Host-seeking Activity of *Ixodes ricinus* in Relation to the Epidemiology of Lyme Borreliosis in Sweden

BY

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Abstract
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This thesis examines seasonal, diel and vertical distribution patterns of activity of host-seeking *Ixodes ricinus* (L.) ticks at three localities in south-central Sweden. In addition, by examining the prevalence of infection in ticks with Lyme borreliosis (LB) spirochetes, *Borrelia burgdorferi* s.l, information for estimating relative LB risk in humans and the effect of control measures directed against this tick vector is provided.

The seasonal activity pattern of *I. ricinus* was, in general, bimodal with peaks of activity in May-June and August-September. Tick densities were generally high at Torö and low at Kungshamn-Morga. The greatest variation in tick density occurred at the sample site level, which indicated a patchy distribution of ticks. The diel activity of adult *I. ricinus* sampled at Bogesund showed a distinct nocturnal activity peak while nymphal ticks exhibited no particular diel variation. At the meadow site, there was a strong negative association between activity of each tick stage and ambient air temperature, and larval ticks also showed a nocturnal activity peak. *I. ricinus* of all stages were present in the vegetation up to at least 140 cm above ground level. At Torö, host-seeking larvae were found at significantly lower levels (below 20 cm) in the vegetation compared to nymphs and adults (50-59 and 60-79 cm, respectively). Vegetation structure is likely to be the main factor governing tick vertical distribution at this locality. The northern limit of the geographical distribution of *I. ricinus* in Sweden corresponds with the southern boundary of the taiga zone, as well as with several other climatic or vegetational isoclines primarily associated with the vegetation period.

The prevalence rates of *Borrelia* spirochetes, recorded by phase-contrast microscopy in host-seeking *I. ricinus*, were 0% in larvae, 5.8-13.1% in nymphs and 14.5-28.6% in adult ticks. The human LB risk, estimated by the number of *Borrelia*-infected nymphs per hectare, was greater at Torö than at Kungshamn-Morga and greater in woodland than in open areas. The risk also possessed a bimodal seasonal pattern similar to that of subadult host-seeking activity. Controlling the number of infected nymphs through de-ticking of reservoir hosts seems not to be an effective control measure in Sweden due to the ubiquitous availability of alternative reservoir hosts.

*Key words: Borrelia burgdorferi*, control, *Ixodes ricinus*, Lyme borreliosis, seasonality.

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Preface

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


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   - (Manuscript to be submitted for publication in *Journal of Medical Entomology*)


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Man förlorar bara en massa tid på att vara punktlig.

Storm P
INTRODUCTION

Ticks continue to earn great attention world-wide despite their relatively low number of species. The main reason for this is related to their role as obligate blood-feeders, parasitizing man and animals. Apart from the negative effects of blood loss, allergies and toxicoses, a vast number of different infectious agents can be transmitted between ticks and their hosts. The variety of these agents is unmatched even by mosquitoes. Some recently discovered diseases of humans seem to have increased to nearly epidemic proportions, e.g. Lyme disease/ Lyme borreliosis (LB\(^1\); Steere et al. 1977) and ticks also severely limit livestock production in many areas of the world. Therefore, it is of great importance to identify the biological factors responsible for the transmission and maintenance of these diseases if meaningful control measures are to be developed.

Systematics and general biology of ticks

The vast majority of all chelicerate arthropods belong to the class Arachnida. The subdivisions of this class include such wellknown groups as scorpions, spiders, harvestmen, mites and pseudoscorpions. The major subclass Acari (mites), is commonly referred to as “mites and ticks”. This is however inconsistent, because of the different levels of classification: all ticks are mites but very few mites are ticks!

The world fauna of ticks (Ixodida) comprises ~850 described species in three families (Sonenshine 1991). The hard ticks (Ixodidae) are the most important family, both with respect to number of species and to veterinary and medical importance. Soft ticks (Argasidae) – which lack the sclerotized scutal shield present in hard ticks, hence their name – have a predominantly tropical or subtropical distribution and are also of some veterinary and medical importance. The third family (Nutalliellidae) comprises only one single species and shares morphological traits with both Argasidae and Ixodidae (Sonenshine 1991).

All ticks have at least four developmental stages: egg, 6-legged larva, 8-legged nymph and adult. In hard ticks there is only a single nymphal instar whereas soft ticks and other members of the Acari possess several (often 3-4). No sexually differentiating characters are present until the adult stage. Soft ticks are usually associated with xeric environments, a concealed, nidicolous behaviour and short feeding periods on multiple hosts (Sonenshine

\(^1\) throughout this thesis, LB will be used as a collective term for both expressions and makes no distinction between the two
1991). Hard ticks, on the other hand, usually occur in more humid environments, quest freely on the vegetation, and feed only one time in each active developmental stage on either one, two or three different hosts. During this prolonged period of blood-feeding in hard ticks (several days), new cuticle is synthesised as to accommodate for the great expansion of body surface (Balashov 1972); in feeding females, the body weight can increase more than 100-fold (Sonenshine 1991). In adult males however, the sclerotized scutal plate that covers the entire dorsal surface restricts any major increase of surface area. The life cycle of an individual tick is completed during a rather extended period of time, ~3 years in the case of *Ixodes ricinus* (L.) in Northern Europe (Lees & Milne 1951; Gray 1991).

Ticks can be found parasitizing virtually any terrestrial vertebrate, and have been found on a number of reptiles and amphibians. Ticks do not primarily detect their host by vision (at least in the genus *Ixodes* which lacks eyes), but rather through sensillae or receptors responding to mechanical, chemical, heat radiative or auditory stimuli (Waladde & Rice 1982). Generally, mating takes place on the host (in Argasidae and most Ixodidae) or off the host (in most *Ixodes* species) prior to the female’s bloodmeal (Sonenshine 1991); *I. ricinus* has been confirmed to mate on- as well as off-host (Gray 1987). Sperm transfer is accomplished via a spermatophore (Oliver 1982). As argasid females mate and feed multiple times, they also go through multiple gonotrophic cycles and thus lay multiple batches of fewer eggs compared to ixodid females. The latter have a single gonotrophic cycle ending by oviposition in ground litter in a single batch of several thousand eggs (Sonenshine 1991).

The *Ixodes ricinus* species complex

In temperate regions of the Northern hemisphere, the four members of the *I. ricinus* complex are among the most wellknown tick species and those directly involved in the transmission of LB (Sonenshine 1993). The complex consists of two Eurasian species with the following geographic distribution: *I. persulcatus* Schulze from Eastern Europe to Japan and *I. ricinus* from Western Europe to Northern Africa. From Poland, the Baltic countries and Eastern Finland and eastwards to Kazakstan both species are present (Sonenshine 1993), but seem to be associated with different biotopes; *I. persulcatus* is less sensitive to desiccation compared to *I. ricinus* and is mainly found in the drier taiga forest environment while the latter species mainly occurs in areas with denser ground vegetation. In the Nearctic, the complex is
represented by *I. scapularis* Say in Eastern America and *I. pacificus* Cooley & Kohls in Western America. There is no overlap in the geographical distribution of these species.

*Life of I. ricinus in Sweden*

Due to the extended longevity of this tick, a considerable amount of time is spent off the host, where the environment imposes a varying degree of threat to the survival of individual ticks. As for several other animals and plants in Sweden, restraints caused by climate become evident as the vegetation period gets shorter to the north. At some point this combined effect of increasing latitude/altitude will prohibit completion of the tick’s life-cycle. This is one of the reasons why *I. ricinus* is absent from the mountain regions in northern Sweden (Jaenson et al. 1994; IV). The effect of altitude on climate and thereby on tick occurrence was confirmed in mountain regions of central Europe. In Bohemia, *I. ricinus* was not able to complete its life-cycle in areas exceeding 700 m a.s.l. (Daniel 1993).

The likewise universal problem of terrestrial arthropods in preventing dehydration also plays an important role for the smaller scale distribution and survival of *I. ricinus*. Although this species has the ability to extract water from a subsaturated atmosphere (i.e, 80-96% relative humidity [R.H.]; Gray 1991; Knülle & Rudolph 1982), the physiological stress of such an activity will reduce the life-span and decrease the chances of finding a host (Daniel 1993). The desiccation tolerance is generally smaller in the larval stage and greater in the adult (Knülle & Rudolph 1982). Throughout its distributional area, *I. ricinus* seems to be associated mainly with woodland areas on richer soils. Such areas will generally have a buffering effect on microclimate with less fluctuations in temperature and R.H, thus enabling ticks to restore their water balance within the well-developed ground vegetation where the content of atmospheric water is usually high (Lees 1948). Additionally, the number of tick hosts is also generally higher in such areas and, obviously, so is the number of potential tick-host contacts.

Only part of the time spent off the host is actually used for active host-seeking behaviour (i.e, questing; Lees & Milne 1951). The main part is allocated for other reasons, such as restoring the water balance, behavioural and morphogenetic diapause or egg-laying (in the case of an adult female). Actively host-seeking ticks climb the vegetation and usually come to rest at the tips of vegetational parts (Lees 1948). After contact with a suitable host, the tick can spend considerable time in search for an acceptable attachment site which is usually located on the host’s extremities or head region (Nilsson 1981). After completion of
the feeding process (from 3-5 days in larval to 7-11 days in adult female ticks; Balashov 1972) the tick detaches and drops to the ground. There is only one blood-meal in each active developmental stage, i.e, as a larva, nymph and adult female tick, respectively. The male does not require blood as an adult (Balashov 1972), but spends the time in search of females. Generally, if the blood-meal is completed in spring or summer, subadult ticks will moult and eggs from fed females will hatch within the next one to three months whereas ticks fed in late summer/autumn will moult the following year due to diapause behaviour (Daniel et al. 1976, 1977; Gray 1982, 1991) induced by the changing photoperiod (Belozerov 1982). The threshold temperature above which activity commences is ~5°C for I. ricinus nymphs and adults (Gray 1984). This temperature is - by meteorological definition - coincident with the onset of spring and the ending of autumn, respectively. Thus, in south-central Sweden, ticks are generally active from March-April to October-November.

**Tick-host relations and LB**

*Borrelia burgdorferi* sensu lato (s.l.) is a complex of (so far) 10 different species/genomic groups of spirochetal bacteria. Three of these, i.e, *B. burgdorferi* sensu stricto (s.s.), *B. garinii* and *B. afzelii*, are implicated as causative agents of LB and each is associated with both different clinical manifestations of the disease and with different tick-host systems (Baranton et al. 1992; Marconi & Garon 1992; Gern & Falco 2000). The significance of the remaining species is unknown, i.e, *B. andersonii* and *B. bissettii* in USA (Marconi et al. 1995; Postic et al. 1998), *B. lusitaniae* and *B. valaisiana* in Europe (Le Fleche et al. 1997; Wang et al. 1997), and *B. japonica*, *B. tanukii* and *B. turdae* in Japan (Fukunaga et al. 1996; Kawabata et al. 1993). The distribution of *B. burgdorferi* s.l. is probably world-wide, but so far verified clinical cases have only been reported from the northern hemisphere, where it is in fact the most common vector-borne disease. In Sweden, 5,000-10,000 cases are estimated to occur each year (Berglund et al. 1995; Tälleklint 1996b). LB is multi-systemic, and affects both skin, heart, joints and the nervous system. The cardinal sign of the disease is erythema migrans (EM), a gradually expanding rash (caused by migrating spirochetes) with – often – subsequent fading in the centre. EM appears on the site of a tick bite some days or weeks after infection (Gern & Falco 2000). In those cases where EM is absent, which is more common in Europe compared to North America, the infection might go unnoticed. However, for diagnosed cases the prognosis is very good, especially if treatment with antibiotics is commenced during the acute phase. Certain domestic animals such as dogs and horses have
also been shown to suffer from manifestations of LB (May et al. 1991). In these cases, problems have mainly been joint-associated. In Sweden, infection is typically caused by nymphal ticks which is the most abundant of the infective stages (since adult female ticks are less abundant and also probably more easily detected and removed).

Although pronounced host-specificity is common within the genus *Ixodes*, all members of the *I. ricinus* complex can be considered “opportunistic” and can potentially use a broad spectrum of host species. The number of host species recorded for *I. ricinus* and *I. persulcatus* total 317 and 241, respectively (Anderson 1991) and for *I. scapularis* nearly 100 (Anderson 1988) rendering these species potentially powerful vectors of disease. Regarding LB, *Borrelia* spirochetes are maintained within several systems of ticks and a certain segment of their hosts capable of acquiring, maintaining and transmitting them, i.e, reservoir hosts. The interchange between these systems is important for understanding the epidemiology of the disease, and apart from the human-biting members of the *I. ricinus* complex, several additional tick species may be of some importance: i) One system, present in both hemispheres, involves *I. uriae* White and several colony-nesting seabirds which have been shown capable of maintaining as well as transmitting spirochetes (i.e, *B. garinii*; Olsén et al. 1993, 1995a). Humans do not generally come into contact with this tick, but *I. uriae* will occasionally bite humans if given the opportunity. ii) A species that readily bite humans and occurs together with *I. ricinus* on Islands in the Baltic Sea is *Haemaphysalis punctata* Canestrini & Fanzago. Spirochetes have been detected in a small (2%) proportion of nymphs of this species (Tälleklint 1996a), but their ability to transmit spirochetes further remains unknown. iii) Ticks that do not bite humans, such as *I. hexagonus* Leach, *I. canisuga* Johnston, *I. trianguliceps* Birula and some additional species, have been shown to harbour *B. burgdorferi* s.l in Europe (Akimov & Nebogatkin 1995; Estrada-Peña et al. 1995; Gorelova et al. 1996). However, as for *H. punctata*, it is not clear whether these ticks also manage to transmit spirochetes to hosts.

In Western Europe, the systems involving *I. ricinus* and its hosts are well studied and presently major contributors to the overall enzootic circulation of LB spirochetes. The reservoir hosts are found among small and medium-sized mammals and occasionally birds. In the UK, grey squirrel (*Sciurus carolinensis* Gmelin) and pheasant (*Phasianus colchicus* L.) seem to be the major reservoirs in some areas (Craine et al. 1997), while red squirrel (*S. vulgaris* L.) is suspected to play a significant role in some areas in Switzerland (Humair & Gern 1998). However in many studies, shrews (*Sorex* spp, *Neomys fodiens* Pennant), voles (*Clethrionomys glareolus* [Schreber], *Microtus agrestis* [L.]), mice (*Apodemus* spp.), hares
(Lepus spp.) and possibly passerine birds (Turdus spp., Erithacus sp.) dominate the mainland area (Gern et al. 1998; Hovmark et al. 1988; Humair et al. 1993; Jaenson & Tälleklint 1996, 1997; Kurtenbach et al. 1995; Matuschka et al. 1990; Olsén et al. 1995b; Tälleklint 1996b). The competency of a certain reservoir host is a function of its density, tick load and infectivity to feeding tick larvae. With reference to a study area in Sweden, density of the common shrew (Sorex araneus L.) and bank vole (C. glareolus) seemed to be the most important factor (Tälleklint et al. 1993). However, immune responses directed against feeding ticks or invading spirochetes are additional factors regulating host competency. For instance, acquired resistance against feeding ticks has been shown to develop in bank voles (Dizij & Kurtenbach 1995), thereby reducing the number of successfully engorged larvae. Many of the primary reservoir hosts, i.e., shrews and rodents, do not pass spirochetes to its progeny and also have a shorter life-span compared with I. ricinus. In order to maintain the infection over time, this means that each generation of hosts have to be reinfected by nymphal ticks. Thus, spirochete-containing nymphs themselves actually become the primary overwintering reservoirs for B. burgdorferi s.l. (Tälleklint & Jaenson 1995).

Although small mammals are usually the main hosts for I. ricinus larvae and a small proportion of nymphs, they do not support feeding by any adult female ticks or by large numbers of nymphs. Instead, these ticks feed on larger host animals such as hares and roe deer (Capreolus capreolus L.). The segment of tick hosts that can support feeding by female ticks is defined as maintenance hosts, and will indirectly determine the production of new larvae through blood-fed females. These larger hosts are also fed upon by larval ticks, and can by themselves support continuous populations of I. ricinus. This was shown to occur on islands in the Baltic Sea where hares were the only tick hosts present, but did nevertheless support both the tick population as well as LB spirochetes (Jaenson & Tälleklint 1996). Areas where the roe deer is the only mammal present are unlikely, but could, at least in theory support tick populations over time. However, roe deer, and cervids in general [e.g, moose (Alces alces L.), red deer (Cervus elaphus L.), white-tailed deer (Odocoileus virginianus [Zimmerman]), sika (Cervus nippon Temminck) and fallow deer (Cervus dama [L.]), have all been shown incapable of transferring LB spirochetes to feeding ticks (Gray et al. 1992, 1996; Jaenson & Tälleklint 1992; Telford III et al. 1988). Therefore, these hosts are not regarded as reservoirs in any tick-host system. On the contrary, in addition to the “diluting” effect on nymphal spirochete prevalence in areas with an overrepresentation of cervids (Gray et al. 1992), even pre-existing spirochetal infection in I. ricinus nymphs has occasionally been shown to vanish after feeding on cervid hosts (Matuschka et al. 1993).
Other pathogenic agents and diseases transmitted by I. ricinus

In Sweden, the viral disease Tick-Borne Encephalitis (TBE) has a yearly incidence of about 50 to 80 human cases (Gustafson 1994). The main reservoirs for the TBE virus are probably small mammals and the distribution seems restricted to certain areas around Lake Mälaren and to some islands in the Baltic Sea. However, the recent changes in the general climate of Sweden may affect this distribution (Lindgren 2000; Lindgren et al. 2000).

Besides LB, several other diseases caused by bacterial agents are known from Sweden. Among pathogens belonging to the spotted-fever group, Rickettsia helvetica (previously described from Switzerland, see Rehacek 1993) have been isolated from Swedish I. ricinus. This pathogen is present in ~20% of nymphal and adult ticks, and could possibly be involved in sudden cardiac failure under certain conditions (Nilsson et al. 1999a, 1999b). Another rickettsia of the genus Ehrlichia cause granulocytic ehrlichiosis in cattle, sheep, dogs and horses. Monocytic ehrlichiosis and human granulocytic ehrlichiosis (HGE) are human diseases transmitted by ticks in Northern America. The HGE agent is probably also transmitted by I. ricinus in Sweden. Another rickettsia (Coxiella burnetii) causes Q-fever in both livestock and humans. In Sweden, this disease occurs on the Island of Gotland and competent reservoirs are found among birds, small mammals and reptiles. In addition to tick bites, the disease agent can also be transmitted via air-borne dust particles associated with tick faeces or infected livestock. Tularaemia is a zoonotic disease among hares and rodents caused by Francisella tularensis. Several routes of infection exist, usually via the bite of a blood-sucking arthropod but also by direct contact, aerial and oral ingestion of infectious material.

The protozoan haemoparasite Babesia divergens usually infects cattle in Sweden, but severe disease in splenectomized humans have also occurred.

Control of tick-borne diseases

Since the discovery in 1982 of I. scapularis ticks acting as vectors of human LB in Northern America (Burgdorfer et al. 1982), research directed at controlling the transmission of spirochetes at the tick-host interface has been intensified. Traditionally, methods aimed at controlling the spread of disease in livestock have primarily included treating the animals with acaricide (Amoo et al. 1993; Hardeng et al. 1992). For wild animals, control by host-targeted applications of acaricide, e.g. permethrin-impregnated cottonballs used as rodent nesting material (Deblinger & Rimmer 1991; Mather et al. 1988) or acaricide automatically applied to
deer visiting food stations (Sonenshine et al. 1996), reduced tick numbers in some cases but
were less effective in others (Daniels et al. 1991; Leprince & Lane 1996; Stafford III 1991b,
1992; V). Acaricidal, insecticidal or desiccating compounds have also been used for large
scale application in the natural habitats of vector tick populations, where several studies show
at least a short-term decrease in tick numbers (Allan & Patrician 1995; Monsen et al. 1999;
Schulze et al. 1991, 1992; Solberg et al. 1992; Stafford III 1991a). On a local scale however,
indirect control of ticks such as habitat modification (e.g. burning, mowing or grazing by
livestock; Bloemer et al. 1990; Fourie et al. 1996; Mather et al. 1993; Schulze et al. 1988)
and restriction of host access by fencing (Bloemer et al. 1990; Daniels et al. 1993; Gray et al.
1992; Stafford III 1993) reduced tick numbers considerably but did not always result in
decreased LB risk. In fact, the efficiency of such control measures are probably highly
dependent on the local tick-host system present. Potential candidates for biological control of
ticks are scarce. The most studied of ixodid parasitoids is the encyrtid wasp Ixodiphagus
hookeri (Howard), associated with several Ixodes species including I. ricinus (Mwangi et al.
1991). As a parasitoid of I. scapularis however, this wasp seems only to be associated with
extremely dense tick populations (Hu et al. 1993; Mather et al. 1987; Stafford III et al. 1996).

Aims of this thesis

In order to better understand how pathogens of humans transmitted by I. ricinus in Sweden
are maintained and how such microparasites circulate (in this case LB) it is crucial to
investigate in natural environments several ecological parameters of this important tick
species, particularly:

a) What is the density range of I. ricinus ticks generally encountered in a south-central
   Swedish locality, and how does the density of the tick population vary temporally and
geographically?

b) What parameters can be used to describe these variations in tick density and what factors
   may be most important in limiting the northern geographical distribution of I. ricinus on a
   macro-geographical scale?

c) How prevalent is Borrelia burgdorferi s.l. in I. ricinus in habitats where this tick has
   become established? How can the risk for human LB be assessed based on such data?

d) What are the main characteristics of high-risk areas for LB in Sweden and can host-
targeted de-ticking by acaricide reduce this risk?
MATERIALS AND METHODS

Study areas

The majority of the field-work consisted of tick sampling, i.e., quantifying the number of actively host-seeking *I. ricinus* present in a natural environment. This was performed in 1988 and 1989 at the Kungshamn-Morga (KM) nature reserve just south of Uppsala (III; Fig. 1), generally once monthly from April/May to September/October, but in 1989 however, sampling was made twice monthly from March to October. Additionally, tick samplings were conducted at Röskär, Bogesund – a peninsula in Lake Mälaren 10 km south of Stockholm – during 1991 to 1993 (I; Fig. 1), and during 1995 (II), respectively. In parallel with the tick samplings at KM in 1988 and 1989, samplings were also made at Torö, an Island of 17 km² situated 57 km south of Stockholm (III; Fig. 1). In 1990 and 1991, trapping of small mammals was also performed at this location (V). Data from tick samplings at three additional localities situated in south-western Sweden were used in paper II (sampled in 1995) and in paper IV (sampled in 1992-95): Änggårdsbergen (City of Gothenburg), Dagsås, situated in the central part of the province of Halland approximately 10 km from the coast and Hallands Väderö, an island three km outside the north-western corner of the province of Skåne.

The vegetation at the sample sites at Torö was mainly mixed coniferous/deciduous woodland. In 1988 and 1989 (III), four rectangular sites of approximately 1500 m² each were sampled. Two of these sites were located in woodland dominated by spruce (*Picea abies* [L.]), and with scattered birch (*Betula pubescens* Ehrh.), aspen (*Populus tremula* L.) and alder (*Alnus glutinosa* [L.]) trees. The ground layer at these sites was mostly dense, and occasionally reached considerable heights where stinging nettle (*Urtica dioica* L.) or loosestrife (*Lysimachia vulgaris* L.) occurred. The remaining two sites were located in an area adjacent to the sea shore and were dominated by alder, reed (*Phragmites australis* [Cav.]),

![Fig. 1. Map of Southern Sweden showing three main study localities (Kungshamn-Morga, Bogesund and Torö) and three additional reference localities (Änggårdsbergen, Dagsås and Hallands Väderö) where I. ricinus ticks were sampled.](image-url)
raspberry (*Rubus idaeus* L.) and stinging nettle. In 1990 and 1991 (IV, V), four circular sampling sites of 1 ha each were sampled. All of these sites were located in the same type of woodland area as described above and were separated with at most 500 m. In 1992 and 1993 sampling was performed in a clearing made for electrical power lines, a narrow transect flanked by mixed spruce/deciduous woodland. The vegetation here consisted of young alder and birch trees, and a high and dense layer of grasses and ferns (II, 'high' vegetation). At KM (III), three biotopes constituting two sites each were sampled: 1) a marshy pasture with scattered aspen trees and low ground vegetation due to grazing by cattle and horses 2) blueberry-spruce forest with less developed ground vegetation and 3) mixed deciduous/coniferous woodland along the north-eastern shore of Lake Ekoln. At the Bogesund locality two rectangular sites of 480 m² each were sampled in 1991-1993 (I) and 1995 (II, 'low' vegetation). One approximately 40-year old pine (*Pinus sylvestris* L.) plantation with a medium-dense ground layer, and an open meadow, respectively. The sampling sessions at Dagsås, Änggårdsbergen and Hallands Väderö in 1992 to 1995 (II, IV) were all performed in broad-leaf (mainly *Fagus sylvatica* L.) forest areas with sparse ground cover.

Most of the primary tick hosts in Sweden, i.e., shrews (*Sorex* spp.), rodents (*C. glareolus, Apodemus* spp.), hares and roe deer are naturally occurring within all areas sampled. Generally, however, quantitative data on host densities for the particular areas have not been available, especially concerning the small and medium-sized mammals. One exception is the Bogesund locality where the population dynamics of roe deer has been extensively studied. This area harboured an extremely dense population of deer, in the autumn of 1992 it reached 0.36 animals/ha (courtesy of Mr. P. Kjellander, Grimsö wildlife research station 1999). According to hunting statistics, a similarly high density (0.3-0.4 animals/ha) of roe deer is suspected to have occurred at Torö during the period 1988 to 1992. The roe deer density at KM at this time was, however unsubstantiated, presumably lower.

**Sampling methods**

The sampling procedure generally adopted (papers I, III, IV, V) was to use blanket-dragging to obtain the number of actively host-seeking *I. ricinus* per unit area, that is, those ticks that attached to a 1.5 m² white, woollen flannel cloth dragged over the ground vegetation and checked every 10 to 20 m. In paper II the same material was used in a dress at ordinary walking pace to obtain a measure of the vertical distribution of host-seeking ticks. In general, the effective area sampled at each sampling occasion ranged from 50 to 250 m², depending on
which study was performed and on the density – and the subsequent time-consuming counting – of host-seeking *I. ricinus* larvae. Sampling was made during daylight hours, mainly between 10 a.m. and 6 p.m. (DST), but was avoided shortly after or during rainfall. In paper I however, *I. ricinus* was sampled at intervals of 1 month, each time during 24 hours. Trapping of small mammals (V) was accomplished using the rodent live trap “Ugglan special”. The traps were baited with apple, carrot and oat cereals and set for one night on each occasion.

![Sampling by blanket-dragging at Bogesund, 1993.](image)

*Fig. 2. Sampling by blanket-dragging at Bogesund, 1993. Photo: L. Tälleklint*

**Detection and identification of the study organisms**

The species status of all ticks brought back to the laboratory was noted before dissection. Those specimens counted but left in the field were still regarded as *I. ricinus* since similar tick species are host-specific and tend only to occur in the nests or burrows of their particular host. Ticks were put in tubes with moistened tissue paper and kept at +4°C. Dissection was performed within six weeks of collection.
The initial method for detecting *Borrelia* spirochetes in collected ticks was by phase-contrast microscopy at 400-500X magnification. The body content of each tick was put into a drop of phosphate-buffered saline on a glass slide and screened for spirochetes for 10 min. By this technique, spirochetes are readily visible and can also be quantified for each individual tick specimen. Quantitative data of this kind was obtained for ticks collected in 1988 and 1989 (unpublished data), but not for those collected in 1990-1992. The slides, with cover slip removed, were then air-dried, fixed in ice-cold acetone and stored at -20°C. Subsequent examination of a subset of the stored slides with indirect immunofluorescence assay (using monoclonal antibody H6831 directed at the outer surface protein B, ospB, of *B. burgdorferi*) revealed a small fraction of these slides as positive (III). *B. afzelii* was later confirmed by PCR from ticks collected at Torö (IV).

The poor result of the immunofluorescence test can be explained by the more recent discovery that the target protein ospB is generally lacking in the *Borrelia* species prevailing in Sweden (i.e, *B. garinii* and *B. afzelii*), and is usually present only in the nominate species *B. burgdorferi* s.s, which was detected in ticks on migrating birds in Sweden (Fig. 3; Olsén et al. 1995b). However, changes in the osp profile can occur after passage of spirochetes through tick vectors and their hosts, so it is possible that the positive slides observed represent spirochetes which have acquired ospB but do not belong to *B. burgdorferi* s.s. The actual status of the *B. burgdorferi* s.l. species occurring in especially Kungshamn-Morga and Bogesund remains unknown, but is likely to consist of *B. garinii* and/or *B. afzelii*.

**Statistical treatment**

When sampling host-seeking ticks, the resulting variable is a measure of ticks per unit area – provided that sampling was performed in a standardised manner. This kind of data is the statistical basis for all material published in this thesis. However, some problems with this kind of measurements that will potentially violate one or more of the critical assumptions of
general parametric methods are evident: i) Counts of individuals can only adopt even numbers and are therefore not truly continuous variables. This will affect the (statistical) distribution, which is generally found non-normal. ii) Due to the host dependence and restricted lateral movement off-host, ticks cannot be considered randomly distributed in the vegetation. In fact, clumped/over-dispersed distribution of ticks is generally the case (Petney et al. 1990). For the above reasons, the statistical procedures generally performed here are those adapted for distribution-free or "non-parametric" data. Even though there are ways to transform data to meet the assumptions of parametric tests, it was found that the success of such activities was often limited to certain subsets of the data, e.g., only to nymphal ticks or to samples collected during a particular year. Similar problems to those discussed above also arise when analysis is performed on frequencies, such as the *Borrelia* infection prevalence in nymphs. In some cases however, the situation was remedied by transformation of original data (IV). In this context, it might have been more appropriate to use the median as a measure of location instead of the arithmetic mean and standard deviation (I-V) which is valid only for continuous, normally distributed data. However, this choice would not affect the outcome of the statistical procedure used.

The following tests were used when evaluating tick density (i.e., ticks per unit area; Sokal & Rohlf 1981): the Kruskal-Wallis test with $\chi^2$-approximation for multiple comparisons between groups (I-III,V) and the Mann-Whitney U-test (V) and Wilcoxon matched pairs test (I) for pairwise comparisons. For comparisons of *B. burgdorferi* s.l. frequencies found in *I. ricinus* nymphs the G-test was used (I,III,V), and when not applicable, the Fisher's exact test (III). A 1-way ANOVA was also performed on arcsine-transformed mean frequencies (IV). Correlation was performed on ranked variables, i.e., the Spearman ranked correlation coefficient ($r_s$) was used (I,II,IV).

The computer software generally used was the statistical package CSS (Statsoft™) and MS Excel 5 add-ins for data analysis.
RESULTS AND DISCUSSION

Temporal and spatial activity patterns of I. ricinus

There are several expressions more or less related to quantity of ticks used in the separate papers of this thesis (i.e, density, activity, abundance, availability, number of) that essentially denote the same thing: the fraction of unfed, host-seeking ticks present in a particular area and moment, that will also attach to the sampling device. By no means do these expressions imply the total number of ticks present in an area, a quantity that is virtually unattainable.

Diel activity (I)

The results of the 24h sampling sessions at Bogesund in 1991-1993 primarily indicated a nocturnal peak activity (i.e, from 23.00-03.00) for all active stages of I. ricinus at the meadow site. However, differences between the six time periods were not significant for nymphs at this site. At the forest site, no significant differences between time periods were detected for any tick stage (Table 1). Generally, subadult ticks (i.e, larvae and nymphs) were more abundant in the forest (82-124 and 3.5-5.3 per 40m², respectively) compared to the meadow (0.6-6.1 and 2.0-4.7, respectively), while adult numbers were similar (ranging from 0.4-0.8; pooled data). When the proportions of ticks sampled in each time interval were compared between sites, those of larval ticks differed significantly (G-test, G=147, d.f.=5 and p<0.0001; Table 1). This indicated a more irregular diel activity pattern in the meadow site, although the peak activity at both sites (6.1 and 124 for meadow and forest, respectively) were recorded during the same time interval (23.00-03.00).

For all developmental stages of ticks sampled in the meadow site, a negative association with ambient air temperature was recorded (Spearman rank correlation, p≤0.005), while a corresponding positive association with R.H. was established only for adult ticks (p<0.05). In the forest site, there was no association between nymphs or adults and these variables, whereas for larvae, the situation was reversed (i.e, a positive correlation with temperature [p<0.05] and a negative correlation with R.H. [p=0.005], respectively). In many cases, macroclimatic variables (e.g, temperature, R.H.) measured at the time of sampling or obtained post-sampling from nearby meteorological stations, will often produce weak or non-existent associations with tick activity data. However, this must not necessarily mean that
Table 1. Mean no. per 40 m² of *I. ricinus* larvae, nymphs and adults (y) and fraction of total sample (%) sampled in meadow and forest sites during six time intervals (DST): I=2300-0259, II=0300-0659, III=0700-1059, IV=1100-1459, V=1500-1859, VI=1900-2259; n= no. of 40 m² drag-samples during 1991-93 at Bogesund, Stockholm.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Biotope</th>
<th>Larvae</th>
<th>Nymphs</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>y</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
<td>Meadow</td>
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</tr>
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<td>II</td>
<td>Meadow</td>
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<tr>
<td>V</td>
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<td>7</td>
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<td>VI</td>
<td>Meadow</td>
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**Sum¹** 98  * 101  ns  100  **  99

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<td></td>
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<td>%</td>
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<td>VI</td>
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<td>16</td>
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**Sum** 99  ns  99  ns  100  ns  99

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<th>Larvae</th>
<th>Nymphs</th>
<th>Adults</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>y</td>
<td>%</td>
</tr>
<tr>
<td>I</td>
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<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Both</td>
<td>26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Both</td>
<td>33</td>
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<td>-</td>
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</tr>
<tr>
<td>V</td>
<td>Both</td>
<td>33</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>Both</td>
<td>41</td>
<td>-</td>
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</tr>
</tbody>
</table>

**Sum** 197  ***  100

¹ Due to rounding error, sums of fractions do not always equal 100%. Significance levels by the Kruskal-Wallis test: ns, p>0.05; *p<0.05; **p<0.01; ***p<0.001

there is no association, but merely that estimates obtained in this way either are too crude, or their interrelationship separated by a time lag.
An example of the latter was shown by Gigon (1985), where a model for *I. ricinus* activity that involved measures of daily R.H. minima during the week before the actual sampling showed a strong predictive value.

The pattern of diel activity recorded for ticks collected at the meadow site is most likely linked to the daily fluctuations of temperature, indicated by the pronounced association with this variable. This is consistent with Belozerov (1982), who reviewed previous studies on the daily rhythms of activity for several tick species. Here, temperature was the main leading factors for tick diel activity, followed by R.H. and solar radiation. At Bogesund, in response to the more adverse microclimatic conditions encountered in open areas (e.g., increased solar radiation during the day and heat escape during the night), the probability of survival of individual ticks may be decreased. An indication of increased mortality of larval ticks was the fact that the catch ratio between larvae and nymphs differed markedly between the open meadow site (close to 1:1) and the forest site (~25:1). Additionally, some previous studies have actually shown that the mortality of *I. ricinus* larvae was higher in an open area compared with a woodland site (Daniel et al. 1976, 1977). Alternative explanations include those regarding sampling efficiency and, although less likely, host-associated differences: i) Due to differences in the vegetation structure between meadow and forest (i.e., denser, grass-dominated vegetation in the meadow) it is possible that a smaller proportion of the host-seeking larvae was available for sampling. ii) For some reason the production of fed female ticks from hosts (and hence host-seeking larvae) was lower in the meadow.

*Vertical distribution (II)*

In contrast to the host-seeking behaviour of *I. scapularis* in North America (which is usually questing at ground level; Ginsberg & Ewing 1989a, 1989b), *I. ricinus* ticks are mainly found questing on the vegetation, i.e., at some distance above ground level (Gigon 1985). Potential questing places are ultimately restricted by vegetation structure, but the gradual decline of atmospheric humidity with increasing height above ground level is likely to affect the longevity of questing ticks depending on their location (Lees 1948). Because of the differential desiccation tolerance of *I. ricinus* ticks, i.e., larvae being the least tolerant and adult ticks the most tolerant (Knülle & Rudolph 1982), a similar vertical distribution of these stages was expected. Additionally, the different "preferences" of larvae, nymphs and adults, respectively, for hosts of different sizes, was also expected to impact tick vertical distribution.
Such a host-size dependent vertical distribution has previously been proposed both for *I. ricinus* (Gigon 1985) and *I. pacificus* adults (Loye & Lane 1988).

In high vegetation, ticks of all stages were recorded from 10 to 140 cm above ground level, while the lowest interval (0-9 cm) could not be sampled. The intervals with the greatest mean number of ticks were 10-19 cm for larvae, 50-59 cm for nymphs and 60-79 cm for adults, respectively (Fig. 4). Here, larvae occurred at a significantly lower level (mean height=50 cm) compared to nymphs (59 cm) or adults (66 cm).

In low vegetation (i.e., from 0 to 80 cm), adult ticks were absent while larvae were sampled from the interval 0-59 (n=96) and nymphs from 0-49 cm (n=5). For larvae, the interval with the greatest mean number was 0-9 cm and mean height=18 cm. The fact that larval *I. ricinus* was generally found to quest at lower heights in the vegetation than did nymphs or adults is similar to the results of Gigon (1985). In that study on Swiss populations of *I. ricinus*, both subadult stages were found closer to the ground (7-11 cm) compared to adult ticks (10-50 cm). However in my study, the mean questing height for larvae

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Fig. 4. Estimated proportion of main hosts’ surface area (shaded bars) and vegetation heights (open bars) in high vegetation at Torö. Lines represent mean numbers per 100 m² of questing *I. ricinus* larvae (dotted), nymphs (broken) and adults (solid) at the vertical range 10-140 cm. Ticks were sampled on eight occasions in 1992.

---

1 absent between 20 and 29 cm
in high vegetation (50 cm) may seem unreasonably high, but is most likely biased due to the exclusion of the interval 0-9 cm. Probably for the same reason the larval to nymphal ratio (1.4:1) in high vegetation appears skewed, both compared with blanket-dragging data from the same area (7.5:1-25:1; V, unpubl. data) as well as with vegetation-free areas (8:1-32:1). In a study by Tälleklint & Jaenson (1994) it was estimated that the majority of host-seeking *I. ricinus* larvae were fed on small, ground-dwelling mammals which is also a strong indication of high larval densities close to the ground, i.e., within the interval 0-9 cm.

**Table 2.** Spearman rank correlations between tick availability, i.e. the numbers of questing *I. ricinus* collected, and the estimated vertical proportion in 10-cm increments of host surface area or vegetation heights.

<table>
<thead>
<tr>
<th>Tick stage</th>
<th>Vegetation</th>
<th>Number of ticks</th>
<th>Host surface area</th>
<th>Vegetation height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva</td>
<td>High</td>
<td>796</td>
<td>0.37</td>
<td>0.96</td>
</tr>
<tr>
<td>Nymph</td>
<td>High</td>
<td>550</td>
<td>0.60</td>
<td>0.80</td>
</tr>
<tr>
<td>Adult</td>
<td>High</td>
<td>35</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Larva</td>
<td>Low</td>
<td>96</td>
<td>-0.14</td>
<td>0.92</td>
</tr>
<tr>
<td>Nymph</td>
<td>Low</td>
<td>5</td>
<td>-0.17</td>
<td>0.45</td>
</tr>
<tr>
<td>Adult</td>
<td>Low</td>
<td>0</td>
<td>No ticks observed</td>
<td>No ticks observed</td>
</tr>
</tbody>
</table>

Ticks were sampled in the interval 0-140 cm above ground level in high and low vegetation types at two localities (Torö and Bogesund) near Stockholm. $r_s$, Spearman rank correlation coefficient. n, no. of vertical increments. ns, not significant at the 0.05 level.

The vertical distribution pattern of *I. ricinus* was also compared to the distribution of vegetational apices, i.e., potential questing places, as well as to the distribution of the potential hosts' surface areas (Fig. 4, Table 2). Estimates of the distribution of vegetational apices were visually quantified for each 10-cm interval, henceforth referred to as vegetation height. The surface areas of "preferred" *I. ricinus* hosts, i.e., the bank vole, yellow-necked field mouse (*A. flavicollis* [Melchior]), wood mouse (*A. sylvaticus* [L.]), common shrew, varying hare (*L. timidus* L.), European hare (*L. europaeus* Pallas) and roe deer; Tälleklint & Jaenson 1994) were calculated by combining available data on mean tick host densities per hectare with the cross-sectional area in cm² (frontal projection) of each host species. Subsequently, the fractions of total host area (7740 cm²/ha) for each height interval were calculated. The results
from ranked correlation between the vertical distribution of ticks and vegetation height showed significant association for larvae and nymphs in high vegetation and for larvae in low vegetation, respectively (Table 2). No association was detected between subadult ticks and host area, or between adult ticks and any parameter. The microclimatic variables measured at the time of sampling, i.e, ambient air temperature and R.H, showed almost no variation between the different measurements on 0, 10, 50 and 100 cm above ground level, respectively. Thus, the variation in these variables was not sufficient to explain any differences in vertical distribution of ticks.

Seasonal activity (unpubl. data relating to III and V)

Studies on the seasonal occurrence of host-seeking *I. ricinus* ticks were conducted at three different biotopes (i.e, open pasture, blueberry-spruce and mixed woodland, respectively) at KM in 1988-1989, and at two different biotopes (semi-open brush/meadow and mixed woodland, respectively) at Torö in 1988-1990. Generally, ticks of all stages were more abundant at Torö compared to KM, as well as more abundant in woodland compared to more exposed areas.

Generally, two types of seasonal activity patterns were distinguished in the case of host-seeking subadults: i) the unimodal pattern characterised by a single peak of activity usually lasting from spring/early summer to late summer/autumn, or one peak occurring either in spring or autumn. ii) the bimodal pattern characterised by decreased activity during the end of June to early August period thus resulting in one spring peak (April-Midsummer), and one autumn peak (late August-September), respectively (Fig. 5). The bimodal pattern prevailed for the subadult ticks sampled in 1988 and 1989 at both KM and Torö. In 1990 however, the numbers of larval ticks were extremely high in both sample sites at Torö. Here, the unimodal pattern dominated activity (i.e, spring peak for area C and autumn peak for area B, respectively), while that of nymphs was mainly bimodal. The activity of adult ticks showed no distinct seasonality, however the numbers sampled were generally low. Some early studies however, suggested the seasonal activity of adult *I. ricinus* to be of a multimodal type (Nass 1975).
Fig. 5. Mean numbers per 100 m² of *I. ricinus* larvae, nymphs and adults in three biotopes at KM (pasture, spruce forest and mixed coniferous/deciduous woodland; left column) and in two biotopes at Torö (shrub/marsh and mixed coniferous/deciduous woodland; right column). The ticks were sampled during March-October 1989.
The modality of seasonal patterns are frequently different between different years and/or areas and in an article by Gray (1991), the modulation of these pattern of *I. ricinus* was reviewed: Firstly, the longevity of ticks in open areas will be short due to higher desiccation rates and a more rapid activation caused by high insolation. Thus, the resulting seasonal activity peaks will tend to be more distinct as well as separated, i.e, a bimodal pattern. In woodland areas however, the longevity of ticks will be more extended which will tend to produce a unimodal pattern. Secondly, climatic parameters will determine the onset and termination, respectively, of tick activity as well as tick development rates. Thirdly, photoperiodic cues regulates the diapause behaviour of ticks and fourthly, host fauna density and composition will affect the density of ticks. The pattern of seasonal appearance of subadult ticks at Torö and KM is most likely related to some of the above parameters, and especially to duration of the vegetation period (IV) and to the density of the host faunas (i.e., roe deer).

**Prevalence of spirochetes in host-seeking ticks**

*Local and seasonal prevalence of spirochetes in I. ricinus (III)*

Several methods for detecting *B. burgdorferi* s.l. spirochetes in ticks can be used. However, all methods are not necessarily comparable to each other. For instance, PCR-based detection is more sensitive, i.e, yields a higher proportion of infected ticks, compared with direct or indirect microscopic methods (moderately sensitive) and cultivation in BSK medium (least sensitive; Hubalek & Halouzka 1998).

All larval ticks examined by phase-contrast microscopy (n=421) were negative for LB spirochetes (Table 3). Similar findings were presented by Tälleklint & Jaenson (1993), where none out of 191 larvae dissected contained spirochetes. In comparison with a European-wide study however, an average of 1.9 % (n=5699, range 0-11 %) of larval *I. ricinus* were positive for *Borrelia* (Hubalek & Halouzka 1998). This would imply that at least in some cases, transmission of spirochetes by the transovarial route does occur. However, the number of pathogens transferred via single eggs is likely to be small. For nymphal ticks, the most abundant of the infective stages, the mean prevalence of spirochetes was similar (~7 %) at KM in both 1988 and 1989, while significantly different (13 and 8 %, respectively) between these years at Torö (Table 3). For adult ticks, the mean *Borrelia* prevalence in males was similar at both KM (15 %) and Torö (14 %), as well as for females collected at these localities.
A significant difference between sexes was only established for adults sampled at Torö in 1988 (12 and 36 % for males and females, respectively). In fact, there is no reason to expect a general difference between sexes in this respect, especially since the number of previous blood-meals ingested are the same. However, in a study by Alekseev & Dubinina (1996) adult *I. persulcatus* kept separately, i.e., without access to the opposite sex, showed lower spirochete prevalences compared with male-female pairs which showed an almost 2-fold increase. The routes of infection in this case are probably venereal from male to female via the spermatophore, and by cannibalistic feeding from female to male. Venereal transmission is also the most likely explanation for the elevated spirochete prevalence in *I. ricinus* females in this study, especially since adult ticks were not kept separately before dissection. The mean *Borrelia* frequencies reported here, both for *I. ricinus* nymphs (9 %) and adults (20 %), are close to the European mean values of 10.8 and 17.4 %, respectively (Hubalek & Halouzka 1998) although considerable variation is present due to different sample sizes, detection methods and composition of host faunas.

Table 3. Prevalence (%) of *Borrelia* infection in *I. ricinus* from two localities in south-central Sweden. Total number of ticks examined in parentheses. The ticks were collected during May-September 1988 and March-October 1989 at both Torö (T) and Kungshamn-Morga (KM).

<table>
<thead>
<tr>
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<td>Larva</td>
<td>0 (147)</td>
<td>0 (53)</td>
<td>0 (189)</td>
<td>0 (32)</td>
<td>0 (336)</td>
<td>0 (85)</td>
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<td>Nymph</td>
<td>12.9 (333)</td>
<td>7.1 (126)</td>
<td>8.5 (622)</td>
<td>6.8 (324)</td>
<td>10.1 (955)</td>
<td>6.9 (450)</td>
</tr>
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<td>Male</td>
<td>12.2 (41)</td>
<td>27.3 (11)</td>
<td>21.4 (14)</td>
<td>6.7 (15)</td>
<td>14.5 (55)</td>
<td>15.4 (26)</td>
</tr>
<tr>
<td>Female</td>
<td>36.4 (22)</td>
<td>16.7 (6)</td>
<td>15.4 (13)</td>
<td>29.4 (17)</td>
<td>28.6 (35)</td>
<td>26.1 (23)</td>
</tr>
</tbody>
</table>

Generally, there were no detectable differences in *Borrelia* prevalence between biotopes sampled at either the KM or Torö locality. However, differences in this parameter were detected within two of these biotopes, i.e., between the two sample sites in mixed forest at Torö (for nymphs and adult males) and in the pasture (for nymphs), respectively. These results may reflect both variation in the monthly incidence of infected ticks as well as clumped distribution of infected ticks. However the seasonal variation, i.e., the difference in proportions of infected ticks between months, was not significant for either KM or Torö. Similar results were obtained from the Stockholm area and despite a tendency towards higher
nymphal prevalences in late spring-early summer (Tälleklint & Jaenson 1996), these differences were not significant by G-test for either of the three years investigated.

Additionally, data from a Czech study showed no significant seasonal variation in *Borrelia* prevalence for either nymphal or adult *I. ricinus* (Hubálek et al. 1994). A clumped distribution of infected ticks seems a likely explanation for the pattern found at KM and Torö, and is also indicated by the large within-biotope variations in numbers of *Borrelia*-infected nymphs (Table 4). Aggregated distribution of both nymphs as well as nymphs infected with *Borrelia* spirochetes was previously described for *I. scapularis* in North-Western America (Telford III et al. 1992). Additionally, the importance of spatial scale in assessing LB risk and the small-scale distribution of infected ticks was also demonstrated for *I. pacificus* nymphs (Tälleklint-Eisen & Lane 1999).

**Table 4.** Estimated infection percentage (no. examined) and mean number of host-seeking, *Borrelia*-infected *I. ricinus* nymphs per hectare of 2 different biotopes at Torö (T) and 3 different biotopes at Kungshamn-Morga (KM) during 1988-1989.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Biotope</th>
<th>1988 % (n)</th>
<th>mean per hectare</th>
<th>1989 % (n)</th>
<th>mean per hectare</th>
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<tbody>
<tr>
<td>T</td>
<td>Mixed 1</td>
<td>12.7 (55)</td>
<td>135</td>
<td>6.6 (211)</td>
<td>73.5</td>
</tr>
<tr>
<td>T</td>
<td>Mixed 2</td>
<td>12.0 (92)</td>
<td>139</td>
<td>13.6 (191)</td>
<td>155</td>
</tr>
<tr>
<td>T</td>
<td>Shrub 1</td>
<td>14.9 (47)</td>
<td>68</td>
<td>1.6 (62)</td>
<td>6.3</td>
</tr>
<tr>
<td>T</td>
<td>Shrub 2</td>
<td>12.9 (139)</td>
<td>222</td>
<td>7.6 (158)</td>
<td>80.7</td>
</tr>
<tr>
<td>KM</td>
<td>Pasture 1</td>
<td>16.7 (12)</td>
<td>12.9</td>
<td>37.5 (16)</td>
<td>29.4</td>
</tr>
<tr>
<td>KM</td>
<td>Pasture 2</td>
<td>0 (17)</td>
<td>0</td>
<td>0 (14)</td>
<td>0</td>
</tr>
<tr>
<td>KM</td>
<td>Spruce 1</td>
<td>0 (18)</td>
<td>0</td>
<td>7.5 (40)</td>
<td>12.2</td>
</tr>
<tr>
<td>KM</td>
<td>Spruce 2</td>
<td>16.7 (12)</td>
<td>21.2</td>
<td>3.5 (57)</td>
<td>10.5</td>
</tr>
<tr>
<td>KM</td>
<td>Mixed 1</td>
<td>6.5 (46)</td>
<td>32</td>
<td>5.9 (118)</td>
<td>52.8</td>
</tr>
<tr>
<td>KM</td>
<td>Mixed 2</td>
<td>9.5 (21)</td>
<td>26</td>
<td>5.1 (79)</td>
<td>22.4</td>
</tr>
</tbody>
</table>

Controlling *I. ricinus* in high-risk areas (IV, V)

LB spirochetes have been shown to occur wherever *I. ricinus* ticks occur (Jaenson 1991). Therefore, a factor obviously limiting the distribution of LB in Sweden is the geographical distribution of this tick species. In Sweden, the northern limit of *I. ricinus* is close to the *Limes Norrlandicus*, an important zoogeographical zone which marks the northern
distribution of several plant and animal populations. To the north of this zone begins the true boreal or taiga zone. In paper IV, the northern limit of the distributional area of *I. ricinus* (Jaenson *et al.* 1994) was compared with several climatic and vegetational isoclines on a national scale. Data show that *I. ricinus* is present in areas with an annual snow cover of 150 days or less and in areas where the duration of the vegetation period exceeds 170 days, respectively. Three additional isoclines showed rather close correspondence with the northern distributional limit of *I. ricinus*: 1) the northern limit of the southern boreal zone (Fig. 6) 2) the average date of first frost = 15 September and 3) the temperature sum = 1100 degree-days. Thus, the importance of increasing latitude/altitude is again demonstrated to be of key importance, reflected in all of the above-mentioned parameters. However, along the east coast of central and northern Sweden *I. ricinus* seem to generally extend its distribution beyond that of nearly all isoclines mentioned above. A likely explanation is that the Baltic Sea has a buffering effect on the climate so that the vegetation period in the area is increased. Additionally, the higher humidity in the atmosphere around the Baltic Sea could positively affect the survival of ticks. Tick survival has been shown to increase in the vicinity of large water bodies.

The basis for evaluating the risk for human LB is usually the number of *B. burgdorferi* s.l. infected *I. ricinus* nymphs present in a particular area, an index which can be extracted from tick field-sampling data. To estimate the actual risk to contract the infection and to develop clinical LB symptoms, however, it is necessary to acquire data on several “post-attachment” parameters such as human immunological status, number of spirochetes transmitted, their virulence etc., factors which cannot easily be quantified. Therefore, the measure of LB risk used in the present papers is defined as the number of infected *I. ricinus* nymphs per unit area, and high-risk areas are thus characterised by large numbers of infected nymphs. In addition, the frequency of visits by people in all study areas were estimated and categorised as low (+), intermediate (++) or high (+++) human utilisation (Table 5). High-risk areas were mainly associated with deciduous or mixed deciduous/coniferous woodland along the south-eastern coast of Sweden (Bedarö/++, Bogesund/+++, Grönö/+ and Torö/++; Table 5), while the lowest numbers of *Borrelia*-infected nymphs per 100 m² (i.e, less than 1.0) were found in two of three localities investigated on the Swedish west coast (Dagsäs/+ and Änggårdbergen/+++), in an inland area close to Uppsala (KM/+++) and in one locality along
Fig. 6. Comparative matching of the estimated distribution of *I. ricinus* in Sweden (shaded) with the northern limit of the south-boreal zone (solid line) and northern limit of the boreo-nemoral zone (hatched line), respectively. Data for vegetational zone distribution from 1961-90 (Raab & Vedin 1995).
### Table 5. Density and infection percentage of *I. ricinus* nympha and *B. burgdorferi* s.l. genomospecies diagnosed at 12 study localities in Sweden including key mammal species and type of vegetation.

<table>
<thead>
<tr>
<th>Study locality [Province]</th>
<th>Long, Latitude</th>
<th>Vegetation/woodland type</th>
<th>Mammals</th>
<th>Human utilisation</th>
<th>Nymphs (NN) per 100 m² (% inf.)</th>
<th>inf. NN per 100m²</th>
<th>B.b. s.l. genosp.***</th>
<th>References*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norrbyskär [Ångerman]</td>
<td>63°33’N, 19°52’E</td>
<td>alder</td>
<td>L. timidus voles, mice, shrews</td>
<td>++</td>
<td>ND (2.4)</td>
<td>ND</td>
<td>Bg</td>
<td>Bergström et al. 1992, Jaenson et al. 1989</td>
</tr>
<tr>
<td>Gårdskär [Uppland]</td>
<td>60°36’N, 17°35’E</td>
<td>mixed**</td>
<td>roe deer, moose, hares, voles, mice, shrews</td>
<td>+</td>
<td>7.9 (6.3)</td>
<td>0.5</td>
<td>Bg</td>
<td>Tälleklint &amp; Jaenson 1996a</td>
</tr>
<tr>
<td>Grönö [Uppland]</td>
<td>60°30’N, 17°45’E</td>
<td>mixed**</td>
<td>-</td>
<td>+</td>
<td>11.7 (28.6)</td>
<td>3.4</td>
<td>Bg</td>
<td>Tälleklint &amp; Jaenson 1996a; Tälleklint et al. 1996</td>
</tr>
<tr>
<td>Kungshamm-Morga [Uppl.]</td>
<td>59°47’N, 17°40’E</td>
<td>spruce</td>
<td>-</td>
<td>++</td>
<td>2.2 (5.8)</td>
<td>0.1</td>
<td>ND</td>
<td>III; Tälleklint et al. 1998</td>
</tr>
<tr>
<td>Bogesund [Uppland]</td>
<td>59°23’N, 18°20’E</td>
<td>deciduous/oak</td>
<td>-</td>
<td>++</td>
<td>19.7 (25.0)</td>
<td>4.9</td>
<td>ND</td>
<td>Tälleklint &amp; Jaenson 1996a, 1996b; Tälleklint et al. 1998</td>
</tr>
<tr>
<td>Torö [Södermanland]</td>
<td>58°50’N, 17°51’E</td>
<td>deciduous</td>
<td>roe deer, (moose), hares, voles, mice, shrews</td>
<td>++</td>
<td>9.3 (9.9)</td>
<td>0.9</td>
<td>Ba</td>
<td>III</td>
</tr>
<tr>
<td>Bedarön [Södermanland]</td>
<td>58°54’N, 17°58’E</td>
<td>mixed**88-89</td>
<td>-</td>
<td>++</td>
<td>12.6 (10.0)</td>
<td>1.3</td>
<td>Ba</td>
<td>Tälleklint et al. 1998; Tälleklint &amp; Jaenson 1996a, 1996b; Tälleklint et al. 1998</td>
</tr>
<tr>
<td>Stora Karlsö [Gotland]</td>
<td>57°17’N, 17°57’E</td>
<td>Juniperus brush</td>
<td>L. timidus</td>
<td>+++</td>
<td>22.1 (11.2)</td>
<td>2.5</td>
<td>1 Bg (in 1 Ir)</td>
<td>Jaenson &amp; Tälleklint 1996</td>
</tr>
<tr>
<td>Hallands Väderö [Skåne]</td>
<td>56°26’N, 12°34’E</td>
<td>deciduous</td>
<td>L. timidus, hedgehog, voles, mice, shrews</td>
<td>+++</td>
<td>16.8 (13.1)</td>
<td>2.2</td>
<td>1 Bg &amp; 1 Ba (in 1 Ir)</td>
<td>1 Bg (in 1 Lt)</td>
</tr>
<tr>
<td>Dagsås [Halland]</td>
<td>57°4’N, 12°30’E</td>
<td>beech</td>
<td>roe deer, voles, mice, shrews</td>
<td>++</td>
<td>4.7 (5.2)</td>
<td>0.2</td>
<td>ND</td>
<td>Bg</td>
</tr>
<tr>
<td>Änggårdsbergen [Västergöt]</td>
<td>57°41’N, 11°57’E</td>
<td>beech</td>
<td>roe deer, voles, mice, shrews</td>
<td>+++</td>
<td>4.2 (16.1)</td>
<td>0.7</td>
<td>Bg</td>
<td>Bg</td>
</tr>
</tbody>
</table>

Reference(s) for a study locality indicates that at least some of the information in this paper has been published previously in the reference(s). **mixed** = mixed coniferous and deciduous vegetation; ***A figure denotes number of positive tick specimens (Ir, *I. ricinus*) or hares (Lt, *L. timidus*); Bb, *B. burgdorferi*; Ba, *B. afzelii*; Bg, *B. garinii*; ND, no data available
The northernmost area investigated was the Island of Norrbyskär (in the Baltic Sea east of Umeå). This is most likely also a low-risk area as indicated by the low spirochete prevalence in nymphs (2.4%; Table 5).

The two components of the risk index, i.e., nymphal density and spirochete prevalence in nymphs, respectively, seemed not to be associated when analysed by ranked correlation, neither for Swedish data alone nor when including data from several European and American studies (IV). However as discussed earlier, the lack of association may be an effect of the non-random distribution of (infected) ticks. The inherent variability in these parameters, i.e., average values of spatially and temporally widely scattered data, might obscure any potential relations. Also, by comparing several different tick-host systems in Europe and North America will certainly add to the above variability.

At Torö, considered a high-risk area for LB, a field experiment was performed in 1990 to 1992 aimed at reducing the numbers of *Borrelia*-infected *I. ricinus* nymphs in natural habitats (V). The approach was to distribute commercially available Damminix™ tubes filled with permethrin-treated cottonballs in the natural habitat of small mammal reservoir hosts. If this cotton was then used by rodents as nesting material (i.e. mice and bank voles), their ectoparasite load and the production of *Borrelia*-infected nymphs from these hosts would likely be reduced. The results showed that the tick load of bank voles was significantly reduced in treated areas compared to control areas but no such difference was detected for yellow-necked field mice (Table 6). No clear differences between treated and untreated areas could be detected regarding the mean numbers of host-seeking nymphs infected with spirochetes. A possible reason for this uncertainty is the significant increase in mean numbers of infected *I. ricinus* in the untreated control areas (2.0, 6.2, 10.6 infected nymphs per 100 m² for 1990, 1991 and 1992, respectively), while the density of infected ticks did not change significantly in the treated areas (6.4, 3.0 and 3.9, respectively).

Treatment with host-targeted acaricide has in several studies been shown to greatly reduce the number of host-seeking *Borrelia*-infected nymphs of *I. scapularis* in North-Eastern America. However the main reservoir host for spirochetes in these areas is the white-footed mouse, a single important host which will also readily use cotton as nesting material. Additionally, the area of treatment in question is often limited to peoples' backyards and is thus more easily saturated with acaricidal material. In Sweden, the ecological situation is more complex. First, there are several alternative reservoir hosts for LB spirochetes, all of which are not likely to respond in the same way to a particular treatment. Second, the vast areas
inhabited by ticks makes it virtually impossible to saturate the environment with, for instance, acaricidal nesting material.

**Table 6.** Mean number of *I. ricinus* larvae per rodent and number of rodents examined (n) of the bank vole (*C. glareolus*) and the yellow-necked field mouse (*A. flavicollis*).

<table>
<thead>
<tr>
<th>Year</th>
<th>Area</th>
<th>No. larvae per vole</th>
<th>n</th>
<th>No. larvae per mouse</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>Treated</td>
<td>0.9</td>
<td>12</td>
<td>21.6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16.3</td>
<td>6</td>
<td>16.5</td>
<td>6</td>
</tr>
<tr>
<td>1991</td>
<td>Treated</td>
<td>3.5</td>
<td>24</td>
<td>1.0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13.0</td>
<td>13</td>
<td>3.2</td>
<td>4</td>
</tr>
</tbody>
</table>

**Conclusions**

The spatial and temporal activity patterns of host-seeking *I. ricinus* seemed to be primarily associated with the physical environment, i.e., with vegetation structure and climatic parameters, and the strength of these associations generally seemed greater for the larval stage and smaller or non-existent for adult ticks. Lower numbers of larvae were generally found in open areas compared to woodland, indicating a lower survival rate of larvae in these areas. The northern limit of the distributional area of *I. ricinus* in Sweden corresponded closely with several climatic isoclines, in particular to those relating to the vegetation period.

The LB-like spirochetes (*B. afzelii* and *B. garinii*) were detected in both nymphal and adult *I. ricinus* while not in larval ticks. The frequency of infection in nymphs did not vary seasonally but occasionally between localities and was not associated with nymphal density. The risk for human LB was estimated as the number of host-seeking, *Borrelia*-infected nymphs per unit area.

The greatest risk for human LB was estimated to occur in southern Sweden, in deciduous or mixed deciduous/coniferous woodland preferably close to the south-east coast. In a high-risk area at Torö where the rodent reservoir was provided with acaricide-impregnated nesting material, no significant reduction in number of infected nymphs could be detected. Thus, control measures aimed controlling the tick infestation rate on only a segment
of the numerous species of tick hosts in areas with many potential hosts are not likely to reduce the overall risk for human LB.

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