Master's Thesis

Seasonality of *Ixodes ricinus* and *Ixodes trianguliceps* tick on the bank vole (*Myodes glareolus*) and on vegetation in Central Finland

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ABSTRACT

Many infectious diseases are zoonoses, that is, they can be transmitted between vertebrate animals and humans. More than a fifth of zoonotic pathogens are transferred from one host to another by vectors, which are often blood-sucking arthropods, such as ticks. In Europe, Lyme borreliosis, spread by *Ixodes ricinus* tick, is the most prevalent tick-borne zoonosis. *I. ricinus* spreads also other zoonotic pathogens, such as tick-borne encephalitis virus (TBEV), *Anaplasma phagocytophilum* bacterium and *Babesia microti* protozoan. Pathogen transmission between ticks occurs as a susceptible tick feeds on an infected host who has become infected while feeding an infected tick. In some cases ticks must co-feed on a host to enable pathogen circulation, why the synchronous seasonal activity of different tick instars is essential. Wild rodents are reservoir hosts for many tick-borne pathogens, having an important role in the circulation of enzootic tick-borne infections. *I. trianguliceps* is a tick species that parasitizes only small mammals and lives in their burrows. It can maintain infections within rodent-tick system in the absence of other tick species. *I. ricinus*, instead, is a generalist species, which searches for a host on vegetation, i.e. quests, and may transfer pathogens e.g. from rodents to humans. In order to understand the circulation of tick-borne pathogens in tick populations and the risks they may cause to humans, it is important to examine the seasonal relationship between ticks and rodents. Very little is known about the population dynamics of ticks in Finland, where climate is strongly seasonal and the abundance of several rodent species varies cyclically. Therefore, my main purpose was to study the seasonal dynamics (between May–September) of *I. ricinus* and *I. trianguliceps* on a common host species, the bank vole, and on vegetation in urban and non-urban forests in Central Finland. In addition, I studied other factors that might affect the tick burden of bank voles. *I. ricinus* was the most common in early summer when larvae and nymphs had the activity peak. *I. ricinus* was abundant at urban sites, whereas at non-urban sites it was rarely found. *I. trianguliceps*, instead, infested voles at all sites. I also found that vole characteristics (sex and age) affected its infestation load, which may suggest that certain individuals harbour the majority of ticks and thus, facilitate the transmission of pathogens. I conclude that humans have the greatest risk to become in contact with ticks in early summer when *I. ricinus* is common on vegetation. Due to the seasonal synchrony of *I. ricinus* larvae and nymphs, the environmental conditions might be favourable to maintain TBEV in Central Finland, which currently occurs mainly in coastal areas in Finland.

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TIIVISTELMÄ

Monet infektiotaudit ovat zoonooseja eli ne voivat siirtyä selkärankaisten eläinten ja ihmisten välillä. Yli 20 % zoonoosien taudinaiheuttajista siirtyy toisen nk. vektorilajin avulla isäntäyksilöstä toiseen. Vertaimevät niveljalkaiset, kuten puutiaiset, toimivat monien taudinaiheuttajien vektoreina. Euroopassa *Ixodes ricinus* puutiaisen levittämä Lymen borrelioosi on yleisin puutiaisvälitteinen zoonoosi. Myös esimerkiksi puutiaisaivokuumevirus (TBEV), *Anaplasma phagocytophilum* bakteeri ja *Babesia microti* alkueläin ovat *I. ricinuksen* levittämiä taudinaiheuttajia. Taudinaiheuttajat siirtyvät puutiaisesta toiseen, kun altis puutiainen ruokailee infektoituneella isäntäyksilöllä, joka on saanut tartunnan infektoituneelta puutiaiselta. Joissakin tapauksissa puutiaisten on ruokailtava isännällä samanaikaisesti taudinaiheuttajan siirtymiseksi, jolloin puutiaisten eri elämänvaiheiden yhtäaikainen aktiivisuus on oleellista. Villijyrsijät toimivat monien puutiaisvälitteisten taudinaiheuttajien ”varastoeläiminä” ja niillä on tärkeä rooli infektioiden ylläpidossa. *I. trianguliceps* on puutiaislaji, joka loisii vain pikkunisäkkäillä ja elää niiden pesissä. Se voi säilyttää infektioita jyrsijäpopulaatioissa *I. ricinuksen* puuttuessa alueelta. *I. ricinus*, joka löytää isännän odottaen kasvillisuudessa, on ns. generalistilaji, joka voi siirtää taudinaiheuttajat jyrsijöistä suurempiin isäntiin, myös ihmisiin. Puutiaisvälitteisten tautien ihmisille aiheuttamien riskien ymmärtämiseksi on tärkeää tutkia puutiaisten ja jyrsijöiden vuorovaikutusta. Puutiaisten populaatiodynamiikasta Suomessa, missä sääolot ovat hyvin vaihtelevat ja useiden jyrsijälajien runsaus vaihtelee syklisesti, tiedetään vähän. Tutkimukseni tarkoituksena olikin tarkastella *I. ricinus* ja *I. trianguliceps* puutiaisen vuodenaikaista esiintymistä (touko–syyskuussa) yleisellä isäntälajilla, metsämyyrällä, ja kasvillisuudessa kaupunki- ja ei-kaupunkimetsissä Keski-Suomessa. *I. ricinus* oli yleisin alkukesällä, jolloin larvoilla ja nymfeillä oli aktiivisuushuippu. *I. ricinus* esiintyi runsaana vain kaupunkimetsissä, kun taas *I. trianguliceps* oli yleinen myös kaupunkialueiden ulkopuolella. Havaitsin myös, että myyrien sukupuoli ja ikä vaikuttivat niiden puutiaiskuormaan, mikä viitannee siihen, että osa yksilöistä kantaa valtaosan puutiaisista helpottaen näin infektioiden säilymistä. Tulosteni mukaan ihmisillä näyttäisi olevan suurin riski joutua kontaktiin puutiaisten kanssa alkukesällä, jolloin *I. ricinus* on yleinen kasvillisuudessa. *I. ricinus* larvojen ja nymfien yhtäaikaisesta aktiivisuudesta johtuen ympäristöolot saattaisivat olla suotuisat TBEV:n säilymiselle Keski-Suomessa, viruksen esiintyessä Suomessa tällä hetkellä lähinnä rannikkoalueilla.

Contents

1. INTRODUCTION

Infectious diseases have a great impact on global health still nowadays. The incidence of many infectious diseases has increased during last decades (Jones et al. 2008) and changes in socio-economic, environmental and ecological conditions yet forward their emergence and transmission (Patz et al. 2004, Weiss & McMichael 2004, Keesing et al. 2010). Habitat destruction, farming and recreation, for instance, increase contacts between wild fauna, livestock and humans. This is noteworthy because zoonotic pathogens, i.e. those transmitted between vertebrate animals and humans, account for more than half of pathogen species known to cause disease in humans (Taylor et al. 2001, Woolhouse & Gowtage-Sequeria 2005). Some zoonoses are transmitted directly (e.g. rabies) or indirectly, for example through contaminated food (e.g. *Salmonella*), from animals to humans. However, more than 20 % of zoonoses are transmitted by vectors, which means that the pathogen is transmitted by other organisms between infected and susceptible hosts (Taylor et al. 2001). Vector-borne diseases impose a great challenge to disease ecology as the population biology of several species needs to be taken into account to understand pathogen dynamics.

Many arthropods, e.g. mosquitoes, flies, fleas and ticks, may act as vectors for zoonotic pathogens. Major human diseases of the developing world, such as malaria, sleeping sickness and leishmaniasis, are arthropod-borne diseases. In Europe, Lyme borreliosis (LB), spread by *Ixodes ricinus* tick, is the most prevalent arthropod-transmitted zoonosis (WHO 2004). *Borrelia burgdorferi* sensu lato (s.l.) (the agents of LB) occur in most European countries, corresponding approximately the distribution of *I. ricinus* (Randolph 2001, Piesman & Gern 2004). *I. ricinus* spreads also other diseases of medical and veterinary significance in Europe, such as tick-borne encephalitis (TBE), granulocytic anaplasmosis and babesiosis. During the last decades the incidences of tick-borne diseases have increased, at least partly because the distribution of *I. ricinus* is expanding (e.g. Randolph 2001, 2004a, Jaenson et al. 2012, Medlock et al. 2013). Pathogen transmission between a tick and a host is enabled as ticks (superorder *Parasitiformes*, suborder *Ixodida*, family *Ixodidae*) take one large blood meal per life stage, i.e. as a larva, nymph and as an adult female, from hosts (Francischetti et al. 2009). Blood meal is a requirement for the larva and nymph to develop to the next stage and, for the female to lay eggs.

The seasonal activity of ticks determines when humans have the greatest risk to become in contact with ticks. When the abundance of questing, i.e. host-seeking, *I. ricinus* ticks on vegetation is at its highest, also the risk that humans get tick bites is high. Questing activity and also tick development rate depend largely on climatic conditions (Perret et al. 2000, Randolph et al. 2002). Consequently, the seasonal population dynamics of *I. ricinus* vary greatly geographically (Korenberg 2000, Randolph et al. 2002), which has a major impact on the pathogen transmission (Randolph et al. 2000). Many tick-borne pathogens transfer between ticks as an uninfected tick, in most cases a larva, feeds on an infected host individual who has become infected while feeding an infected tick (typically a nymph) (Randolph et al. 1996). Thus, for example, the geographical variation in the seasonal activity of *I. ricinus* larvae and nymphs appears to explain the focal distribution of TBE in Europe (Randolph et al. 2000). In regions where TBE is present, larvae and nymphs are active simultaneously, often in late spring or early summer (Randolph et al. 2000). In the areas TBE is not present, larvae are active later in the summer, often a couple of months after the activity peak of the nymphs (Randolph et al. 2000).

I. ricinus feeds on several host species including many mammalian species, such as e.g. ungulates, lagomorphs and rodents, as well as bird species and some reptiles (Milne

1949). Larvae and nymphs feed mainly on smaller mammals whereas adult females infest principally large hosts such as deer and livestock (Milne 1949). However, some tick species, like *I. trianguliceps*, parasitize only small rodents and shrews during each life-stage (Cotton & Watts 1967, Randolph 1975b). These nidicolous ticks do not quest above ground but live in the nests and/or burrows of their hosts and thus, they do not become in contact with humans. *I. trianguliceps* has been found, however, to be important as maintaining tick-borne pathogens in small mammal populations in the absence of *I. ricinus* (Bown et al. 2003, 2008). *I. trianguliceps* may even be, at least regionally, the principal vector for *Anaplasma phagocytophilum* bacterium and *Babesia microti* protozoan (Bown et al. 2008). Bown et al. (2008), for instance, found that the exclusion of deer from the study area reduced the amount of *I. ricinus* in the area but did not have an effect on the *I. trianguliceps* burden or on *A. phagocytophilum* and *Ba. microti* infection prevalences in small rodents. As opportunity enables *I. ricinus* can act as a bridge-vector, i.e. transfer pathogens also to other host species, including humans (Bown et al. 2008).

The persistence of tick-borne pathogens requires competent reservoir host species that can effectively infect feeding ticks and thus, support pathogen circulation (reviewed by Piesman & Gern 2004). For example ungulates, such as deer, feed large numbers of *I. ricinus* but they do not transmit pathogens to ticks (e.g. Jaenson & Tälleklint 1992). Small mammals, such as mice, voles and shrews, have been highlighted to have a key role in tick-borne disease dynamics (Gern et al. 1998). Small mammals feed large numbers of immature ticks and pathogens are easily transferred from many small mammals to ticks (Humair et al. 1999). Furthermore, small mammals usually exist at high densities and are almost ubiquitous animals. It has been suggested that LB risk in North America, for instance, may vary depending on the abundance of a common rodent species, white-footed mouse (*Peromyscus leucopus*) (Ostfeld et al. 2006), which is the most competent reservoir host that infects 40–90 % of feeding ticks (Mather et al. 1989, Schmidt & Ostfeld 2001). Among small mammals in Europe, at least *Apodemus* mice, field vole *(Microtus agrestis)*, bank vole (*Myodes glareolus*) and common shrew (*Sorex araneus*) appear to be important reservoir species for many tick-borne pathogens (Humair et al. 1999, Bown et al. 2003, 2006, 2008, 2011, Pérez et al. 2012).

Ticks are usually highly aggregated on rodent populations (Randolph 1975a, Randolph et al. 1999, Kiffner et al. 2011). This is a common phenomenon for parasites among hosts (Shaw et al. 1998) and for example Randolph et al. (1999) found that approximately 20 % of mice and voles fed more than half of collected immature ticks. The aggregation might be a result of several factors. Firstly, the distribution of ticks in their habitat may be patchy, particularly for larvae, which arise from egg masses of even thousands of eggs and do not move much, resulting a highly clumped occurrence (Ostfeld et al. 1996). Consequently, a host individual that collects larvae is likely to collect many of them simultaneously. Secondly, host activity is thought to be very important. Older individuals appear to be more infested by *I. ricinus* ticks than younger ones, which might due to greater activity of older rodents (Tälleklint & Jaenson 1997, Kiffner et al. 2011). Furthermore, male voles and mice harbour usually more ticks than females (Cotton & Watts 1967, Ostfeld et al. 1996, Tälleklint & Jaenson 1997). This can partly be due to more wide-ranging movements of males, which increase the probability of contacting ticks (Kikkawa 1964, Ostfeld et al. 1996). Thirdly, it has been also shown that testosterone levels of males may increase their vulnerability to ticks (Hughes & Randolph 2001). Aggregation is prerequisite for the transmission of tick-borne pathogens, especially for those with short infectious period, such as TBE virus (TBEV) (Randolph et al. 1996). Thus, the small proportion of highly parasitized hosts is responsible for most of the transmission events in tick-borne pathogens.

In order to understand the circulation of tick-borne pathogens in tick populations and the risks they may cause to humans, it is important to examine the relationship between ticks and their rodent hosts. However, very little is known about the population dynamics of ticks in Finland, where climate is strongly seasonal and the abundance of several small rodent species fluctuates multiannually as well as seasonally. *I. ricinus* is a widespread tick species in Finland (Öhman 1961) while the distribution of *I. persulcatus*, another questing species, is limited to few areas (Jääskeläinen 2011). *I. trianguliceps* is the only reported tick species parasitizing only small mammals in Finland (Ulmanen 1972). The purpose of my Master's thesis was to study the seasonal dynamics of *I. ricinus* and *I. trianguliceps* on vegetation and on a very common host species, the bank vole, in Central Finland. My main objectives were to 1) examine the seasonal variation of different developmental stages of ticks between May and September on vegetation and on voles and 2) clarify the factors that affect the numbers of ticks feeding on voles. This study provides important information in order to evaluate exposure of humans to ticks and prerequisites for the circulation of tick-borne pathogens.

2. MATERIALS AND METHODS

2.1. Study rodent species

Bank vole is the most abundant rodent species in Finland and common in many parts of Europe. Bank vole prefers forest habitats and the diet consists mainly of berries, seeds, buds and fungi (Bjärvall & Ullström 1985). In Central Finland, bank vole reproduces two to three times between May and September and litter size ranges between 2 and 10 pups (Koivula et al. 2003). Pups from the first litter can reproduce already during the same year. Mature females are territorial (Koskela et al. 1997), whereas males occupy large home ranges and move longer distances than females, especially in summer (Kikkawa 1964).

In Central Finland, bank vole populations typically fluctuate in a cyclic manner with very high population peaks every 3–4 years followed by steep declines, i.e. crashes, in population density (Kallio et al. 2009). Within a year, the population density is usually the lowest in spring, when the populations consist mostly of old overwintered individuals, and the highest in autumn and populations consist mainly of individuals that have been born during the same year (Kallio et al. 2010).

2.2. Study sites and timing of the fieldwork

The fieldwork was carried out in Jyväskylä, in Central Finland. There were altogether 16 study sites (for locations see Appendix 1) and each site had two transects (lines) (1A, 1B; 2A, 2B etc., altogether 32 transects), which were separated approximately by 50–70 meters or a forest road. Eight of the study sites (sites 1, 2, 5, 6, 9, 10, 13 and 14) were located near settlement ("urban areas") and the other eight sites (sites 3, 4, 7, 8, 11, 12, 15 and 16) were more remote ("non-urban areas"), where settlement was very sparse. All the sites were forest areas, most of which were dominated by spruce (*Picea abies*) or mixed forest with spruce, pine (*Pinus sylvestris*) and/or birch (*Betula*). At two sites other transect was in pine-dominated forest. Most typical grasses were, for instance, *Vaccinium myrtillus*, *V. vitis-idaea*, *Maianthemum bifolium*, *Linnaea borealis* and *Oxalis acetosella*. The sites were chosen so that they would have been represented favourable habitats for the bank vole.

The fieldwork, i.e. vole trappings and the collection of questing ticks, was done in each site five times with ~ 4 weeks interval between mid May and late September 2012.

Four study sites were trapped simultaneously, and every week 4 to 8 sites were studied. Hence, it took approximately 3–4 weeks to carry out one fieldwork session covering all sites. Tick collection was usually done within one week (before or after) from the vole trapping. The collection of questing ticks was not carried out at session 3 (July–early August) at four sites (sites 13–16) due to sickness and adverse weather.

2.3. Study procedures

2.3.1. Vole trapping and examination

In every study site both transects had ten multiple-capture live traps (Ugglan Special mouse trap, Sweden). Traps were placed at 10–15 m intervals near burrows or otherwise in sheltered places. Traps were taken to study sites 2–6 days before the trapping session and baited with sunflower seeds, but not set. This usually enhances the possibility that voles find and enter the traps during the trapping session. After the pre-feeding, the traps were baited again (with sunflower seeds and a piece of potato) and set (between 1 and 3 p.m.). Traps were checked on the following morning (from 9 a.m.) and captured bank voles were taken into laboratory facilities at the university. Removed traps were replaced with empty ones and all traps were checked again the following morning, when they were also removed from the field.

Other rodent species (field vole, northern birch mouse (*Sicista betulina*) and yellow-necked mouse (*Apodemus flavicollis*)) were also trapped but they were released without an examination because of too low numbers to have enough significant comparative data. However, all the captured rodents were counted. Also, shrews (common shrew, pygmy shrew (*Sorex minutus*) and Eurasian water shrew (*Neomys fodiens*)) were always released if they had not died in the trap. Their numbers were calculated, too.

For the half of the urban (sites 2, 5, 10 and 13) and non-urban (sites 3, 8, 11 and 16) sites sunflower seeds were added to burrows and holes near every trap place (half a bucket per transect) when the traps were removed. The aim was to try to attract more bank voles due to potentially low numbers of individuals (the year 2012 was expected to be low in vole abundance).

In the laboratory, bank voles were kept individually in standard mouse cages with food, water and paddings. The body mass, head width and the sex of each bank vole were recorded. Voles were examined through for ticks in bright lighting. Ears and the region of snout, where most ticks occur (e.g. Cotton & Watts 1967), were searched especially carefully. All ticks were removed from the voles with forceps, stored in 70 % ethanol and frozen for later examination. Voles were marked with numbered earmark in the first trapping session (in May–early June), while a microchip implant was placed under the skin to ensure better persistence from the second trapping session (June onwards). Males, juvenile females and adult females that were clearly not pregnant were released at the point of capture as soon as possible (at latest the day after the capture). Other females were kept in the laboratory until they reproduced or confirmed not to be pregnant. Two to four toes of the newborn pups were cut for later individual recognition. After this a mother was released with its pups at the point of capture. The cage was left slightly open in the woods in a sheltered place so that mothers could carry pups under cover themselves (Oksanen et al. 2002). Animals were trapped and handled in accordance with Finnish welfare regulations on animal care.

2.3.2. Collection of questing *I. ricinus* ticks

Questing *I. ricinus* ticks were caught by dragging a white flannel blanket (1 m x 1 m) across the vegetation surface. At the urban areas, the flag was dragged typically for 0.4 km (range 0.3–0.5 km) on both transects and attached ticks were collected from the blanket with forceps for every 30 m. At the non-urban areas corresponding numbers were 0.5 km and 50 m except one transect (4A) where dragging was done only 0.3 km due to rough terrain. The walking distance to vole trapping transects was at maximum 0.1 km, but usually much shorter. Sampling was conducted generally between 9 a.m. and 4 p.m. (a few times between 10 a.m. and 8 p.m.). Dragging was not done on windy or rainy days. Ticks were stored in 70 % ethanol and frozen for later examination.

2.3.3. Observation of mammalian host species

All the mammalian host animals of the ticks that were observed at the study sites or near them (maximum distance approximately 200 m) during the field days were counted. These data were not used in the analyses but were employed as approximate information for evaluating the results.

2.3.4. Identification of ticks

Identification of tick species was done using a stereo and a light microscope and morphological identification keys (Arthur 1963, Filippova 1977, Snow 1978). To further confirm the species identification, a sample of individuals that were morphologically identified as *I. ricinus* (7 individuals) and *I. trianguliceps* (3 individuals) were examined with molecular methods. DNA was extracted from ticks using alkaline digestion method (Bown et al. 2003). PCR amplification of a fragment of the 16S rRNA gene of ticks was performed as described earlier (Caporale et al. 1995) and the amplicons were sequenced and 8 sequences were obtained. Sequence identity was determined by BLAST search against the NCBI Nucleotide database. The sequences confirmed the morphological identifications.

Ticks were separated according to their developmental stage. Thus, the number of larvae, nymphs, females and males of each species was counted.

2.4. Statistical analyses

To investigate the factors associated with the occurrence of ticks on bank voles and on vegetation, a generalized linear mixed model (GLMM) approach was used. GLMMs make possible to take several factors into consideration simultaneously. Furthermore, these models enable to control pseudoreplication by taking into account potential correlations between observations from the same site or from the same host individual. Analyses were started from a maximum model and the least significant predictors were removed one by one. In some cases, however, it was reasonable to include also non-significant predictors in the models to enable a comparison to other results.

Parasites typically show aggregated distribution in the host population with a small proportion of host individuals carrying large numbers of parasites (Shaw et al. 1998). This

was seen also in my data (Figure 1). In order to model the tick infestation load on bank voles, negative binomial distribution (log link function) was used. Negative binomial model describes the quantity of ticks an individual bank vole carries in relation to the independent variables. Modelling was done separately for larvae and nymphs of each tick species, whereas female and male ticks were excluded from the analyses due to the low number of animals found on the bank voles. Although the number of feeding *I. ricinus* nymphs was also low, they were analyzed because of their significance in pathogen transmission. The following fixed effects, i.e. predictors, were employed: 1) location of the trapping site (urban or non-urban area), 2) trapping month (= session), 3) sex of the vole, 4) head width of the vole, 5) number of trapped bank voles per site per month (= session) and 6) number of other trapped small mammals (rodents and shrews combined) per site per month (= session). Session 5 (September) was selected to be in the intercept, i.e. as a point of comparison for the other sessions, due to notably higher number of captured bank voles in September than early summer and thus, resulting smaller errors. The head width was used as a proxy for the age of bank vole, as their head width increases with age (Kallio et al. in press). The positive effect of extra food on the numbers of captured bank voles has been taken into account by using the number of bank voles as a predictor. The individual code of the bank vole and the trapping site were used as random effects. As *I. ricinus* was not found in all sites, only the sites where bank voles were infested by this species were included in analyses of *I. ricinus* infestation on voles.

The abundances of different instars of ticks on individual bank voles were examined with bivariate correlation (Pearson). The relationships between the numbers of feeding larvae and nymphs of a same and a different tick species were analyzed. Only the sites where bank voles were infested by *I. ricinus* were included in these correlation analyses.

To examine the effect of the month to the numbers of *I. ricinus* nymphs and females (stages that bite humans and may transfer pathogens) questing on the vegetation, negative binomial distribution with log link function was used. September was employed as a point of comparison for other months. The sum of nymphs and females was employed as a dependent variable and the site as a random effect. Tick abundance from each trapping transect (line), instead of site, was used as a target of examination because at some sites the two transects differed in walked distances. Accordingly, distance walked (in kilometers) was used as an offset variable to take into account the differences in walked distances between the transects. Only the data from the sites where *I. ricinus* were caught from the vegetation were included in the analysis.

Linear regression model was used to analyse how the quantity of questing *I. ricinus* on the vegetation explained the *I. ricinus* burden on the bank voles. The analyses were done in two ways: a) using the number of questing ticks (per 100 m^2) per site as a predictor and the mean number of feeding ticks per site as a dependent variable and b) using the number of questing ticks (per 100 m^2) per site per month as a predictor and the mean number of feeding ticks per site per month as a dependent variable. The analyses were done separately for *I. ricinus* larvae and nymphs. All the sites where *I. ricinus* were found from the vegetation and/or on the bank voles were included in these analyses.

All statistical analyses were performed by using IBM SPSS Statistics-program (version 20).

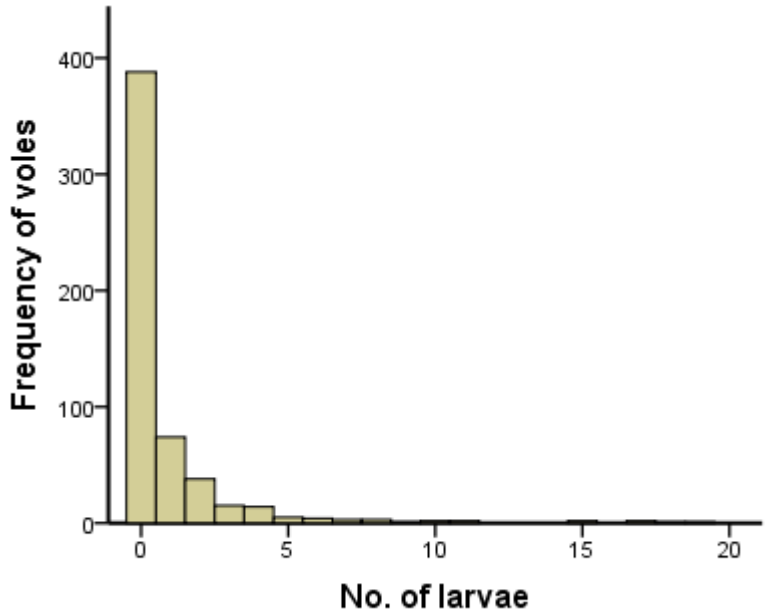

Figure 1. Typical aggregated frequency distribution of parasites on hosts. As an example *Ixodes trianguliceps* larvae parasitizing bank voles (from the present data).

3. RESULTS

3.1. Captured bank voles and parasitizing ticks

During the study period 407 bank voles were captured. Of these, 113 individuals were recaptured one to four times. Mean number of captures for all bank voles was 1.4. The total number of bank vole observations was 573. Furthermore, 909 shrews, 76 yellow-necked mice, 18 field voles, 4 northern birch mice and 4 weasels (*Mustela nivalis*) were captured. Weasels were not included in the analyses.

Two tick species, *Ixodes trianguliceps* and *I. ricinus*, were found on the bank voles (Figure 2). In total, 961 *I. trianguliceps* and 333 *I. ricinus* were discovered. *I. trianguliceps* parasitized 52.2 % of the bank voles and *I. ricinus* 19.7 % (information was not available for 16 voles). Both tick species fed on 9.3 % of the voles. The number of ticks on bank voles and the proportion of voles infested by each life-stage of both tick species separately are shown in Table 1.

I. trianguliceps was found on the bank voles at all 16 study sites, whereas *I. ricinus* parasitized voles only at the sites 1–3, 5–10 and 12 (i.e. in 10 out of 16 sites). At these ten sites, 30.9 % of the bank voles were infested by *I. ricinus*. Bank voles were the most commonly infested with *I. ricinus* at sites 1, 2, 5 and 6.

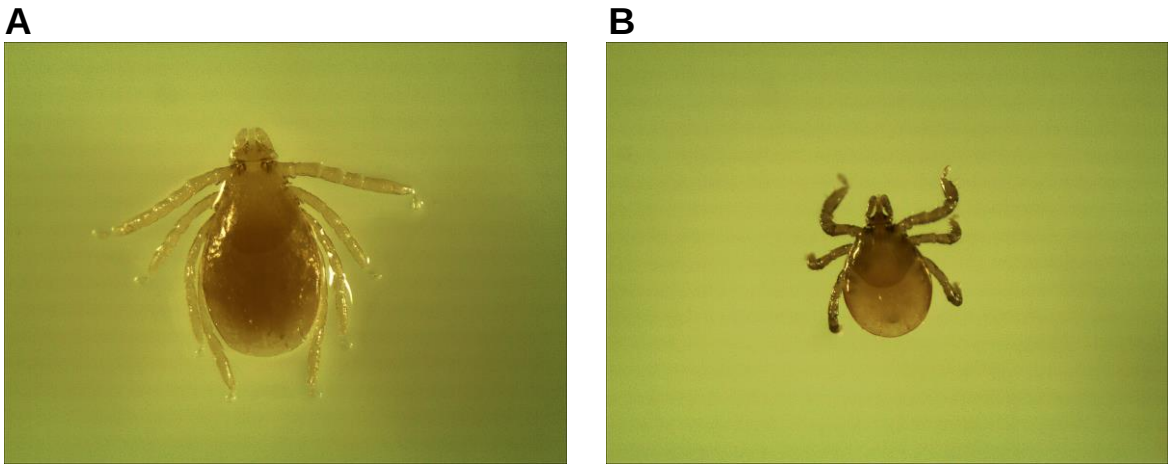

Figure 2. a) *Ixodes trianguliceps* nymph and b) *I. ricinus* larva. NB: Pictures are not on a scale of 1:1 and not on the same scale with each other.

Table 1. Infestation of each life-stage and total infestation of *Ixodes trianguliceps* and *I. ricinus* on bank voles. Minimum and maximum numbers (range) infesting individual hosts, total numbers (sum) found on the voles, percentages of voles infested and mean numbers of ticks per vole and per parasitized vole. (The number of bank voles is 557, except for mean per parasitized vole the number is 290 for *I. trianguliceps* and 110 for *I. ricinus*), (SD = standard deviation).

Ixodes trianguliceps	Range	Sum of ticks	% voles infested	Mean per vole (SD)	Mean per parasitized vole (SD)
Larvae	0—19	503	30.2	0.90 (2.33)	1.71 (3.01)
Nymphs	0—12	388	33.4	0.70 (1.49)	1.33 (1.84)
Females	0—6	67	6.5	0.12 (0.56)	0.23 (0.76)
Males	0—2	3	0.4	0.01 (0.10)	0.01 (0.13)
Total	0—21	961	52.2	1.73 (3.04)	3.30 (3.54)
Ixodes ricinus					
Larvae	0—27	301	18.9	0.54 (1.94)	2.74 (3.62)
Nymphs	0—4	31	3.6	0.06 (0.34)	0.28 (0.72)
Females	0—1	1	0.2	0.00 (0.04)	0.01 (0.10)
Males	0—0	0	0.0	0.00 (0.00)	0.00 (0.00)
Total	0—29	333	19.7	0.60 (2.12)	3.03 (3.94)

3.2. *I. ricinus* and *I. trianguliceps* on bank voles

The numbers of feeding *I. ricinus* larvae and nymphs on the bank voles were both influenced by the location, with voles at urban sites were more infested than voles at non-urban sites (Table 2). The trapping month influenced significantly the larval infestation load ($F = 8.381$, $df1 = 4$, $df2 = 345$, $P < 0.001$). As compared to September, bank voles had

more *I. ricinus* larvae in June and less in August (Table 2, Figure 3A). Month was not a significant factor for nymph abundance (F = 1.012, df1 = 4, df2 = 345, P = 0.401), although they were slightly more common during summer than in September (Table 2, Figure 3B). In addition, host individual's sex affected the *I. ricinus* infestation, male voles having more larvae and nymphs than female voles. The effect of the age (measured as head width) was positive, thus, older (= larger) bank voles having more both *I. ricinus* larvae and nymphs than younger individuals.

Table 2. Model estimates (estimated coefficient and standard error [SE]), *t*-values and P-values for final generalized linear mixed models (negative binomial) of *Ixodes ricinus* larvae (a) and nymphs (b) on bank voles. Significant P values are in italics. The number of bank voles is 353.

Tick stage	Variable	Coefficient	SE	*t*-value	P-value
a) Larvae	Intercept	-6.421	2.485	-2.583	*0.010*
	Location				
	Non-urban	0[a]			
	Urban	1.767	0.620	2.851	*0.005*
	Month				
	May	0.155	0.523	0.296	0.767
	June	1.037	0.321	3.235	*0.001*
	July	-0.301	0.374	-0.805	0.421
	August	-1.025	0.365	-2.805	*0.005*
	September	0[a]			
	Sex				
	Female	0[a]			
	Male	0.766	0.256	2.989	*0.003*
	Head	0.481	0.193	2.485	*0.013*
	Random effect:	Estimate	SE	Z-value	P-value
	Var(Code)	0.000	0.000	0.350	0.726
	Var(Site)	0.647	0.405	1.596	0.110
b) Nymphs	Intercept	-6.557	2.632	-2.491	*0.013*
	Location				
	Non-urban	0[a]			
	Urban	0.677	0.298	2.271	*0.024*
	Month				
	May	0.735	0.552	1.330	0.184
	June	0.598	0.407	1.472	0.142
	July	0.719	0.414	1.740	0.083
	August	0.585	0.380	1.539	0.125
	September	0[a]			
	Sex				
	Female	0[a]			
	Male	0.816	0.288	2.831	*0.005*
	Head	0.424	0.204	2.078	*0.038*
	Random effect:	Estimate	SE	Z-value	P-value
	Var(Code)	0.000	-	-	-
	Var(Site)	0.000	-	-	-

0[a] = Set to zero because the parameter was as a point of comparison

While the location of the study site did not affect the numbers of *I. trianguliceps* larvae feeding on bank voles, feeding nymphs were significantly more common at non-urban sites than at urban sites (Table 3). The trapping session influenced both the larval (F

= 6.661, df1 = 4, df2 = 546, P < 0.001) and nymphal (F = 6.586, df1 = 4, df2 = 545, P < 0.001) infestation load. As compared to session 5 (= September), bank voles had less *I. trianguliceps* larvae in session 3 (= July–early August) and 4 (= August) (Table 3, Figure 3C). Nymphs were more common in every session compared to session 5 (Table 3, Figure 3D). The age of the vole had negative effect on larvae and, thus, younger bank voles had more *I. trianguliceps* larvae than older individuals. In addition, the vole sex was significant for nymphs, with males having more *I. trianguliceps* nymphs than females. The number of bank voles captured from the site during the trapping session had positive influence on nymphal infestation and the number of other small mammals also affected positively both on the number of feeding larvae and nymphs.

Table 3. Model estimates (estimated coefficient and standard error [SE]), *t*-values and P values for final generalized linear mixed models (negative binomial) of *Ixodes trianguliceps* larvae (a) and nymphs (b) on bank voles. Significant P values are in italics. The number of bank voles is 555.

Tick stage	Variable	Coefficient	SE	*t*-value	P value
a) Larvae	Intercept	5.579	1.640	3.403	*0.001*
	Location				
	Non-urban	0[a]			
	Urban	-0.024	0.185	-0.132	0.895
	Session				
	1	0.217	0.362	0.599	0.550
	2	-0.252	0.241	-1.044	0.297
	3	-1.119	0.251	-4.466	*<0.001*
	4	-0.881	0.233	-3.788	*<0.001*
	5	0[a]			
	Sex				
	Female	0[a]			
	Male	-0.006	0.167	-0.036	0.972
	Head	-0.437	0.126	-3.472	*0.001*
	Small mammals	0.043	0.014	3.061	*0.002*
	Random effect:	Estimate	SE	Z-value	P-value
	Var(Code)	0.000	-	-	-
	Var(Site)	0.000	-	-	-
b) Nymphs	Intercept	-1.378	1.721	-0.801	0.424
	Location				
	Non-urban	0[a]			
	Urban	-0.886	0.307	-2.884	*0.004*
	Session				
	1	1.653	0.471	3.506	*<0.001*
	2	1.264	0.290	4.366	*<0.001*
	3	0.827	0.278	2.980	*0.003*
	4	1.055	0.259	4.073	*<0.001*
	5	0[a]			
	Sex				
	Female	0[a]			
	Male	0.618	0.171	3.624	*<0.001*
	Head	-0.119	0.126	-0.945	0.345
	Bank voles	0.069	0.026	2.599	*0.010*
	Small mammals	0.040	0.019	2.166	*0.031*
	Random effect:	Estimate	SE	Z-value	P-value
	Var(Code)	0.000	-	-	-
	Var(Site)	0.168	0.100	1.682	0.092

0[a] = Set to zero because the parameter was as a point of comparison

Small mammals = Abundance of other small mammals than bank voles

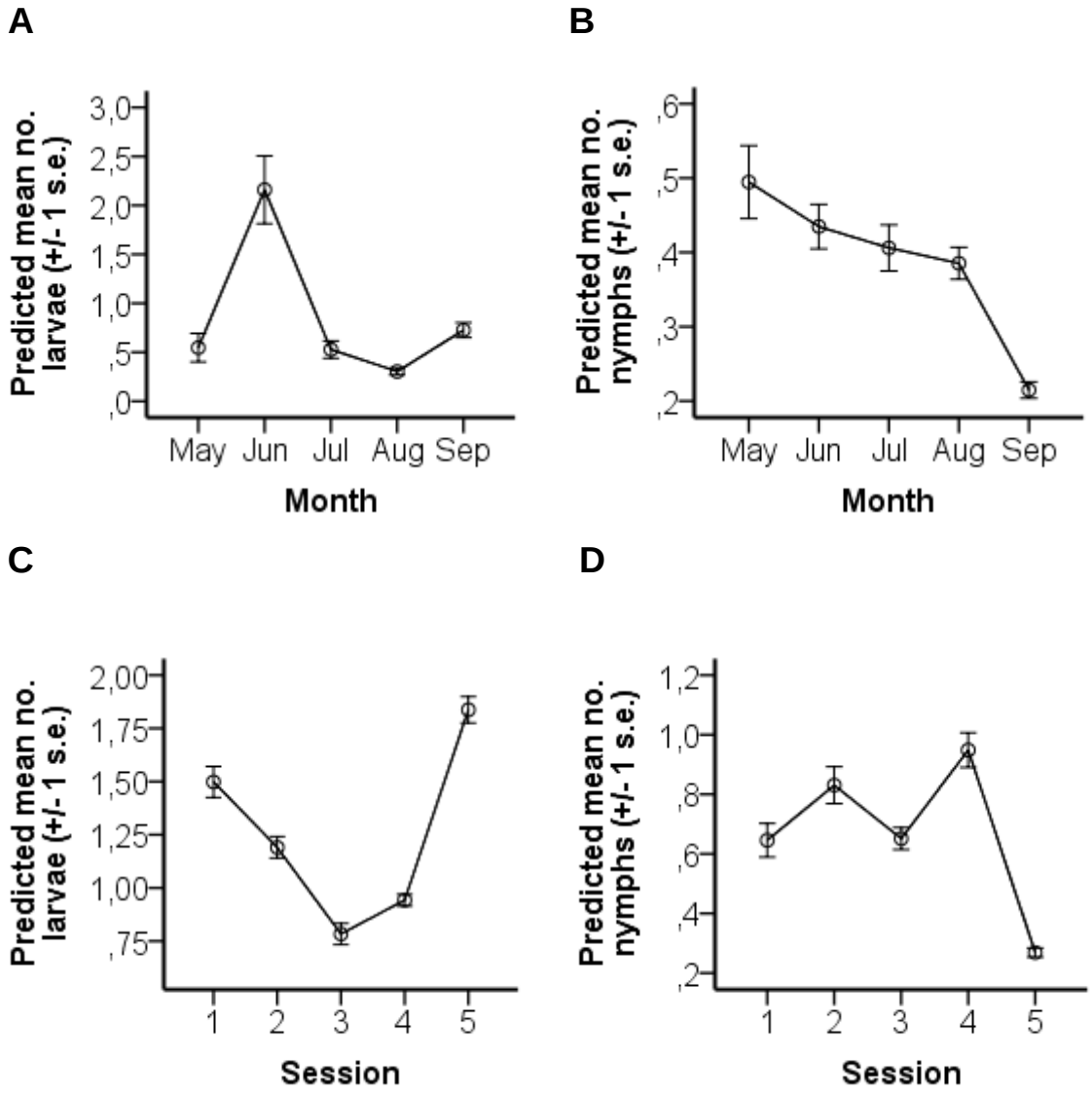

Figure 3. Predicted number (mean ± 1 standard error [s.e.]) of a) *Ixodes ricinus* larvae, b) *I. ricinus* nymphs, c) *I. trianguliceps* larvae and d) *I. trianguliceps* nymphs on bank voles in relation to the study session. (The number of voles for *I. ricinus* is 353 and for *I. trianguliceps* 555).

The numbers of feeding larvae and nymphs on the bank voles correlated positively among the same tick species for both *I. ricinus* (Pearson correlation: r = 0.473, n = 353, P < 0.001) and *I. trianguliceps* (r = 0.132, n = 353, P = 0.013), but different tick species did not interact (Table 4).

Table 4. Pearson correlations (correlation coefficient [r] and P-value) of the numbers of infesting *Ixodes trianguliceps* and *I. ricinus* (larvae and nymphs separately) on the bank voles. Significant P values are in italics. The number of bank voles is 353.

		I. tri nymphs	I. ric larvae	I. ric nymphs
I.tri larvae	r-value	0.132	0.025	-0.022
	P-value	*0.013*	0.634	0.686
I.tri nymphs	r-value		0.028	-0.017
	P-value		0.603	0.752
I.ric larvae	r-value			0.473
	P-value			*<0.001*

I. tri = *Ixodes trianguliceps*
I. ric = *Ixodes ricinus*

3.3. Questing *Ixodes ricinus*

Questing ticks that were collected from the vegetation were predominantly *I. ricinus* ticks. In total, 226 *I. ricinus* larvae, 247 nymphs, 46 females and 49 males were obtained. Two *I. trianguliceps* nymphs were collected with flags but they were not included in the analyses. Questing *I. ricinus* were obtained from the sites 1, 2, 4–9 and 12 (9 sites), of which *I. ricinus* was regularly caught only at sites 1, 2, 5 and 6.

Questing *I. ricinus* were observed during each month during the study period (May–September). Larvae and nymphs were common early summer (in May–June), after which their quantity reduced (Figure 4). The quantity of females was highest in August. As a whole the month did not influence on the total number of *I. ricinus* nymphs and females questing on the vegetation ($F = 2.001$, $df1 = 4$, $df2 = 82$, $P = 0.102$). However, as compared to September, more nymphs and females were collected in May ($P = 0.020$) and the result was almost positively significant also for June ($P = 0.054$) (Table 5).

Table 5. Model estimates (estimated coefficient and standard error [SE]), *t*-values and P values for final generalized linear mixed model (negative binomial) of the sum of questing *Ixodes ricinus* nymphs and females. Significant P values are in italics. The number of sample times is 87.

Variable	Coefficient	SE	*t*-value	P value
Intercept	-1.410	0.824	-1.711	0.091
Month				
May	1.898	0.800	2.372	*0.020*
June	1.537	0.785	1.959	0.054
July	0.468	0.825	0.567	0.572
August	0.707	0.813	0.869	0.387
September	0[a]			
Random effect:	Estimate	SE	Z-value	P-value
Var(Site)	2.707	1.630	1.661	0.097

0[a] = Set to zero because the parameter was as a point of comparison

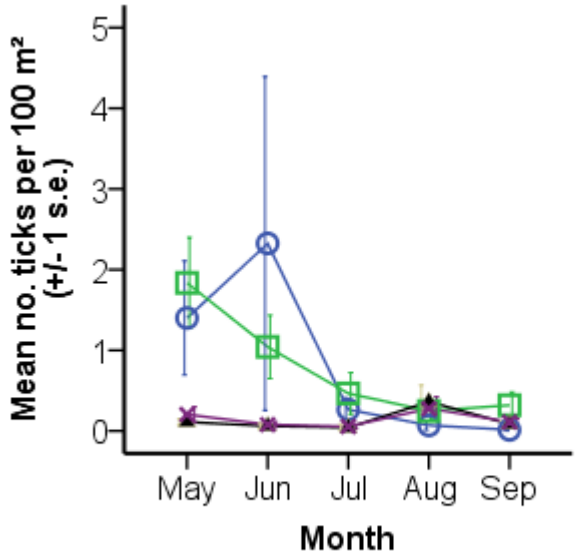

Figure 4. The seasonal patterns of mean numbers (± 1 standard error [s.e.]) of questing *Ixodes ricinus* larvae (○), nymphs (□), females (Δ) and males (X) per 100 m² between May and September, (n = 87).

3.4. The quantity of questing and parasitizing *I. ricinus*

The abundance of ticks feeding on bank voles was examined in relation to the abundance of ticks caught from the vegetation. The larval *I. ricinus* burden on the bank voles was positively associated with the number of questing *I. ricinus* larvae both per site (all sessions combined) and per site per month (Table 6). Thus, the higher abundance of larvae on the vegetation the higher number of feeding larvae. As to nymphs, also both associations were positive significantly, although the prediction per site per month was weak (Table 6).

Table 6. Linear regression models between the numbers of questing and feeding *Ixodes ricinus* larvae and nymphs per site and per site per month. Significant P values are in italics.

	Tick stage	Regression line	R^2	F value	df	P value
Site	Larvae	y = 0.463+0.051x	0.602	13.624	1, 9	*0.005*
	Nymphs	y = 0.007+0.019x	0.568	11.825	1, 9	*0.007*
Site*Month	Larvae	y = 0.429+0.235x	0.675	107.790	1, 52	*<0.001*
	Nymphs	y = 0.026+0.073x	0.169	10.581	1, 52	*0.002*

4. DISCUSSION

4.1. Summary of the main results

In Central Finland, bank voles are parasitized by two tick species, *Ixodes ricinus* and *I. trianguliceps*, while ticks collected from the vegetation are predominantly *I. ricinus* ticks.

I. ricinus was found from 11 out of 16 study sites, of which only at 4 sites *I. ricinus* was common both on the bank voles and on the vegetation. *I. trianguliceps*, instead, was discovered from voles at all 16 study sites.

The occurrence of ticks on the bank voles and on the vegetation varied depending to the study month between May and September. *I. ricinus* (both larvae and nymphs) were the most common in early summer (May–June). As to *I. trianguliceps*, larvae were the most common in early summer and in autumn, whereas nymphs occurred rather constantly before a clear crash in autumn.

I. trianguliceps parasitized 52 % and *I. ricinus* 31 % of the bank voles in the sites the ticks were found. The numbers of feeding ticks on bank voles were influenced by the location of the study site, month (= session) and the sex and age of the vole. Moreover, the abundance of bank voles and other small mammals captured at the site at the same month had an effect on the *I. trianguliceps* burden in bank vole individuals.

4.2. Tick infestation on the bank voles

This study supports previous findings that the bank vole is a host species for *I. ricinus* and *I. trianguliceps* ticks (e.g. Humair et al. 1993, Bown et al. 2003, Pérez et al. 2012). While all life stages (larva, nymph, female) of *I. trianguliceps* fed on bank voles, predominantly larval *I. ricinus* infested voles, as has been seen among small mammals in general (Humair et al. 1999, Randolph et al. 2002, Bown et al. 2006). The main explanation is the questing behaviour of *I. ricinus*: larvae quest low on the vegetation and thus, encounter small mammals that move in the litter layer (Mejlon & Jaenson 1997). Instead, only few *I. ricinus* nymphs were found on the bank voles even at sites where nymphs were rather common on the vegetation. It is probable that *I. ricinus* nymphs, questing higher on the vegetation than larvae (Mejlon & Jaenson 1997), mainly parasitized larger hosts, such as birds and squirrels (Craine et al. 1995, Olsén et al. 1995). Craine et al. (1995) found that the grey squirrel (*Sciurus carolinensis*) was a more important host for nymphs than small mammals throughout the year in Great Britain. In addition, for example hares (*Lepus* spp.) may carry a lot of *I. ricinus* nymphs (Tälleklint & Jaenson 1997), although during this study, hares were observed less frequently than squirrels. All the life-stages of *I. trianguliceps*, instead, were common on the bank voles. Proportionally *I. trianguliceps* nymphs parasitized even a bit more voles than *I. trianguliceps* larvae (33.4 % and 30.2 %). This could be due to longer feeding time of nymphs than that of larvae. In addition, *I. trianguliceps* females infested 6.5 % of the bank voles. These results are consistent with the ecology of *I. trianguliceps*, which lives in the nests and/or burrows of its small mammalian hosts and does not quest on the vegetation (Cotton & Watts 1967, Randolph 1975b).

I observed that vole individual characteristics had an effect to its tick burden. Firstly, I found that male bank voles had more *I. ricinus* larvae and nymphs than on females. In many previous studies male rodents have also been found to have higher burden of questing tick species compared to females (Ostfeld et al. 1996, Tälleklint & Jaenson 1997). Male voles and mice move longer distances than females (Kikkawa 1964, Ostfeld et al. 1996) and thus, they encounter more questing ticks. Furthermore, it has been suggested that high testosterone level of males, in addition to increasing locomotory activity, may reduce their resistance to ticks (Hughes & Randolph 2001). In addition to the sex, the age of the bank vole was significant, older voles having more *I. ricinus* larvae and nymphs than younger ones, which is likely explicable by the greater activity of older individuals (see also Tälleklint & Jaenson 1997, Bown et al. 2008). The number of larval *I. trianguliceps*,

however, was greater on young bank voles and decreased with age. Younger voles spend more time than adults in the nest or near it, which increases their likelihood collect nidicolous larvae. Furthermore, it may be that it is easier for larvae to grasp thinner skin of young voles, which could explain why the number of feeding *I. trianguliceps* nymphs did not depend on the age of the vole. Randolph (1975a) observed, however, that mature male wood mice (*Apodemus sylvaticus*) fed more larvae than immature males (see also Nilsson 1974). In addition, according to my results, male bank voles had more *I. trianguliceps* nymphs than female voles, which could reflect the greater activity of males also underground. Male bank voles have been found to be more infested with both *I. trianguliceps* larvae and nymphs than females in previous studies (Cotton & Watts 1967, Bown et al. 2003). In this study, the sex did not affect the larval burden, which could be explained by the same kind of behaviour of young male and female voles. Nevertheless, it must take into consideration that individual behavior depends simultaneously on many factors, such as age, sex, breeding status and available resources. Thus, more information is needed to evaluate precisely the key rodent individuals for tick and tick-borne pathogen dynamics.

The abundance of bank voles captured at the site at the same session had a positive influence on the number of feeding *I. trianguliceps* nymphs on the bank voles. Furthermore, the abundance of other small mammals influenced positively on the larval and nymphal *I. trianguliceps* burden on the bank voles. *I. trianguliceps* infests only small mammals and thus, it is presumable that the abundances of small mammals control mainly the abundance of *I. trianguliceps*. Small mammal abundance had been high year before (at 2011), at least for the bank vole (Metla 2012), and thus, plenty of nourishment for ticks. Therefore, it is probable that lots of next life-stages of *I. trianguliceps* occurred in the study year. Other captured small mammals were mostly shrews, which are significant hosts for *I. trianguliceps* (Bown et al. 2011). Bown et al. (2011) found that common shrews carried higher numbers of *I. trianguliceps* larvae than field voles and that the vast majority of nymphs infesting shrews were *I. trianguliceps* species. In addition, it must be taken into consideration that with higher rodent density individuals' home ranges tend to be smaller (Ostfeld et al. 1996) and it appears to decrease contacts with ticks and thus, their tick burden (Ostfeld et al. 1996, Kiffner et al. 2011). On the other hand, higher density of small mammals increases the success of individual ticks finding a host and thus, higher proportion of ticks may feed (Ostfeld et al. 1996).

4.3. Seasonal questing and feeding activity of *Ixodes ricinus*

I found that the month had an effect the occurrence of larval *I. ricinus* infesting bank voles and *I. ricinus* questing on the vegetation. The highest larval infestation burden on bank voles was in June. Often the population density of small mammals increases towards the end of the breeding season (late summer/autumn), as was observed also here, and the high host density might 'dilute' the larval infestation rate at individual host level in the late summer compared to the early summer (Ostfeld et al. 1996, Ostfeld & Keesing 2000a,b). In addition, the number of questing *I. ricinus* larvae predicted fairly well the larval *I. ricinus* burden on the bank voles, which supports the timing of the main larval activity period at the study year. *I. ricinus* larvae parasitize mainly small mammals (Milne 1949) and thus, it is likely that high larval abundance on the vegetation results in high larval infestation rate on small mammals. The blanket dragging might not have given, however, the entirely right picture on the amount of larvae on the vegetation, as larvae quest mainly close to the ground (Mejlon & Jaenson 1997). Therefore, their abundance could have been underestimated more when the vegetation became longer, the blanket not reaching the

lowest parts of the vegetation. Furthermore, there is a risk that larvae have been missed as they occur highly aggregated in the field (Ostfeld et al. 1996). Craine et al. (1995) observed questing *I. ricinus* larvae only from June to September even if larvae were present on hosts almost the whole year (see also Pérez et al. 2012). Thus, small mammals that move in the litter layer might be better indicator of larval seasonal abundance than flag dragging.

As to the feeding *I. ricinus* nymphs, their number did not vary with the month, although they were slightly more common during summer than in September. Furthermore, the questing *I. ricinus*, of which the greatest part was nymphs (only the sum of nymphs and females were analyzed), had the highest abundance in the early summer, after which the quantity reduced. I found that the numbers of *I. ricinus* nymphs on the vegetation predicted fairly well the numbers of nymphs on bank voles per site. However, when including also the month in the model the infestation level of the bank voles was less well predictable. This might be due to several reasons. Firstly, nymphs occur usually highly aggregated on few rodent individuals and because the bank vole abundance was very low in early summer, it may be that those individuals were not just captured. Secondly, it is possible that climatic conditions, which had not taken into account in this study, affected to the questing height of the nymphs (Randolph & Storey 1999). Consequently, the probability to pick up nymphs with the blanket and nymphs probability to become in contact with the bank voles may have varied during the study period. Randolph & Storey (1999) found, for instance, that when conditions became drier, the number of questing *I. ricinus* nymphs in lower layers of the vegetation increased and thus, their number on small rodents increased. On the other hand, in humid conditions nymphs may quest so high on vegetation that they rarely infest small mammals. In addition, under too dry conditions only few *I. ricinus* larvae quest and feed, possibly because of becoming quiescent (Randolph & Storey 1999). Thus, it would be important to include also temperature and humidity estimates to the study for understanding better *I. ricinus* seasonal dynamic patterns (e.g. Randolph & Storey 1999, Perret et al. 2000, Randolph et al. 2000, Randolph 2004b, Dobson et al. 2011). Climatic conditions were measured also during this study period but the data were too weak to be analyzed.

According to this study, it seems that *I. ricinus* larvae and nymphs both have the main activity time in early summer in Central Finland. In North and Central Europe the seasonal abundance peak of *I. ricinus* larvae has been found to be either unimodal, with a peak around May–July or August–September or bimodal with peaks around May–June and August–September, seasonality pattern varying even at the same area between years (Humair et al. 1993, Craine et al. 1995, Tälleklint & Jaenson 1997). Nymphs have been observed to have the activity peak in spring or early summer in North and Central Europe and small resurgence may occur in autumn (Perret et al. 2000, Walker 2001, Randolph et al. 2002). However, Craine et al. (1995), for instance, observed a rather constant peak of questing nymphs from June to October in Great Britain (see also Randolph et al. 2002 for larvae). The seasonal synchrony of larvae and nymphs is believed to be associated with a rapid fall in autumnal temperature why unfed larvae pass the winter in quiescence and become active with nymphs in the next spring (Randolph et al. 2000). Most tick-borne pathogens, including TBEV, *B. burgdorferi* s.l., *A. phagocytophilum* and *Ba. microti*, are transmitted between tick individuals through a rodent, i.e. an uninfected larva becomes infected while feeding on an infected rodent who has become infected while feeding an infected tick (typically a nymph) (Randolph et al. 1996). I found that the abundance of *I. ricinus* larvae on the bank voles correlated positively with the number of feeding *I. ricinus* nymphs, which could suggest that certain individuals harbour vast majority of ticks and thus, facilitate the transmission of tick-borne pathogens. For the circulation of short-lived

TBEV synchronous activity is essential: the uninfected larvae need to feed at the same time on the same rodent individual with an infected nymph (co-feeding) (Randolph et al. 1996, 2000). Similarly, Pérez et al. (2012) found that *I. ricinus* larvae that fed simultaneously with nymphs were more often infected with *B. burgdorferi* s.l. than larvae feeding without nymphs. However, with *B. burgdorferi* s.l. hosts can remain infected even months and thus, co-feeding is not as significant as for short-lived pathogens (Randolph et al. 1996). Although TBEV is not known to exist in Central Finland (Lindquist & Vapalahti 2008), the seasonal synchrony of immature tick stages could support its circulation in ticks in Jyväskylä area.

4.4. Seasonal feeding activity of *Ixodes trianguliceps*

Seasonal feeding activity of *I. trianguliceps* larvae on bank voles was bimodal with one peak in early summer, followed by a trough, and the other, higher peak in autumn. Similar seasonal pattern of larvae appears in previous studies on *I. trianguliceps* (e.g. Cotton & Watts 1967, Randolph 1975b, Bown et al. 2003). Usually rodent population density increases towards autumn, as in this study and, thus, the major feeding period of larvae coincides with high host abundance (Randolph 1975b). Furthermore, I observed that the number of nymphs on bank voles was quite stable during the whole summer, following by a rapid decline in autumn. Also studies carried out in Great Britain have shown similar pattern with the high abundance of nymphs in summer and a rapid autumnal decline (Cotton & Watts 1967, Randolph 1975b, Bown et al. 2003). As with *I. ricinus*, the coincident seasonal activity of *I. trianguliceps* instars is important for the transmission of tick-borne pathogens. The seasonal synchrony was observed here to be only partial, nymph activity reducing considerably during the main larval activity period (in autumn). However, it can be sufficient to maintain pathogens in a rodent-tick system, as Bown et al. (2003) observed with *A. phagocytophilum*. They discovered a high aggregation of ticks among rodents, which is likely to enhance the transmission of this short-lived pathogen. I also found that the abundance of *I. trianguliceps* larvae on the bank voles correlated positively with the number of feeding *I. trianguliceps* nymphs, which might suggest, similarly as with *I. ricinus*, that certain individuals harbour vast majority of ticks and thus, facilitate the transmission of tick-borne pathogens. *I. trianguliceps* has been discovered to be able to maintain pathogens, at least *A. phagocytophilum* and *Ba. microti*, among small mammal populations in the absence of *I. ricinus* (Bown et al. 2003, 2008). Thus, *I. trianguliceps*, which I found to parasitize bank voles more commonly than *I. ricinus*, may have an important role in the circulation of enzootic tick-borne infections also in Central Finland. In addition, around 9 % of the bank voles harboured both tick species. *I. ricinus* that infests various vertebrates, can act as a bridge-vector, i.e. transfer pathogens also to other host species, including humans (Bown et al. 2008).

4.5. Areal differences in the occurrence of ticks

I. trianguliceps was discovered from voles at all 16 study sites. *I. ricinus*, instead, was found at 11 study sites, of which at 4 sites (sites 1, 2, 5 and 6) it was commonly observed both on the vegetation and on the bank voles. Furthermore, I found that the numbers of feeding *I. ricinus* larvae and nymphs on the bank voles were influenced by the location of the site so that at urban sites voles were more infested than at non-urban sites. At most urban sites red squirrels (*S. vulgaris*) and European hares (*L. europaeus*), which are important hosts especially for *I. ricinus* nymphs (Craine et al. 1995, Tälleklint & Jaenson 1997), were observed and small mammals regularly trapped. Hence, the presence of other host animals might explain the presence and abundance of *I. ricinus* in an area. It must be

taken into account, however, that at few study sites *I. ricinus* was not found frequently (sites 3 and 10) or not at all (site 14) even though small mammals were abundant and squirrels and/or hares observed. It may be that ticks had just not spread to these sites or that the abundance of medium-sized mammals is not large enough to support tick populations. In addition, the significance of other host animals among the sites, such as birds (Olsén et al. 1995) and deer (Vor et al. 2010) that were not examined in this study, should be studied, too. Deer act as important hosts especially for *I. ricinus* females and the occurrence of deer may determine a lot *I. ricinus* distribution and abundance (e.g. Bown et al. 2008, Jaenson et al. 2012).

It is also possible that adverse climatic conditions explain partly the low quantity or absence of *I. ricinus* at some sites even though hosts are frequent. Although all the study sites were forest habitats, at the few sites at least one transect differed rather notably from the most typical habitat type and thus, possibly also their temperature and humidity conditions. In addition, the height of the vegetation might have affected the vertical distribution of ticks on the vegetation (Mejlon & Jaenson 1997). On lower and higher vegetation humidity and temperature conditions likely differ and thus, ticks have to quest at different heights because of differences in desiccation risk (Randolph & Storey 1999). Consequently, the blanket may have caught ticks differently from different forest habitat types due to tick behaviour but also vegetation structure itself, i.e. reaching ticks more efficiently from low vegetation. Therefore, in further studies these perspectives should be taken into account as well. It appears, nevertheless, that urban sites in Central Finland may be potential high risk areas for humans because of the generality of *I. ricinus* and the presence of *B. burgdorferi* s.l., with ~35 % infection prevalence among questing *I. ricinus* nymphs (unpublished data, see also Junttila et al. 1999).

The location of the site influenced the numbers of *I. trianguliceps* nymphs feeding on the bank voles so that at non-urban sites voles were more infested than at urban sites. As *I. trianguliceps* parasitizes only small mammals (Cotton & Watts 1967), it is likely that differences in the community composition of small mammals and in their abundances between the sites affect the infestation load of the bank voles (see also part 4.2.). It might also be that at urban sites, where *I. ricinus* occurs more commonly than at non-urban sites, the amount of *I. trianguliceps* is reduced through the competition with *I. ricinus*.

4.6. Conclusions

In this study, tick dynamics on vegetation and on rodents were explored for the first time in Finland. Based on these results, humans have the greatest risk to become in contact with ticks in early summer when questing *I. ricinus* are the most common on the vegetation. Nymphs especially are seen as a great threat because of the small size and thus, more difficult to perceive on the skin than adult ticks. In Finland humans appear, however, to visit forest areas more often in the mid-summer and autumn than in early summer, for example because of the berry picking and hunting, which can reduce contacts with ticks. It is possible that female *I. ricinus* are common late summer (Figure 4) but their quantity is lower than that of nymphs. My results suggest that due to the seasonal synchrony of *I. ricinus* larvae and nymphs, the environmental conditions might be favourable to maintain TBE also in Central Finland. However, the spread of the virus from the focal TBE-areas may be unlikely because an infected nymph, who has encountered the infection while feeding on an infectious rodent as larva, should feed for example on a bird to be moved long distance. After this, it feeds as adult and thus, unlikely on a rodent through which transmission could happen. It must also be taken into account, however, that this study encompassed May–September during one year only. Furthermore, the climatic conditions

in Finland vary a lot between the seasons. Moreover, the TBE persistence is also dependent on other factors, such as tick abundance, which might be lower even in the high *I. ricinus* areas in Jyväskylä than in areas TBE is endemic. Thus, the long-term data are needed for understanding better the seasonal activity pattern of *I. ricinus*.

Certain sites appear, according to this study, to be clearly risk areas for humans with the high abundance of *I. ricinus* ticks. These sites were urban forests near settlements and recreation areas, whereas in non-urban areas only few *I. ricinus* were found. The grasses near waterways are usually viewed as good tick habitats in Finland. In this study, however, all the study sites were woodlands chosen to represent favourable habitats for the bank voles and, it seems that these areas may pose as well a high tick infestation risk for humans and companion animals (dogs and cats).

Bank vole is a very abundant rodent species in Finland and was, in this study, found to be a host for both larval and nymphal *I. ricinus*. Therefore, being a competent reservoir species, bank voles may support endemic cycles of tick-borne pathogens also in Finland. In addition, I found that bank voles harboured all life-stages of the *I. trianguliceps*, which may alone maintain pathogen cycles. Because at some sites both *I. trianguliceps* and *I. ricinus* co-occurred, enzootic infections maintained in a rodent-*I. trianguliceps* cycle could escape into other host species, including humans, via *I. ricinus*. It seems that certain bank voles may harbour the vast majority of ticks. The factors causing aggregation are very essential elements to try to understand and, possibly to control the transmission of tick-borne pathogens.

In Finland the bank vole, as well as some other rodent species, have a clear cyclic population dynamics. The last abundance peak of the bank vole in South and Central Finland, although locally modest, occurred in the autumn 2011 and in the study year (2012) populations were low but recovered slightly during the summer (Metla 2012). Therefore, there might have been more hosts available for larvae in 2011 and thus, larvae and nymphs were common in the study year 2012. Because tick abundances highly depend on rodent densities, the long-term data would be essential to estimate the effect of rodent population fluctuations on the tick abundance and to predict years that may pose a high risk for humans to become in contact with ticks.

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APPENDIX 1

The study sites located in Jyväskylä, Central Finland.

Maps' background: © Maanmittauslaitos