6. Summary of positive PCR reactions to virus pathogens divided by tick species of ticks collected from northern Sweden in 2018

Species	*Ixodes ricinus*	*Ixodes persulcatus*	*Ixodes trianguliceps*	*Hyalomma marginatum*	Total
TBEV	7	0	0	0	7
UUKV	32	0	0	0	32

Protozoa

There were 4.08 % (58 ticks) of ticks collected from northern Sweden (n=1,421) that resulted to be positive at PCR reactions to *Babesia venatorum* which could divide in tick species groups as follow: 54 ticks were identified as *Ixodes ricinus* and 4 ticks as *Ixodes persulcatus*. There were 0.28 % (4 ticks) of the ticks showing positive PCR reactions to *Babesia microti*, all 4 ticks were *Ixodes ricinus*. While 0.49 % (7 ticks) of the ticks detected positive PCR reactions to *Babesia divergens/B. capreoli* which all 7 ticks belonged to *Ixodes ricinus* as shown in *Table 7*.

Table 7. Summary of positive PCR reactions to protozoa pathogens divided by tick species of ticks collected from Northern Sweden in 2018

Species	*Ixodes ricinus*	*Ixodes persulcatus*	*Ixodes trianguliceps*	*Hyalomma marginatum*	Total
Babesia venatorum	54	4	0	0	58
Babesia microti	4	0	0	0	4
Babesia divergens/B. capreoli	7	0	0	0	7

Schmallemberg virus

There were 0.21% (3 ticks) of ticks collected from northern Sweden (n=1,421) that resulted to be positive at PCR reactions to Schmallemberg virus .

Discussion

There are few data regarding the presence of ticks and tickborne pathogens in northern Sweden. To our knowledge, this is the largest collection of ticks from animals and humans organized in this geographical area. Regarding tick species composition, it is confirmed the dominant role of *I. ricinus*, the sheep tick. It is difficult to compare the distribution and abundance recorded in this study since no previous studies were performed in a similar way before. It can be stated that in the present study *I. ricinus* has been collected not only close to the coastline but also in the mainland of northern Sweden. This could be a consequence of climate change as well as could reflect the collection method, i.e. these specimens are mainly not collected from the environment and therefore their exact site of questing is difficult to record.

Even if the Taiga tick *I. persulcatus* had already been recorded in Sweden (Jaenson et al., 1994, 2016), in the present study this tick species has been found in a broader geographical area; traditionally the distributional area of *I. ricinus* has been seen as a barrier to the expansion of *I. persulcatus* to the western areas of Europe since hybrids of these two species are infertile (Kovalev et al., 2016; Bugmyrin et al., 2016, 2015). More studies are needed to observe what will happen if in the future the trend observed in the present study compared to previous study will be confirmed, i.e if the Taiga tick will become more abundant in the western limit of its distributional area.

In the present study it is also reported the most northernmost collection of *Hyalomma marginatum* ever recorded; this, together with the finding of around 30 *Hyalomma* adult ticks in central and southern Sweden during the same time of our collection, is most likely another sign of the effects of climate change (Grandi et al., 2020).

Regarding TBP, the average presence of *Borrelia* in collected ticks is similar to that observed in other studies in Sweden (Henningsson et al., 2019; Jaenson et al., 2018). It is interesting that samples collected from people had a higher prevalence of these bacteria compared to those collected in dogs and cats. Again, to our knowledge this is the first big collection of ticks from dogs and cats in Sweden in general and in northern Sweden in particular, so it is difficult to draw any conclusion about trends in infection spreading or prevalence.

Interestingly, ticks collected from dogs and cats were more frequently carrying bacteria like those of the genus *Anaplasma* and *Rickettsia*; the first one (*A. phagocytophilum*) is a known pathogen of veterinary relevance and therefore its presence in these specimens could signal the presence of risks not only to human but also to veterinary health in this region.

Protozoan of the genus *Babesia* were more frequently found in ticks removed from people, and this deserves particular attention since human babesiosis is sometimes observed as a potential emerging human pathogen (Bläckberg et al., 2018; Svensson et al., 2019).

The presence of TBEV outside the areas of major incidence for this disease in the country (Stockholm archipelago, as well as some districts in central and southern Sweden) opens new scenarios and requires both a follow up in the upcoming years as well as an update of the risk maps. Unfortunately, the PCR reaction included in FLUIDIGM was only capable to detect the western European strains, therefore we do not know if other ticks (Taiga tick in particular) were carrying other variants of this virus. This will be assessed in further studies.

Regarding other viral pathogens since they were found in very few samples is difficult to draw any conclusion if they are emerging pathogens and how much do they represent a risk for human health. In fact, Uukuniemi virus had already been found in ticks in Sweden (Gondard et al., 2018), while Schmallemberg virus has been found in dipteran vectors in Sweden before (but not in ticks); if confirmed, the presence of Schmallemberg virus is also deserving more and deeper studies in the future.

All these results – despite they still need a final confirmation for at least some of the samples – represent a fundamental baseline for future studies in this geographical area, that probably will experience some of the most dramatic effects related to climate change. Based on these results it is possible to say that the risk for the community to become exposed and infected to the most important TBP (*Borrelia*, TBEV, *Rickettsia* and *Anaplasma*) is present even in northern Sweden, to a greater extent than it was believed.

FLUIDIGM has been again a powerful tool to screen many samples for many TBP at the same time, but in the future it might be possible to concentrate the investigations on the pathogens found in the present study in order to gather a deeper knowledge on those TBPs actually present in the country.

Acknowledgements

Karin Ullman (National Veterinary Institute, SVA) is acknowledged for laboratory assistance regarding processing of ticks and PCR assays; the staff at the Section for Parasitological Diagnostics and at the Section of Molecular Diagnostics at the Department of Microbiology SVA is acknowledged respectively for laboratory assistance in tick identification and for laboratory assistance regarding extraction of nucleic acids from the specimens. Prof. Ann Albihn and Anna Omazic from the Department of Chemistry, Environment and Food Hygiene, SVA, are acknowledged for project coordination; Galina Ganchenko and Ann Högberg from the Department of Chemistry, Environment and Food Hygiene, SVA are acknowledged for excellent assistance in registration and processing of the samples. Giulio Grandi, my supervisor, is acknowledged for his advice and patient guidance throughout this study.

References

Arthur, DON.R., 1963. British ticks. London: Butterworths.

Arthur, D.R., 1952. Ticks collected from birds in Sweden. Ann. Mag. Nat. Hist. Ser. 12, 305–308.

Barker, S.C., Murrell, A., 2004. Systematics and evolution of ticks with a list of valid genus and species names. Parasitology 129 Suppl, S15-36.

Belova, O.A., Burenkova, L.A., Karganova, G.G., 2012. Different tick-borne encephalitis virus (TBEV) prevalences in unfed versus partially engorged ixodid ticks – Evidence of virus replication and changes in tick behavior. Ticks Tick-Borne Dis. 3, 240–246. https://doi.org/10.1016/j.ttbdis.2012.05.005

Bishop, R., Musoke, A., Morzaria, S., Gardner, M., Nene, V., 2004. Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology 129 Suppl, S271-283. https://doi.org/10.1017/s0031182003004748

Bläckberg, J., Lazarevic, V.L., Hunfeld, K.-P., Persson, K.E.M., 2018. Low-virulent Babesia venatorum infection masquerading as hemophagocytic syndrome. Ann. Hematol. 97, 731–733. https://doi.org/10.1007/s00277-017-3220-6

Bonnet, S., Jouglin, M., L'Hostis, M., Chauvin, A., 2007. Babesia sp. EU1 from Roe Deer and Transmission within Ixodes ricinus. Emerg. Infect. Dis. 13, 1208–1210. https://doi.org/10.3201/eid1308.061560

Brinck, P., Johnels, A., Lundholm, B., Svedmyr, A., von Zeipel, G., Zetterberg, B., 1967. Small Mammals in Sweden as Hosts of Tick-Borne Encephalitis Virus and Vagrant Ectoparasites. Oikos 18, 124–134. https://doi.org/10.2307/3564640

Bugmyrin, S.V., Belova, O.A., Bespyatova, L.A., Ieshko, E.P., Karganova, G.G., 2016. Morphological features of Ixodes persulcatus and I. ricinus hybrids: nymphs and adults. Exp. Appl. Acarol. 69, 359–369. https://doi.org/10.1007/s10493-016-0036-3

Bugmyrin, S.V., Belova, O.A., Ieshko, E.P., Bespyatova, L.A., Karganova, G.G., 2015. Morphological differentiation of Ixodes persulcatus and I. ricinus hybrid larvae in experiment and under natural conditions. Ticks Tick-Borne Dis. 6, 129–133. https://doi.org/10.1016/j.ttbdis.2014.11.001

Chitimia-Dobler, L., Schaper, S., Rieß, R., Bitterwolf, K., Frangoulidis, D., Bestehorn, M., Springer, A., Oehme, R., Drehmann, M., Lindau, A., Mackenstedt, U., Strube, C.,

Dobler, G., 2019. Imported Hyalomma ticks in Germany in 2018. Parasit. Vectors 12, 134. https://doi.org/10.1186/s13071-019-3380-4
Colwell, D.D., Dantas-Torres, F., Otranto, D., 2011. Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. Vet. Parasitol. 182, 14–21. https://doi.org/10.1016/j.vetpar.2011.07.012
Cotté, V., Bonnet, S., Le Rhun, D., Le Naour, E., Chauvin, A., Boulouis, H.-J., Lecuelle, B., Lilin, T., Vayssier-Taussat, M., 2008. Transmission of Bartonella henselae by Ixodes ricinus. Emerg. Infect. Dis. 14, 1074–1080. https://doi.org/10.3201/eid1407.071110
Dantas-Torres, F., 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. Int. J. Parasitol. Parasites Wildl., Including articles from the inaugural conference on "Impact of Environmental changes on Infectious Diseases (IECID)" 4, 452–461. https://doi.org/10.1016/j.ijppaw.2015.07.001
De, J. la F., Estrada-Pena, A., Venzal, J.M., Kocan, K.M., Sonenshine, D.E., 2008. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Front. Biosci. J. Virtual Libr. 13, 6938–6946. https://doi.org/10.2741/3200
de la Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A.G., Estrada-Peña, A., Johnson, N., Kocan, K.M., Mansfield, K.L., Nijhof, A.M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllón, N., Vancova, M., Golovchenko, M., Grubhoffer, L., Caracappa, S., Fooks, A.R., Gortazar, C., Rego, R.O.M., 2017. Tick-Pathogen Interactions and Vector Competence: Identification of Molecular Drivers for Tick-Borne Diseases. Front. Cell. Infect. Microbiol. 7, 114. https://doi.org/10.3389/fcimb.2017.00114
Dobler, G., Erber, W., Schmitt, H.-J., 2018. Tick-borne encephalitis (TBE). Global Health Press Pte Limited.
Estrada-Peña, A., Mihalca, A.D., Petney, T.N., 2018. Ticks of Europe and North Africa: A Guide to Species Identification. Springer.
Filippova, N.A., 1977. Ixodid ticks (Ixodinae). Fauna USSR New Ser 4 316.
Golovljova, I., Vene, S., Sjölander, K.B., Vasilenko, V., Plyusnin, A., Lundkvist, Å., 2004. Characterization of tick-borne encephalitis virus from Estonia. J. Med. Virol. 74, 580–588. https://doi.org/10.1002/jmv.20224
Gondard, M., Michelet, L., Nisavanh, A., Devillers, E., Delannoy, S., Fach, P., Aspan, A., Ullman, K., Chirico, J., Hoffmann, B., van der Wal, F.J., de Koeijer, A., van Solt-Smits, C., Jahfari, S., Sprong, H., Mansfield, K.L., Fooks, A.R., Klitgaard, K., Bødker, R., Moutailler, S., 2018. Prevalence of tick-borne viruses in Ixodes ricinus assessed by high-throughput real-time PCR. Pathog. Dis. 76. https://doi.org/10.1093/femspd/fty083
Grandi, G., Chitimia-Dobler, L., Choklikitumnuey, P., Strube, C., Springer, A., Albihn, A., Jaenson, T.G.T., Omazic, A., 2020. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick-Borne Dis. 101403. https://doi.org/10.1016/j.ttbdis.2020.101403
Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., Sattelle, D.B., de la Fuente, J., Ribeiro, J.M., Megy, K., Thimmapuram, J., Miller, J.R., Walenz, B.P., Koren, S., Hostetler, J.B., Thiagarajan, M., Joardar, V.S., Hannick, L.I., Bidwell, S., Hammond, M.P., Young, S., Zeng, Q., Abrudan, J.L., Almeida, F.C., Ayllón, N., Bhide, K., Bissinger, B.W., Bonzon-Kulichenko, E., Buckingham, S.D., Caffrey, D.R., Caimano, M.J., Croset, V., Driscoll, T., Gilbert, D., Gillespie, J.J., Giraldo-Calderón, G.I., Grabowski, J.M., Jiang, D., Khalil, S.M.S., Kim, D., Kocan, K.M., Koči, J., Kuhn, R.J., Kurtti, T.J., Lees, K., Lang, E.G., Kennedy, R.C., Kwon, H., Perera, R., Qi, Y., Radolf, J.D., Sakamoto, J.M., Sánchez-Gracia, A., Severo, M.S., Silverman, N., Šimo, L., Tojo, M., Tornador, C., Van Zee, J.P., Vázquez, J.,

Vieira, F.G., Villar, M., Wespiser, A.R., Yang, Y., Zhu, J., Arensburger, P., Pietrantonio, P.V., Barker, S.C., Shao, R., Zdobnov, E.M., Hauser, F., Grimmelikhuijzen, C.J.P., Park, Y., Rozas, J., Benton, R., Pedra, J.H.F., Nelson, D.R., Unger, M.F., Tubio, J.M.C., Tu, Z., Robertson, H.M., Shumway, M., Sutton, G., Wortman, J.R., Lawson, D., Wikel, S.K., Nene, V.M., Fraser, C.M., Collins, F.H., Birren, B., Nelson, K.E., Caler, E., Hill, C.A., 2016. Genomic insights into the Ixodes scapularis tick vector of Lyme disease. Nat. Commun. 7. https://doi.org/10.1038/ncomms10507

Henningsson, A.J., Asgeirsson, H., Hammas, B., Karlsson, E., Parke, Å., Hoornstra, D., Wilhelmsson, P., Hovius, J.W., 2019. Two Cases of Borrelia miyamotoi Meningitis, Sweden, 2018. Emerg. Infect. Dis. 25, 1965–1968. https://doi.org/10.3201/eid2510.190416

Heyman, P., Cochez, C., Hofhuis, A., van der Giessen, J., Sprong, H., Porter, S.R., Losson, B., Saegerman, C., Donoso-Mantke, O., Niedrig, M., Papa, A., 2010. A clear and present danger: tick-borne diseases in Europe. Expert Rev. Anti Infect. Ther. 8, 33–50. https://doi.org/10.1586/eri.09.118

Hillyard, P.D., 1996. Ticks of North-West Europe. Keys and notes for identificationof species., Synopses of the British Fauna (New Series). Field Studies Council, Shrewsbury, UK.

Hovius, J.W.R., de Wever, B., Sohne, M., Brouwer, M.C., Coumou, J., Wagemakers, A., Oei, A., Knol, H., Narasimhan, S., Hodiamont, C.J., Jahfari, S., Pals, S.T., Horlings, H.M., Fikrig, E., Sprong, H., van Oers, M.H.J., 2013. A case of meningoencephalitis by the relapsing fever spirochaete Borrelia miyamotoi in Europe. Lancet 382, 658. https://doi.org/10.1016/S0140-6736(13)61644-X

Jaenson, T., Tälleklint, L., Lundqvist, L., Olsen, B., Chirico, J., Mejlon, H., 1994. Geographical Distribution, Host Associations, and Vector Roles of Ticks (Acari: Ixodidae, Argasidae) in Sweden. J. Med. Entomol. 31, 240–56. https://doi.org/10.1093/jmedent/31.2.240

Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden. Parasit. Vectors 5, 8. https://doi.org/10.1186/1756-3305-5-8

Jaenson, T.G.T., Petersson, E.H., Jaenson, D.G.E., Kindberg, J., Pettersson, J.H.-O., Hjertqvist, M., Medlock, J.M., Bengtsson, H., 2018. The importance of wildlife in the ecology and epidemiology of the TBE virus in Sweden: incidence of human TBE correlates with abundance of deer and hares. Parasit. Vectors 11. https://doi.org/10.1186/s13071-018-3057-4

Jaenson, T.G.T., Värv, K., Fröjdman, I., Jääskeläinen, A., Rundgren, K., Versteirt, V., Estrada-Peña, A., Medlock, J.M., Golovljova, I., 2016. First evidence of established populations of the taiga tick Ixodes persulcatus (Acari: Ixodidae) in Sweden. Parasit. Vectors 9, 377. https://doi.org/10.1186/s13071-016-1658-3

Kovalev, S.Y., Golovljova, I.V., Mukhacheva, T.A., 2016. Natural hybridization between Ixodes ricinus and Ixodes persulcatus ticks evidenced by molecular genetics methods. Ticks Tick-Borne Dis. 7, 113–118. https://doi.org/10.1016/j.ttbdis.2015.09.005

Manilla, G., 1998. Fauna d'Italia. Acari, Ixodida. Ed. Calderini Bologna, 280.

Mazelier, M., Rouxel, R.N., Zumstein, M., Mancini, R., Bell-Sakyi, L., Lozach, P.-Y., 2016. Uukuniemi Virus as a Tick-Borne Virus Model. J. Virol. 90, 6784–6798. https://doi.org/10.1128/JVI.00095-16

Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., Bødker, R., Fach, P.,

Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. Front. Cell. Infect. Microbiol. 4. https://doi.org/10.3389/fcimb.2014.00103
Nielsen, E.M., Andersen, M.T., 2003. Detection and Characterization of Verocytotoxin-Producing Escherichia coli by Automated 5′ Nuclease PCR Assay. J. Clin. Microbiol. 41, 2884–2893. https://doi.org/10.1128/JCM.41.7.2884-2893.2003
Nilsson, A., 1988. Seasonal occurrence of Ixodes ricinus (Acari) in vegetation and on small mammals in southern Sweden. Ecography 11, 161–165. https://doi.org/10.1111/j.1600-0587.1988.tb00795.x
Papa, A., Zelená, H., Papadopoulou, E., Mrázek, J., 2018. Uukuniemi virus, Czech Republic. Ticks Tick-Borne Dis. 9, 1129–1132. https://doi.org/10.1016/j.ttbdis.2018.04.011
Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.-E., Raoult, D., 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev. 26, 657–702. https://doi.org/10.1128/CMR.00032-13
Parola, P., Raoult, D., 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 32, 897–928. https://doi.org/10.1086/319347
Portillo, A., Santibáñez, P., Palomar, A.M., Santibáñez, S., Oteo, J.A., 2018. ‘Candidatus Neoehrlichia mikurensis’ in Europe. New Microbes New Infect. 22, 30. https://doi.org/10.1016/j.nmni.2017.12.011
Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Špitalská, E., Kazimírová, M., 2014. Ixodes ricinus and Its Transmitted Pathogens in Urban and Peri-Urban Areas in Europe: New Hazards and Relevance for Public Health. Front. Public Health 2. https://doi.org/10.3389/fpubh.2014.00251
Svensson, J., Hunfeld, K.-P., Persson, K.E.M., 2019. High seroprevalence of Babesia antibodies among Borrelia burgdorferi-infected humans in Sweden. Ticks Tick-Borne Dis. 10, 186–190. https://doi.org/10.1016/j.ttbdis.2018.10.007