Ticks and Tick-borne Diseases 7 (2016) 208–215

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Assessing the abundance, seasonal questing activity, and *Borrelia* and tick-borne encephalitis virus (TBEV) prevalence of *Ixodes ricinus* ticks in a Lyme borreliosis endemic area in Southwest Finland

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form
14 September 2015
Accepted 15 October 2015
Available online 24 October 2015

Keywords:
Ixodes ricinus
Borrelia burgdorferi
Borrelia miyamotoi
Biotope distribution
Tick abundance
Tick-borne encephalitis virus

ABSTRACT

Studies have revealed that *Ixodes ricinus* (Acari: Ixodidae) have become more abundant and their geographical distribution extended northwards in some Nordic countries during the past few decades. However, ecological data of tick populations in Finland are sparse. In the current study, *I. ricinus* abundance, seasonal questing activity, and their *Borrelia* spp. and tick-borne encephalitis virus (TBEV) prevalence were evaluated in a Lyme borreliosis endemic area in Southwest Finland, Seili Island, where a previous study mapping tick densities was conducted 12 years earlier. A total of 1940 ticks were collected from five different biotopes by cloth dragging during May–September 2012. The overall tick density observed was 5.2 ticks/100 m^2 for nymphs and adults. Seasonal questing activity of ticks differed between biotopes and life stages: bimodal occurrences were observed especially for nymphal and adult ticks in forested biotopes, while larvae in pastures exhibited mostly unimodal occurrence. Prevalence of *Borrelia* and TBEV in ticks was evaluated using conventional and real-time PCR. All samples were negative for TBEV. *Borrelia* prevalence was 25.0% for adults ($n = 44$) and the minimum infection rate (MIR) 5.6% for pooled nymph samples (191 samples, 1–14 individuals per sample; 30/191 positive). No *Borrelia* were detected in pooled larval samples (63 samples, 1–139 individuals per sample). Five species of *Borrelia* were identified from the samples: *B. afzelii*, *B. burgdorferi* s.s., *B. garinii*, *B. valaisiana* and *B. miyamotoi*. In Finland, *B. valaisiana* and *B. miyamotoi* have previously been reported from the Åland Islands but not from the mainland or inner archipelago. The results of the present study suggest an increase in *I. ricinus* abundance on the island.

Introduction

The castor bean tick *Ixodes ricinus* (Linnaeus, 1758) is a tick species of the family Ixodidae (Acari) commonly found in Europe. Its distribution covers most of the continent, extending from southern Italy up to latitudes of 66°N in northern Fennoscandia (Gray et al., 2009; Nilsson, 1988). It has long been acknowledged as an important vector for a wide variety of pathogens of medical and veterinary importance. Those commonly reported in Europe and of particular medical interest in Finland are *Borrelia burgdorferi* s.l. causing Lyme borreliosis and the tick-borne encephalitis virus (TBEV) (Hubálek and Halouzka, 1997; Lindquist and Vapalahti, 2008; Mansfield et al., 2009).

Surveys of long-term changes in the geographical distribution and abundance of *I. ricinus* in northern Europe have been conducted especially in Sweden, where a northwards shift in geographical distribution and an increase in abundance have been observed over the past few decades (Jaenson et al., 2012; Lindgren et al., 2000; Tälleklint and Jaenson, 1998). These changes have been attributed

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http://dx.doi.org/10.1016/j.ttbdis.2015.10.011
1877-959X/

to various effects of climate change (Jaenson et al., 2012; Lindgren et al., 2000). Climate change can affect *I. ricinus* distribution and abundance through changes in the abundance of important host animals such as roe deer (*Capreolus capreolus* Linnaeus) (Dobson and Randolph, 2011; Jaenson et al., 2012; Jaenson and Lindgren, 2011; Mysterud and Østbye, 2006; Rizzoli et al., 2009), milder winters and extended spring and autumn seasons in the northern hemisphere (Gray et al., 2009; Medlock et al., 2013), and faster tick developmental rates due to higher average temperatures (Jaenson and Lindgren, 2011; Sonenshine and Roe, 2014). The seasonal questing activity patterns of *I. ricinus* are highly variable in relation to tick life stage and environmental conditions. Unimodal and bimodal activity patterns have both been reported from tick populations in northern Europe (Craine et al., 1995; Gray, 2008; Nilsson, 1988). The seasonal questing activity of ticks has not been the focus of studies in Finland before.

Environmental conditions affect tick occurrence by influencing host-seeking possibilities and tick mortality. *I. ricinus* are sensitive to desiccation and require high relative humidity (>80 RH %) in microhabitats at ground level to survive off-host periods (Herrmann and Gern, 2010; Medlock et al., 2013; Stafford, 1994). Off-host periods include periods of host seeking, transstadial development after blood meals, behavioral and morphogenetic diapause, and rehydration (Herrmann and Gern, 2010; Medlock et al., 2013; Perret et al., 2003; Sonenshine and Roe, 2014). The compositions of field and ground layers are therefore critically important in determining the survival of ticks, principally due to their effects on the humidity of microhabitats (Guerra et al., 2002; Lindsay et al., 1998; Mejlon and Jaenson, 1997). Humidity retaining microhabitats are typically most abundant in forested areas where, in addition to the shade provided by tree canopy, thick layers of litter and mosses commonly accumulate. Indeed, data from studies of *I. ricinus* habitat distribution show that forested areas typically have higher relative abundances of ticks than more open areas. However, this is likely influenced by other factors as well, such as host animal abundance (Lindström and Jaenson, 2003; Mejlon and Jaenson, 1993; Mäkinen et al., 2003; Walker et al., 2001). As *I. ricinus* is the primary vector of *B. burgdorferi* s.l. and the tick-borne encephalitis virus in Europe, its preference for certain environments has a direct effect on the risk of humans getting infected in the respective environments (Gustafson et al., 1995; Jaenson et al., 2009).

The annually reported number of clinically diagnosed cases of Lyme borreliosis in Finland has been increasing since the mid-1990s. Reporting of Lyme borreliosis is mandatory in Finland, and all serologically confirmed cases are reported to the National Infectious Diseases Register (NIDR) of the National Institute for Health and Welfare. In 1995 there were 345 reported cases, whereas the number of cases was 1704 in 2013. While some of this increase is likely attributed to increased knowledge of ticks and disease symptoms among citizenry, whether these alone can explain the greatly increased numbers is unclear. Occurrence of *B. burgdorferi* s.l. reportedly coincides with *I. ricinus* distribution in Sweden (Gustafson et al., 1995). Therefore, it seems likely that changes in *I. ricinus* abundance and distribution will affect the occurrence of *B. burgdorferi* s.l. and other tick-borne infections in Finland as well. Recent tick-related studies in Finland have mostly been focused on the emergence, occurrence and prevalence of tick-borne diseases (Junttila et al., 1994, 1999; Jääskeläinen et al., 2006, 2010; Mäkinen et al., 2003; Vera et al., 2014; Wilhelmsson et al., 2013). However, ecological studies of tick phenology, abundance and distribution from this region are sparse.

The main aims of the current study were: (1) to provide data on *I. ricinus* abundance and *Borrelia* and TBEV prevalence on the island of Seili in Southwest Finland, which is an area highly endemic for *I. ricinus* and *B. burgdorferi* s.l., and (2) to investigate preferred biotopes and seasonal questing activity patterns of *I. ricinus* on the island.

Materials & methods

Field studies

Field studies were conducted during May–September 2012 on the rural island of Seili (60°14′4″N, 21°57′7″E; surface area 1.6 km^2), located in the inner archipelago of southwestern Finland. Five different biotopes commonly found on the island were chosen for sampling of ticks: coniferous forest, deciduous forest, alder thicket, meadow and pasture.

Biotopes were classified as follows: coniferous forests were forests dominated by *Picea abies* L. and *Pinus sylvestris* L. with field layers clearly dominated by *Vaccinium myrtillus* L. and ground layers by mosses and needle litter. Deciduous forests were forests dominated by *Betula pendula* Roth, *Betula pubescens* Ehrh. and *Corylus avellana* L. with field layers dominated by grasses and ground layers with patches of mosses, *V. myrtillus* and leaf litter. Alder thickets were forested wetlands dominated by *Alnus glutinosa* L. with diverse field layers mostly dominated by *Filipendula ulmaria* L. and ground layers of mixed litter. Meadows were treeless areas with field layers of true grasses, mostly *Dactylis* sp., *Festuca* sp. and *Deschampsia* sp. Pastures were fenced grazing areas with flora similar to meadows. Cattle have been grazing these pasture areas in rotation during summers since 2008. Each pasture area was grazed during the study.

Tick sampling

Three 50 m study transects were placed in each biotope, for a total of 15 transects. Ticks were sampled by dragging a 1.0 m^2 cotton cloth along the 50 m study transects. The three transects of each biotope were chosen from different areas, so they were spatially separate. Each transect was further divided into three approximately 16.7 m subsections. These subsections were dragged separately to decrease the loss of attached ticks due to brushing off.

Each transect was dragged once a week (i.e., a total of 750 m^2 per week) from week 21 in May to week 36 in September, for a total of 15 weeks. No dragging was done during week number 28 due to rainy weather. For this same reason, five transects (one per biotope) were missed in week 25. Temperature and relative humidity were measured for each transect from approximately 10 cm above ground level after each dragging with a handheld data logger (EL-USB-2-LCD RH/Temp data logger from DATAQ Instruments). Saturation deficit (SD) was calculated from the temperature and humidity data with the formula presented in Perret et al. (2000) (see Table S1 for temperature, relative humidity, and saturation deficit values).

All ticks attached to the cloth were collected using tweezers. Larvae, nymphs, and adult males and females were separated and placed in 1.5-ml Eppendorf tubes. Each adult male or female was placed in its own tube, all nymphs from a single subsection of a transect in one tube (1–14 individuals per tube) and all larvae from an entire transect in one tube (1–139 individuals per tube). The ticks were stored in a cooler (+7 °C) and later transported alive to the Department of Biology in the University of Turku for deep freezing (−80 °C).

Sample preparation and tick species determination

DNA and RNA were extracted from the frozen tick samples between June 2013–February 2014 using NucleoSpin® TriPrep–kits (Macherey-Nagel, Germany), following the protocol in the kit manual (Rev. 04, February 2012; available from