12. Lyme disease in Europe: facts and no fiction

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Abstract

Lyme disease is an emerging disease in the Netherlands and elsewhere in Europe. *Ixodes ricinus* L. ticks infected with Lyme disease-causative agents have been found throughout Europe. Remarkable are the large differences in infection rates between different regions. It is supposed that the observed heterogeneity in infected tick numbers is characteristic of the way *Borrelia* species are acquired and transmitted by *I. ricinus* ticks. One factor directly affecting microbial communities in the tick gut, including populations of *Borrelia* species, is host blood. Smaller animals such as birds and rodents, often called 'reservoir hosts', play an especially important role in the transmission of *Borrelia* species between vector and hosts. Local environmental aspects may largely impact these groups of smaller animals and modulate their populations. Factors such as soil, vegetation, predation and human interference can have a large influence on local and temporal fluctuations of reservoir hosts and supposedly also on *Borrelia*-species-infected tick numbers. Local variations in these factors cause differences in infection rates in ticks and we propose that large fluctuations in infected tick numbers may occur at a small scale: so-called 'hot spots' of infected ticks. The concept of hot spots needs further attention, as it is important for the control of infected ticks in natural areas via local and integrative approaches.

Keywords: *Ixodes* tick species, *Borrelia* species, emerging diseases, Lyme borreliosis, infected tick distribution, biological control

Introduction

As mentioned in one of the most fundamental works on ticks 'The Biology of Ticks' (Sonenshine 1991), the first records of ticks as nuisances to humans date back hundreds of years BC, and were described by ancient Greek and Roman authors. Linnaeus first recorded ticks as species in his Systema Naturae of 1758. Ticks have been found encapsulated in fossil amber at least 50 million years old. Only since the early 1900s were ticks known to transmit diseases to cattle and humans. Although the symptoms of Lyme disease, also called Lyme borreliosis, were first described in Europe in 1883 (Matuschka *et al.* 1995), no link was made with ticks as vectors until the late 20th century, when it was revealed that the major vectors of Lyme borreliosis were species belonging to the genus *Ixodes*. The causative agent *Borrelia burgdorferi* was originally isolated in the USA in 1982 and a link was made between this pathogen and the Lyme disease symptom known as erythema migrans in the town of Old Lyme in 1975 (Burgdorfer *et al.* 1982). In Europe, *Borrelia* species have been detected in various *Ixodes* tick species that were obtained from museum specimens dating back to 1896 (Hubbard *et al.* 1998). Although historical evidence provides information on infected ticks up to about a century ago, genetic variation within *Borrelia* species isolates and *Ixodes scapularis* populations indicate that this relationship must have existed over a much longer period, at least since the last ice-age. Similar relationships are also expected between European *Ixodes* tick vectors, which are closely related to their Nearctic counterparts, and *Borrelia* species occurring in ticks, although it must be taken into account that European *Borrelia* species diversity is higher than found in the USA (Kurtenbach *et al.* 2006).

By the late 20th century, Lyme borreliosis had developed into the most prevalent tick-borne zoonosis in the northern hemisphere. The primary vectors of Lyme borreliosis are hard ticks belonging to the *Ixodes ricinus* species complex, which is widely distributed over the Palearctic as well as the Nearctic regions of the northern hemisphere. In North America, the westerly distributed black legged tick (*I. pacificus*) and the deer tick (*I. scapularis*) are the primary vectors of Lyme borreliosis (Piesman *et al.* 1999). In Eurasia, the sheep tick *I. ricinus* and the more easterly-distributed taiga tick *I. persulcatus* are considered to be primary vectors of Lyme borreliosis, although other tick species may also play a role in the enzootic cycle of the Lyme borreliosis agent, but have been rarely linked to clinical cases of Lyme disease (Gray 1998). *I. ricinus* also acts as a vector of other tick-borne diseases such as babesiosis, human granulocytic ehrlichiosis and tick borne encephalitis (Gray 2002), which are not as generally prevalent as Lyme borreliosis and limited to certain geographic regions (Piesman *et al.* 1999).

Lyme borreliosis, which can be acquired by the bite of an infected tick, is a disease with a wide array of symptoms, possibly causing skin, heart, joint and neurological disorders (Stanek and Strle 2003). An early symptom of infection is erythema migrans, a migrating, reddish skin rash that occurs in most, but not all infections after the bite of an infected tick. Different *Borrelia* species cause different symptoms in humans and not all *Borrelia* species have been proven to be pathogenic for humans. Occasionally, clinical symptoms have been observed in various domestic animals.

In this chapter, the emerging character of Lyme borreliosis will be discussed from the ecological perspective of *I. ricinus*. We will dicuss the aetiology of the Lyme-causative agents belonging to the *Borrelia* species, with an emphasis on the heterogeneous distribution of infected ticks in natural areas and possible methods of reducing incidences of Lyme borreliosis. Occasionally, other *Ixodes* species will be mentioned, because parallels in *Borrelia* species infections exist between Palearctic and Nearctic *Ixodes* species.

Ecology of *Ixodes ricinus*

The primary vector of Lyme borreliosis in Europe, *I. ricinus* (Figure 1) has a three-stage lifecycle: larva, nymph and adult, which is typical for all species of the hard tick family, *Ixodidae*.

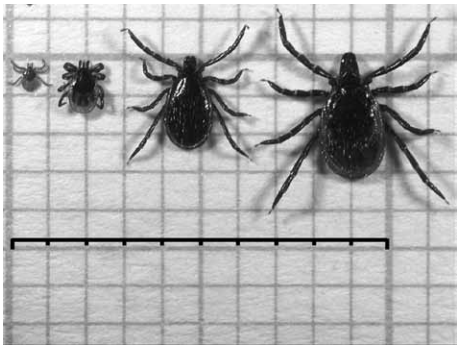

Figure 1. The three developmental stages of the common sheep tick Ixodes ricinus. *From left to right: larva; nymph; adult male; adult female. The scale bar indicates 1 cm.*

I. ricinus ticks depend strictly on a blood meal for moulting and entrance into the next developmental stage. The host is found by specific foraging behaviour called questing, during which the tick detects various cues from the host, such as CO_2, skin volatiles, movement and heat (Sonenshine 1991). In contrast to many other blood-feeding arthropods, *I. ricinus* ticks have a limited action radius and spatial dispersion is mainly dependent on transportation via the host. When questing, *I. ricinus* ticks climb into the vegetation and wait for suitable hosts. When a host passes, the tick grasps its fur and crawls to preferred feeding sites such as ears, neck and the skin between the legs. The *I. ricinus* attaches to the skin and uses specialised mouthparts (Figure 2) and salivary excretions to anchor itself to the skin, where it remains undetected until full engorgement (Sonenshine 1991). Engorgement usually lasts between 3 and 7 days.

Mating usually takes place within the vegetation (Sonenshine 2004) during questing or in some cases on the host. Females normally oviposit 3-27 days after feeding, and can lay 500 to 3000 eggs. Eggs are preferably deposited in leaf litter on the forest floor, and can remain there for months. Development of all tick life stages and eggs is optimal at temperatures between 8 and 11 ºC. Once hatched, larvae prefer small rodents and birds for feeding, but they occasionally also feed on larger hosts (Gray 1998) including humans (Gray 2002). Occurrence of larvae is usually restricted to lower vegetation because of their host preference and susceptibility to desiccation, this in contrast to nymphs and adults, which reside higher in vegetation (Mejlon and Jaenson 1997). The occurrence of larvae close to the ground makes it difficult to collect them for research. The most commonly applied method is to collect ticks from the vegetation by dragging with a cotton blanket (Figure 3).

One must realise that the numbers of larvae collected will in general be underestimated in comparison with those of nymphs and adults. For example, in our studies at several locations within the Netherlands, no larvae were captured in a pine forest over a 5-month period, whereas many were found feeding on rodents in the same forest during that time. After their first blood meal, which takes generally 3-5 days, larvae will retreat into the forest floor and will develop into nymphs, which takes between 24 days to sometimes several months, depending on seasonal and climatic conditions.

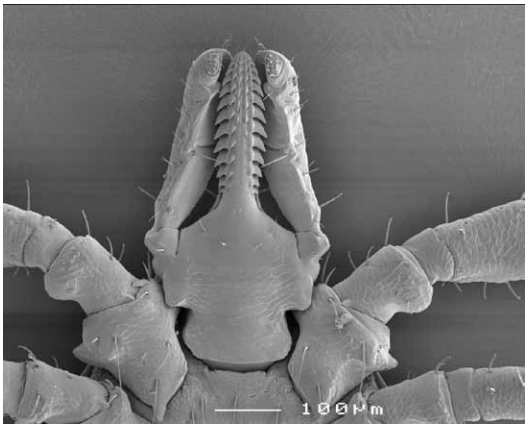

Figure 2. Scanning electron micrograph of nymphal Ixodes ricinus *hypostome (picture by A. van Aelst).*

Figure 3. Blanket dragging method used for collection of questing ticks in vegetation (picture by W. Takken).

The newly developed nymphs have a much wider host range than larvae, from small rodents and birds to reptiles and various ungulates. A wider host range in combination with relatively less susceptibility to desiccation allows nymphs to quest higher in the vegetation than larvae (Mejlon and Jaenson 1997). Once attached to a host, nymphs will feed during approximately 4-7 days, followed by a next moulting period in the forest floor, which can last up to 1 year before they appear as sexually mature adults. Adult ticks are generally found higher in the vegetation in comparison to both other stages and can even be found up to 1.5 meters above the forest floor, which is about the height of an ungulate (Mejlon and Jaenson 1997). Adult ticks have a different host range than larvae and nymphs, and prefer larger animals such as hedgehogs, hares and ungulates (Gray 1998), whereas they are rarely found on smaller rodents. These larger hosts are important to complete the tick life cycle and therefore these large animals are called 'reproduction' hosts. For all stages, questing periods are usually in spring, early summer and autumn depending on photoperiod, temperature and humidity. The duration of the life cycle is flexible because of temperature-dependent differences in development and the possibility to enter into an inactive stage, such as developmental diapause or quiescence during unfavourable climatic conditions or absence of hosts. The entire life cycle from egg to adult is usually between 2 and 4 years but may take up to 7 years, mainly depending on local climate and day length at different latitudes and altitudes. *I. ricinus* thrive in climates with cooler summers and milder winters, which explains why this species mainly occurs in the northern hemisphere (Gray 1998). A study on tick phenology in Western Europe revealed that all tick cohorts (i.e. individuals of the same developmental stage and age) always entered the next developmental stage during autumn (Randolph *et al.* 2002). Furthermore, this study revealed that all tick stages showed clear peaks in questing activity that were attributed to hatching or transition from inactive to active stages. In general, larvae were found questing from early May until the end of August, with a peak in the summer. Nymphs were generally found questing from mid February until the end of November, peaking between March and July, while adults were found questing throughout the year, without any clear peak in questing activity. These observations correspond with those found in the Netherlands, where

larvae were most active from May until late November, nymphs from March until November and adults throughout the year with lowest activity during the winter (Smit *et al.* 2003).

Factors important for *I. ricinus* activity are temperature and relative air humidity. In general, ticks become active when the relative air humidity is above 80% and the temperature 4 ºC or higher. Commonly, the saturation deficit is used as a parameter for tick activity. The saturation deficit combines air temperature with humidity and represents the 'drying power' to which ticks are exposed. Ticks are usually found to be active at saturation deficits of 4 mm Hg and higher (Perret *et al.* 2000). However, the susceptibility of ticks to desiccation not only depends on the saturation deficit, but also on the stored energy in tick bodies. It was found that ticks were more active at lower saturation deficits shortly after a blood meal (Randolph and Storey 1999). This can be explained by the fact that ticks can actively obtain water from the air and for this they need sufficient energy, which they can obtain from the nutrients in blood.

It is expected that *I. ricinus* ticks are more active during the night because the saturation deficit is optimal and many nocturnal host animals are active. It is plausible that ticks are at least as active at night as during the day (Mejlon and Jaenson 1997). However, most studies were done during daytime, and one may wonder whether tick numbers obtained are representative for the populations present in the areas studied. Daytime collection can lead to a serious underestimation of tick abundance (Randolph and Storey 1999). This was also confirmed by our observations, made in a forest near the village of Renkum, in The Netherlands, where higher tick numbers of all stages were found at night than during the day. However, most studies have been done in order to study the transmission of Lyme borreliosis-causative agents to humans and therefore daytime collection of ticks is more appropriate because most people visit natural areas during the day and not at night.

For the life cycle of *I. ricinus*, climatic conditions, vegetation cover, composition of the forest floor (soil and leaf litter layer), and the presence of suitable hosts are the most important parameters. It is clear that these parameters will fluctuate in time and space and thus it can not be expected that tick activity and abundance would be the same for all places at all times. This has important consequences for the transmission of tick-borne pathogens, because tick numbers fluctuate in time and space.

Borrelia species and transmission to hosts

Lyme borreliosis-causative agents are the most important pathogens transmitted via *Ixodes* spp. to humans. A group of closely-related species belonging to the bacterial genus *Borrelia* is responsible for Lyme disease. This complex consists of three main clusters: Lyme borreliosis *Borrelia* species (consisting of approximately 12 species); new world Tick Borne Relapsing Fever *Borrelia* (TBRF) and old world TBRF *Borrelia*. The TBRF agents are widely distributed over the northern hemisphere and are mainly transmitted via body lice and ticks (Rebaudet and Parola 2006), and will not be further discussed in this chapter.

Globally, 33 species are known within the *Borrelia* species complex (http://www.bacterio.cict.fr/b/borrelia.html (Accessed 4 September 2007). At least seven of these are known to occur in Europe: *B. afzelii*, *B. burgdorferi* sensu stricto, *B. garinii*, *B. valaisiana*, *B. lusitaniae, B. spielmanii* and *B. bisettii. B. spielmanii* and *B. bisettii* have been recently described (Derdáková and Lencáková 2005). We cannot exclude that new *Borrelia* species will be found in the near future, for example species

that have remained undetected to date, or more virulent strains of already known species that are newly formed through horizontal gene transfer (Kurtenbach *et al.* 2006). Within the *Borrelia* species complex, interspecies genetic variation is large, especially in *B. garinii*, which can be subdivided into several genospecies based on differential expression of the outer surface protein A (Osp-A). *Borrelia afzelii*, *B. burgdorferi* sensu stricto and *B. garinii* are in particular responsible for symptoms in humans (Stanek and Strle 2003), whereas the role of the other species in the development of Lyme borreliosis symptoms is more disputable. When dealing with disease transmission via *I. ricinus* ticks, one usually refers to the Lyme borreliosis-causative species of the *Borrelia* complex, whereas the other *Borrelia* species are often ignored. The term '*Borrelia* species' is preferred above '*B. burgdorferi* sensu lato', because it covers all species within the *Borrelia* species complex, including the ones that are supposed to be non-pathogenic and those from which pathogenic traits have not yet been elucidated.

Once inside *I. ricinus* ticks, the *Borrelia* bacteria establish in the midgut and can thus be transferred to the next developmental stage of the tick. In principle, tick infection with *Borrelia* can take place with each blood meal, which explains why the highest incidences of *Borrelia* infections are generally found in adults, followed by nymphs and lowest in larvae. Cells of *Borrelia* present in the blood meal can colonise the tick midgut, and from there cells migrate via the haemolymph to the salivary glands. Outer surface proteins (Osp) of *Borrelia* and TROSPA molecules expressed by the tick play an important role in the establishment of the bacteria and its migration from the midgut to the salivary glands. OspC was recently found to be indispensable in this process (Goettner *et al.* 2006, Fingerle *et al.* 2007). Once present in the salivary glands, other molecular mechanisms including factors expressed by the tick can affect the transmission of the *Borrelia* species as well as attachment of the tick to the host (Hovius *et al.* 2007). The parasite only begins to migrate to the salivary glands when the tick begins to feed; the time needed for migration of *Borrelia* from the midgut to the salivary glands in the tick differs per species: in *I. scapularis*, it was found to take place within 36-48 hours (De Silva and Fikrig 1995). However, for *B. afzelii* in *I. ricinus*, dissemination from the midgut to the salivary glands is much faster and can result in *Borrelia* transmission within 24 hours (Crippa *et al.* 2002). Therefore, it is generally advised to remove attached ticks within 24 hours after attachment.

From many different studies, it has become clear that there is a relationship between certain hosts and *Borrelia* species. For example, *B. afzelii, B. burgdorferi s.s.* and the *B garinii* Osp-A 4 genotypes are mainly associated with rodents, while *B. valaisiana* and other Osp-A types of *B. garinii* are more associated with different bird species (Kurtenbach *et al.* 2002). The suitability of particular hosts for the various *Borrelia* species may be explained by differences in host immune responses for different *Borrelia* species. In Europe, at least nine small and seven medium-sized mammals have been found able to transmit *Borrelia* species to *I. ricinus* ticks and are therefore considered reservoir hosts (Gern *et al.* 1998). Furthermore, mainly rodents (Humair *et al.* 1999) and birds (Hanincová *et al.* 2003) account for the natural circulation of the *Borrelia* species. Many reproduction hosts, including common hosts such as ungulates (Tälleklint and Jaenson 1994, Telford *et al.* 1988) and rabbits (Matuschka *et al.* 2000) are generally unsuitable as reservoirs. However, other reproduction hosts can serve as reservoirs, e.g. pheasants (Kurtenbach *et al.* 1998). These hosts are fed upon by all stages of the tick, and therefore could maintain a *Borrelia*-infected tick population in the absence of other reservoirs and reproduction hosts.

Another infection route for ticks can take place through co-feeding. Here, hosts that are poor reservoirs can acquire *Borrelia* species in the bloodstream near a feeding infected tick. Uninfected

ticks feeding nearby could thereby pick up *Borrelia* species without the need of an systemic infection in the host (Ogden 1997). Such incidents can be expected to contribute significantly to the infection prevalence in tick populations, especially if co-feeding occurs in a combination of infected nymphs and uninfected larvae, which can sometimes feed in aggregations of over 50 larvae in one feeding area, such as the neck, the nose or the ears, together with one or more nymphs (F. Gassner, unpublished data).

Finally, a final process that may contribute to the prevalence *B. burgdorferi* s.l. in an area is transovarial transmission; it has not been established, however, that vertically-transmitted *Borrelia* species in larvae are infective to new hosts. This is certainly a phenomenon that needs further study, as 1.9% of larvae are infected Europe-wide (Rauter and Hartung 2005).

The role of various animal groups has major implications on the transmission of Lyme borreliosis-causative agents in nature. The group of smaller animals, such as birds and rodents, requires particular attention because of their presumed role in the transmission of Lyme borreliosis, ultimately leading to infections in humans.

The detection of *Borrelia* species in ticks and animal tissues

To investigate the behaviour of different *Borrelia* species in ticks and vertebrates it is important to distinguish the various Lyme disease-causing *Borrelia* species. Nowadays, a myriad of detection techniques are available to screen for the presence of different bacterial species in biological samples. However, *Borrelia* species need special attention for the following reasons: (1) *Borrelia* bacteria cannot always be recovered from biological samples by culturing, in spite of the fact that these cells are still often viable; (2) cells from different *Borrelia* species closely resemble each other and it is hard to differentiate between the species, especially those that are involved in Lyme borreliosis. Therefore, dark-field microscopy and cultivation-based detection methods are not always suitable for specific detection of *Borrelia* species in biological samples. Culture-independent detection techniques such as molecular (PCR-based) and serological techniques are therefore more appropriate. A commonly applied technique used for the detection of *Borrelia* species in tick samples is the so-called reverse line blot method (Rijpkema *et al.* 1995, Schouls *et al.* 1999). Reverse line blot detection is based on the extraction of total DNA from ticks, followed by PCR with *Borrelia* genus-specific primers, which incorporate biotin into the PCR product. The PCR products are hybridised to a membrane with *Borrelia* species-specific probes. This method has appeared to be applicable in many different studies involving *Borrelia* species infection in ticks, but it has some drawbacks. First, only a limited number of *Borrelia* species can be determined in a single sample and second, this method is qualitative and not quantitative. Because many different pathogens are supposed to be present in ticks, besides *Borrelia* species, it is often important to measure the relative abundance of all pathogens in the same sample. Therefore, detection in quantitative multiplex settings may be more appropriate. Although these techniques have not been applied in tick research yet, it is expected that they will soon become available in our laboratories and will provide deeper insight into interactions within microbial communities in ticks.

Microbial communities in *Ixodes ricinus*

Microbial communities in tick guts may influence the establishment and reproduction of invading pathogens, such as those belonging to the *Borrelia* species complex, in ticks. The microbial composition of the *I. ricinus* midgut may be influenced by various environmental factors (Figure 4).

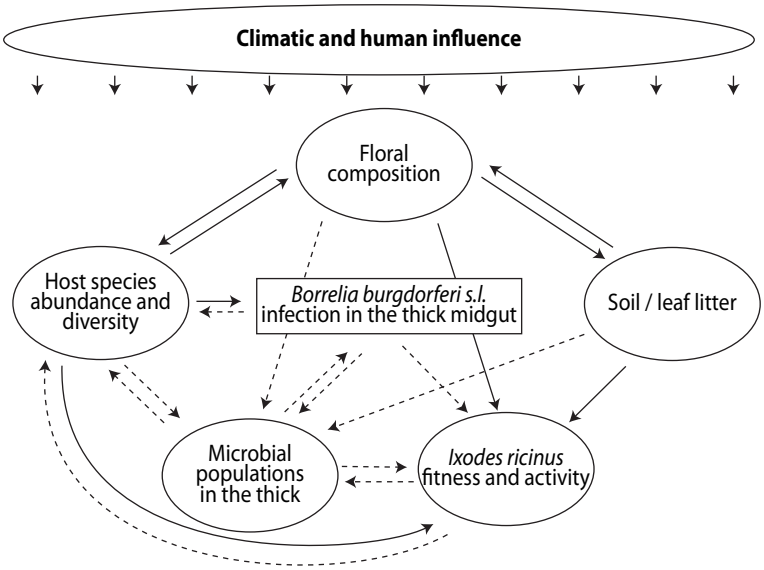

Figure 4. Natural factors affecting microbial composition and Borrelia *species establishment in* Ixodes ricinus *ticks. Solid lines indicate direct effects on microbial communities and dotted lines presumptive indirect effects.*

The most important factor influencing microbial communities in ticks is host blood. Blood composition and micro-organisms present in the blood will determine the microbial composition of tick gut communities. Because ticks are rather immobile, it is expected that local variation in animal hosts will strongly determine the microbial composition in ticks. Other environmental factors such as vegetation, soil type, predation and human activities are also important because these factors can locally influence the composition of small animal populations. The effect of local parameters on the microbial constitution of tick guts and their possible effect on pathogen invasion and establishment have so far rarely been explored; they could, however, explain more in detail why large fluctuations in *Borrelia* infection rates occur in different regions around the world.

An illustrative example of the indirect effect of vegetation on the occurrence of *Borrelia* species in ticks is shown in a study done on acorn mast. Acorn mast is a periodical phenomenon in which oak populations simultaneously produce larger than average amounts of acorns. A modelling study done in North America and based on 15 years of field observations indicated that a mast season can clearly affect reservoir host populations, resulting in higher than normal tick numbers and *Borrelia* infection rates (Ostfeld *et al.* 2006b). Because acorn mast is a common phenomenon in Europe as well, it is expected that it will have the same effect here. Not only does acorn mast sustain host populations, but also the presence of leaves, fruits and other seeds can cause large temporal fluctuations in tick densities through the alteration of host densities. Differences in *Borrelia* infection rates in ticks from areas with different plant communities has been found (Kampen 2004). Therefore, vegetation type must be considered as an important factor affecting *I. ricinus* tick populations and the *Borrelia* species inside these ticks.

Soil and leaf litter composition of forest floors may also be important factors affecting *Borrelia* infection rates in ticks. Soil type has a direct effect on plant composition and thus indirectly affects

local animal communities. However, the leaf litter layer is an important environment for ticks as well, because ticks lay their eggs there and they also spend a considerable portion of their life span in this layer, e.g. for transstadial development. Size, pH and humidity of the leaf litter layer are therefore important factors directly affecting the success of tick reproduction and thus *Borrelia* infection rates in ticks.

As indicated in Figure 4, the role of many factors affecting *I. ricinus* - *Borrelia* species interactions are not yet fully understood. The most prominent one is the direct effect of the various micro-organisms present in ticks on tick survival. Although speculative, a limited number of studies have focused on this interaction and we must take into account that the presence of certain micro-organisms could be responsible for changes in tick reproduction and behaviour (Lefcort and Durden 1996). This has already been shown for several other pathogens in numerous arthropod vectors (Hurd 2003) and requires further attention in tick research.

Spatial and temporal variations in *Borrelia* infection rates in ticks

Local environmental factors have a strong influence on the survival and reproduction of ticks. However, an increase in tick numbers does not necessarily lead to higher infection rates. To understand how *Borrelia* species are able to accumulate in reservoir hosts and ticks, it is important to understand at which spatial and temporal scale the levels of infection rates vary in ticks.

Large spatial variations in *Borrelia* infection rates were found in different countries in Europe (Ferquel *et al.* 2006, Jouda 2004, Kampen 2004, Smit *et al.* 2003, Wielinga *et al.* 2006) and locally in North America (Van Buskirk and Ostfeld 1998). In a Europe-wide meta analysis study, large differences in infection rates were demonstrated (Rauter and Hartung 2005). Especially the difference between Western and Eastern European countries was striking: 14% tick infection rates in Eastern Europe and 24% tick infection rates in Western Europe. Infection rates in ticks ranged from low (1.5% in Slovenia) to very high (49% in Slovakia and Switzerland). Furthermore, only two out of 110 studies included in this meta analysis showed uninfected ticks, which emphasises that *Borrelia* infections in ticks are found almost all over Europe. Of all *Borrelia* species found, *B. afzelii* and *B. garinii* were the most common ones. However, these were not the most dominant species found in all studies. For example, a study in Germany revealed that *B. valaisiana* was more abundant than *B. garinii* and *B. afzelii* (Kampen 2004). It is questionable whether this meta study fully reflects the actual *Borrelia* infection rates throughout Europe, as most, if not all studies were performed in regions with a history of Lyme borreliosis. Within the Netherlands, differences in *Borrelia* infection rates in ticks were found at smaller spatial scale, infection rates varied per region, and were between 5 and 30% (de Boer *et al.* 1993, Rijpkema and Bruinink 1996, Rijpkema *et al.* 1994, 1996, Smit *et al.* 2003, Wielinga *et al.* 2006). Infected tick rates may vary locally, e.g. per forest. What is the minimal scale-level at which differences in *Borrelia* infections occur? One study revealed that tick densities and *Borrelia* infection rates fluctuated within a single woodland (Zeman 1999). Is it possible that incidences of high infection rates occur very locally, e.g. at a scale of a few square meters? These so-called 'hot spots' may explain why such variation exists between and within woodlands. Furthermore, it is important to understand how humans acquire Lyme borreliosis in natural areas and also to understand how the causative agents spread through natural areas and may disappear again. The question is why variations in tick infection rates at smaller spatial scales are usually not published in detail.

The existence of such a variation in numbers of infected ticks is fascinating and deserves further attention. From an ecological perspective, it may explain the role of reservoir hosts and the effect of ecological interactions of such populations on the spread of Lyme borreliosis. Practically, 'hot spots' may be important for the application of control measures aimed at reducing the spread and accumulation of pathogens belonging to the *Borrelia* species complex and possibly also of other pathogens present in *I. ricinus* ticks.

An extreme scenario: The Netherlands

A survey of erythema migrans and tick bite cases in The Netherlands showed a simultaneous, striking increase over a period of 10 years (Hofhuis *et al.* 2006). This study, performed country-wide, shows remarkable regional differences (Figure 5).

On average, the incidence of erythema migrans nearly tripled from 39 cases per 100,000 inhabitants in 1994 to 103 cases per 100,000 inhabitants in 2005. At the same time, tick-bite consultations at hospitals and general practitioners also increased, from 191 per 100,000 inhabitants in 1994 to 446 per 100,000 inhabitants 2005. The latter observation is most likely an underestimation, as many people do not seek medical help for a tick bite.

To date, there is no clear scientific explanation for this dramatic increase. There is a lack of nation-wide tick population and *B. burgdorferi* s.l. monitoring, which is common for most European countries. The available data of infected tick populations in The Netherlands do not show extreme differences with other European countries and vary between 5 and 30% with temporal as well as spatial differences (de Boer *et al.* 1993, Rijpkema and Bruinink 1996, Rijpkema *et al.* 1994, 1996, Smit *et al.* 2003, Wielinga *et al.* 2006). To date, no long-term study has been conducted, but it is expected that the infection rate of ticks may increase (W. Takken, unpublished data), with high local variations. Apart from an increase in populations of infected ticks, an increase in recreation, the creation of ecological corridors connecting nature areas, changes in host abundance and recent milder winters may have caused the increase in Lyme borreliosis. Perhaps the infection prevalence is locally boosted by the occurrence of transmission hotspots, as discussed earlier in this chapter.

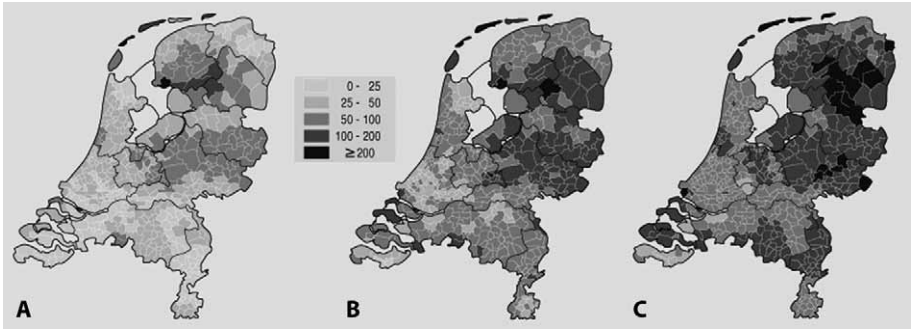

Figure 5. Incidence of erythema migrans as reported by general practitioners in The Netherlands in 1994 (A), 2001 (B) and 2005 (C) per 100,000 inhabitants. (Reproduced with permission from Hofhuis et al. *2006).*

The European distribution of *Borrelia burgdorferi* s.l. and *Ixodes ricinus* overlaps in residential areas

A possible explanation for the observed increase in *Borrelia* incidence in several European countries can be explained by the fact that infected *I. ricinus* ticks are found in highly populated areas. For example, an internet survey in The Netherlands in 2006 and 2007 revealed that many people acquired a tick bite in their own gardens (W. Takken, personal communication). Europe-wide, infected *I. ricinus* are found in recreational areas near or in cities (Table 1).

This phenomenon brings people more into contact with infected ticks; but, how has this developed? For example, did we build our cities in areas where infected ticks were already present, or did they advance into these areas after the expansion of the city boundaries? If city parks and private gardens have been newly constructed, the ticks present would have had to be brought in by various hosts. These urban hosts could be, for example rats, mice, squirrels and various birds such as thrushes and blackbirds (Humair *et al.* 1998, Humair and Gern 1998, Kurtenbach *et al.* 1998, Matuschka *et al.* 1997).

The question remains whether we are expanding our living and recreation areas into tick habitats, or whether the ticks and their pathogens are advancing into human territory. A combination of both may also be true, but it shows that monitoring of tick and *B. burgdorferi* s.l. populations in residential areas is of great importance for monitoring and predicting Lyme borreliosis.

Table 1. Lyme borreliosis vector Ixodes ricinus in European urban areas.

Country	Urban area type	Reference
Finland	Urban recreational areas	Junttila (1999)
Germany	City outskirts, recreational sites and private gardens	Maetzel *et al.* (2005)
Poland	Recreational sites near city	Michalik *et al.* (2003)
The Netherlands	Urban park	Wielinga *et al.* (2006)
United Kingdom	Urban park	Guy and Farquhar (1991)
	Recreational site	Robertson (2000)

Lyme borreliosis and climate change

Over the past few decades, especially in the past few years, climate change has become a very popular topic in the media, in science and for the general public. Evidently, the climate is changing, with various effects worldwide, and threatening human health through direct effects of thermal stress and extreme weather events. Many other indirect effects can be noted, such as the effect on agriculture, air pollution and changes in various infectious (vector-borne) diseases (McMichael *et al.* 2006). In Europe, climate change has been predicted to cause increased drought in the southern parts, and warmer winters with wetter summers in more northern countries. To date,

the effects of climate change are most prominently seen in increased winter temperatures and the increased length of the growing season (Lindgren and Jaenson 2006).

As described earlier in this chapter, local climatic conditions are important for the survival and behaviour of *I. ricinus* in a direct as well as indirect way (Figure 4). Consequently, the effects of climate change can be expected to have amplified effects on populations of infected ticks as climate affects several life stages of the tick at the same time. These effects can be positive as well as negative, for example considering habitat suitability for the vector. A recent modelling study that correlated habitat suitability showed that climatic factors such as temperature and summer rainfall can have different effects depending on the region; some areas were predicted to face stable or increasing *I. ricinus* densities and other areas showed opposite trends (Estrada-Pena and Venzal 2006).

Climatic conditions depend on the latitude and altitude of an area and thereby determine the boundaries of Lyme borreliosis-endemic regions. With a changing climate, these boundaries are expected to shift to other altitudes and latitudes. For *I. ricinus,* an increase in distribution and abundance has been linked to climate change in various studies (Daniel *et al.* 2003, Hubálek 2005, Lindgren *et al.* 2000).

Because an increase in ticks is positively correlated to the incidence of Lyme borreliosis (Hubálek *et al.* 2003, Stafford *et al.* 1998), climate change that increases tick distribution and density is expected to increase the incidence of Lyme borreliosis (Bennet *et al.* 2006). Additionally, the behaviour of the general public should also be taken into account, as warmer summers generally increase the exposure of people to ticks, if an increase in tick numbers coincides with an increase in recreational activity (Joss *et al.* 2007, Mavin *et al.* 2005). Recently, a report by WHO has also stressed the role of climatic change in Europe (Lindgren and Jaenson 2006), but concluded that the emergence of Lyme borreliosis cannot exclusively be attributed to climatic factors. Based on climate changes alone, the distribution of *I. scapularis* and thereby the incidence of Lyme borreliosis in North America has been predicted to increase substantially in the coming decades (Brownstein 2005). Further research is needed in Europe to make reliable predictions.

Control of ticks in nature

Regarding the emerging character of Lyme borreliosis and other tick-borne diseases such as tick borne encephalitis (see Chapter 11), tick control is still a relatively neglected subject in Europe. Surprisingly little scientific attention is paid to direct control of ticks in Europe compared to the USA and Africa, where ticks transmit livestock diseases which can cause severe economic damage regionally (Jongejan and Uilenberg 2004).

In Europe, little information is available on the annual economic damage caused by tick borne diseases, taking into account, e.g. damage to livestock, health-care costs for humans as well as domestic animals, and economic loss due to a decrease in income from recreation in known endemic areas. An accurate assessment, together with the alarming signals described earlier in this chapter, could lower the threshold for European governments to take action.

Tick control does not necessarily need to be aimed at large-scale applications of chemical pesticides such as dichloro-diphenyl-trichloroethane (DDT) or various pyrethroid pesticides. Nowadays, Integrated Pest Management (IPM) is widely applied in agriculture and may be adopted

for application in nature as well. Several strategies can be combined to control ticks; e.g. biological agents in combination with forestry management practices. Biological control of ticks can be done with entomopathogenic fungi, which have been shown to be effective against *I. scapularis* (Benjamin *et al.* 2002, Kaaya and Hassan 2000). Application of these fungi has not been tested before on a large scale, but may offer opportunities in an IPM-like approach.

Other tick control strategies are: the use of insecticide-impregnated nesting materials with which the host may make contact, four posters[12] and bait boxes with a chemical or biological control agent. These methods are already applied and offer opportunities to control ticks in various developmental stages (Lane 2001, Ostfeld *et al.* 2006a). The control of potential reservoirs for *Borrelia* species and vegetation management are alternative measures to control ticks, although these measures are more invasive (Buskirk 1995, Wilson *et al.* 1988). Other methods, such as trapping ticks with pheromones or other chemical attractants, would be most effective on the host, for example by preventing ticks from mating on a reproduction host (Sonenshine 2004). The effect of other types of traps may be limited due to the relatively short-range motility of ticks from the place where they moulted compared to, for example, free-flying mosquitoes. An IPM-like approach may be the best option to control ticks in nature. However, most methods are in an experimental stage and not yet ready for larger-scale applications in Europe.

Apart from control focussing on ticks and tick hosts, reducing the risk of Lyme disease can also be focussed on education and information of the general public, including professional groups such as forest workers. Indeed, the general public has heard of Lyme disease, but very few people know exactly what the risks are. For example, two common misconceptions are that ticks can jump like a flea and that they drop from trees. Knowing that ticks will only cling if one touches it in its questing position, and that ticks rarely drop from trees and generally do not climb up higher than approximately 1.5 meters (Mejlon and Jaenson 1997) can alter peoples' behaviour and awareness.

One effective way to inform the general public is through the media, through which information about times of high risk can be given, together with methods for personal protection and basic information on Lyme borreliosis (Lindgren and Jaenson 2006). For example, tick removal needs clear instructions as it can be a precarious action, due to the strongly barbed tick hypostome (Figure 2) and inadequate removal of the tick may cause faster transmission of the disease agent.

A potentially interesting measure could be a short tick warning item that could be embedded in national weather forecasts, in newspapers, on radio, television and the internet during high-risk periods. Information on personal protection could also be focussed on risk groups such as forest workers and (youth) groups. Possibly, once local hotspots are identified, local warnings in newspapers and at the entrances of nature areas could also be effective.

Concluding remarks

Lyme disease is emerging and difficult to control. The causative agents and the primary vectors of Lyme borreliosis are present in many countries in Europe. Occasional high incidences of *Borrelia* infections in ticks reveal the heterogeneous nature of these infections in *I. ricinus* ticks. It is

[12] Four posters are bait stations with four pesticide-impregnated bristles which rub the ears, antlers and neck of deer that are attracted to the food offered. Four posters are commercially available in the USA.

important to understand the local environmental factors affecting the accumulation of *Borrelia* species in ticks and their natural hosts in order to develop tick control measures in nature. There is a clear need for long-term studies that describe year-round fluctuations in ticks and their pathogens. Additionally, spatial heterogeneity needs to be further assessed in order to assess the risk of Lyme borreliosis. Microbial communities associated with ticks may play an important role in the modulation of *Borrelia* populations in the vector. The role of various microbial species in the establishment and reproduction of *Borrelia* species in *I. ricinus* ticks is so far unexplored and may reveal new opportunities to combat Lyme borreliosis.

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