Erythema Migrans in Primary Health Care

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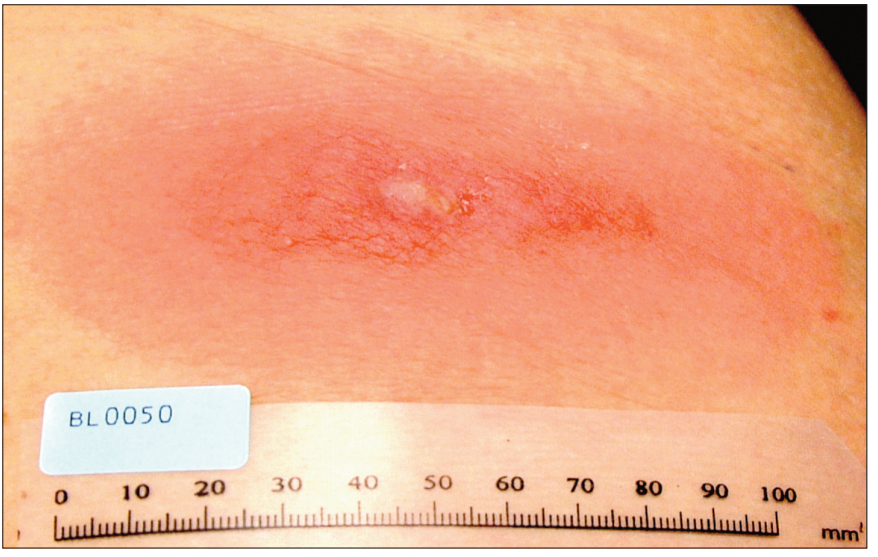

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Erythema Migrans in Primary Health Care

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Abstract

Lyme borreliosis (LB) is the most common vector-borne disease in the northern hemisphere, and southern Sweden is a highly endemic area. In over 70% of the cases, LB is represented by the non-disseminated cutaneous form erythema migrans (EM). This thesis has its focus on EM from a primary health care perspective in southern Sweden, including aspects on epidemiology, the clinical picture, gender differences and the climate.

In paper III, an annual mean incidence rate over 460 cases of EM per 100,000 inhabitants was found in the county of Blekinge. Over 98% of the cases were treated in primary health care and almost every second case occurred during the vacation months of July and August. In paper I, individuals in southern Sweden treated and recovered from EM were followed during a period of 5 years. Annually, 1% were 'reinfected', i.e. had a new infection with LB during the follow-up. This is significantly higher than the prevalence of LB in the area in 1992-1993 of 0.07% ($p < 0.001$), indicating that individuals with a former infection are at a higher risk of LB.

In paper IV studying patients with EM, where genospecies were confirmed by PCR, 74% of the patients were infected with *B. afzelii* and 26% were infected with *B. garinii. B. garinii* seemed to cause more intense local and systemic inflammatory reactions than *B. afzelii.* Totally, 45% (38/85) of the lesions were annular, 46% (39/85) were non-annular and 9% (8/85) were atypical. An interaction between gender and genospecies was found that has not been described before. Men to a higher extent than women developed annular EM while women more often developed non-annular EM, if infected with *B. afzelii.* Surprisingly, time duration from tick bite to diagnosis was not found to have an effect on the clinical appearance of the EM.

In paper II, antibiotic treatment of EM with pcV and doxycycline was found to be highly effective and no cases of disseminated LB were found in patients followed for 5 years.

The incidence rate of EM was significantly higher in women than men (506/100,000 vs 423/100,000 $p < 0.001$) and especially women over 50 years were affected (paper III). Additionally, significantly more women than men were reinfected, 6% and 1%, respectively ($p < 0.01$). All infected women were over the age of 44 years and they were tick-bitten to the same extent as the men (paper I). Immunological differences might have an impact on, and explain, the observed gender differences.

The seasonal incidence rates of EM varied considerably. Different climate factors were found to influence the EM incidence rates (paper III).

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To my grandfather

List of publications

This thesis is based on the following publications, which will be referred to by their Roman numerals.

I. **Bennet L,** Berglund J. Reinfection with Lyme borreliosis: A retrospective follow-up study in southern Sweden. *Scand J Infect Dis* 2002;34:183–6.

II. **Bennet L**, Danell S, Berglund J. Clinical outcome of erythema migrans after treatment with phenoxymethyl penicillin. *Scand J Infect Dis* 2003;35:129–47.

III. **Bennet L**, Halling A, Berglund J. Increased incidence of Lyme borreliosis in Southern Sweden following mild winters and during warm, humid summers. *Eur J Clin Microbiol Infect Dis* 2006;25:426–32.

IV. **Bennet L**, Fraenkel CJ, Garpmo U, Halling A, Ingman M, Ornstein K, Stjernberg L, Berglund J. Clinical appearance of erythema migrans caused by *Borrelia afzelii* and *Borrelia garinii* – effect of the patient's sex. *Wien Klin Wochenschr* 2006;118/17-18:531-7.

Cover illustration
A non-annular erythema migrans
Photograph: Louise Bennet

Photographs in this thesis were taken by Louise Bennet and Johan Berglund.

Abbreviations

ACA	acrodermatitis chronicum atrophicans
B.	*Borrelia*
BL	borrelia lymphocytoma
CI	confidence intervals
ELISA	enzyme-linked immunosorbent assay
EM	erythema migrans
I.	*Ixodes*
IRR	incidence rate ratio
LA	Lyme arthritis
LB	Lyme borreliosis
PCR	polymerase chain reaction
s.l.	the term ‘sensu lato’ refers to all *Borrelia* genospecies within the same *Borrelia burgdorferi* complex
s.s.	the term ‘sensu stricto’ refers to the specific *Borrelia burgdorferi* sensu stricto genospecies
TBE	tick-borne encephalitis

Contents

Introduction

Lyme borreliosis (LB) is a zoonosis caused by the borrelia spirochete and transmitted to humans by the *Ixodes* tick. It is the most common vector-borne disease in the northern hemisphere. The multisystemic illness most commonly involves the skin but may also disseminate to the nervous system, joints and heart.

Historical notes

The first case report on a migrating annular skin lesion at the site of a former tick bite was described in 1910 by the Swedish dermatologist Arvid Afzelius. He called the skin lesion 'erythema migrans' (EM) [Afzelius, 1910]. However, already in 1883 in Germany a case report with idiopathic progressive skin atrophy was described that was later characterised and named as 'acrodermatitis chronica atrophicans' (ACA) [Buchwald, 1883; Herxheimer & Hartmann, 1902]. An association between EM and meningitis was first reported in 1930 [Hellerström, 1930], and during the 1940s a German dermatologist, Bannwarth, described a syndrome, which was later named after him, with lymphocytic meningitis, cranial nerve paralysis and radicular pains [Bannwarth, 1941 & 1944]. In 1949 a Swedish dermatologist, Thyresson, described the successful treatment of ACA with penicillin, and nine years later his colleague Hollström showed the beneficial effects of penicillin on EM [Thyresson, 1949; Hollström, 1958].

In 1948 a Swede named Carl Lennhoff described spirochete-like structures in biopsy specimens of patients with EM [Lennhoff, 1948]. His report was first disputed and then ignored, and it was not until 1981 and 1982 during tick/rickettsial surveys on Long Island in New York, that the spirochete was detected unexpectedly in ticks [Burgdorfer et al., 1982], and it was later identified as a new spirochete within the genus *Borrelia* shown to be the causative agent of LB [Johnson et al., 1984].

The tick – the vector of Lyme borreliosis

Ticks are related to spiders and belong to the class of *Arachnida* and together with mites they are subclassified as *Acari.* They differ from insects by lacking wings, mandibles, antennae and – except in the larvae stage – by having four pair of legs instead of three. The fauna of ticks comprises around 850 described species in three families [Sonenshine, 1991]. The soft ticks, the *Argasidae,* are mainly found in subtropic and tropic climates, while the hard-bodied ticks, the *Ixodidae*, mainly are found in temperate climate in the northern hemisphere. The third family, the *Nutalliellidae*, comprises only a single species and shares morphological traits with both *Argasidae* and *Ixodidae* [Sonenshine, 1991].

The *Ixodidae* ticks consists of 13 genera of which the genus *Ixodes* is the most common vector of the borrelia spirochete. The vector transmitting LB spirochetes to humans includes four tick species of the *Ixodes ricinus (I. ricinus)* complex, i.e. the castor bean tick, *I. ricinus*, and the taiga tick, *I. persulcatus*, in Eurasia, the black-legged tick, *I. scapularis* and the western black-legged tick, *I. pacificus*, in North America [Sonenshine, 1993; Eisen & Lane, 2002]. In Eurasia the castor bean tick and the taiga tick overlap and seem to be associated with different biotopes, as *I. persulcatus* is mainly found in the dryer taiga forest and is less sensitive to desiccation than *I. ricinus*, which is mainly represented in areas with higher humidity and denser ground vegetation [Mejlon, 2000].

The ticks have four development stages: egg, larvae, nymph and adult, and the life cycle is, in the case of *I. ricinus*, completed in about 3 years [Lees & Milne, 1951; Balashov, 1972; Gray, 1991]. The tick must feed in order to complete its life cycle but the tick feeds only once in each development stage. Sexual differentiation does not occur until the tick moults to the adult stage [Mejlon, 2000], and the mating places can occur on as well off host prior to the blood meal of the female [Sonenshine, 1991]. For adult females, a large blood meal is required to lay eggs and continue the developmental cycle and during a blood meal the body weight can increase more than 100-fold [Sonenshine, 1991]. The adult males generally do not require a large blood meal, and after mating they die [Balashov, 1972].

Ticks can feed on any terrestrial vertebrate but larvae and nymphs feed primarily on small hosts such as birds, lizards, hedgehogs and a variety of small and medium-sized mammals, while adults feed on medium-sized and large mammals [Jaenson et al., 1994]. Deer are important hosts for ticks and are also reported to be responsible for maintaining the tick abundance, by feeding adult females before they mate and lay eggs [Gray et al., 1992; Wilson et al., 1985; Jaenson, 1991; Fish & Dowler, 1989]. In southern Sweden the abundance of roe deer has increased in the last 150 years from initially only 100 animals located in the south to the current amount of over 1 million [Wahlström, 1995]. However, studies indicate that roe deer are incapable of infecting ticks with *B. burgdorferi* spirochetes [Telford et al., 1988; Jaenson & Tälleklint, 1992], instead a variety of mammals and small birds act as reservoir hosts [Tälleklint & Jaenson, 1993; Olsén et al., 1993].

Pathogen transmitting ticks posses ‘vector competence’, which means that a tick species can acquire spirochetes when feeding on an infective host, pass them transstadially and, subsequently transmit the spirochetes to a susceptible host while feeding [Eisen & Lane, 2002]. To be considered a ‘reservoir host’, the vertebrate species must be a source of infection for ticks. It must also fulfil the following transmission criteria that define a vector: it must be fed on by infected vector ticks, it must take up a critical number of infectious agents during an infectious bite, it must allow the pathogen to multiply and to survive for some time in its body and it must allow the pathogen to find its way into other feeding ticks. Each of these four steps are crucial to make a tick host an active link in the chain of transmission [Kahl et al., 2002].

The tick will incidentally feed on humans who are to be considered as dead-end hosts, unable to provide significant spirochetemia necessary for transmission of the microorganism. All stages of *I. ricinus* may bite humans, but studies indicate that nymphs are involved more often than either larva or adults, and thus are responsible for most cases of zoonotic disease that ticks transmit to humans such as LB, TBE and ehrlichiosis [O'Connell et al., 1998; Maiwald, 1998; Åsbrink & Hovmark, 1990]. Larvae seldom contains spirochetes since transovarial transmission rarely occurs in *I. ricinus* ticks [Bellet-Edimo, 1997].

The spirochete Borrelia burgdorferi sensu lato – the causative agent of Lyme borreliosis

The order *Spirochaetales* consists of two families and five genera [Canale-Parola, 1984]. The three genera *Treponema, Borrelia* and *Leptospira* contain spirochetes that can infect humans. The *Treponema* are more closely related to *Borrelia* than the *Leptospira* [Canale-Parola, 1984]. The *Borrelia* genus is named after the French bacteriologist A. Borrel (1867–1936) and the borrelia spirochetes are named after Dr Burgdorfer who discovered the aetiological agent of Lyme borreliosis in ticks [Burgdorfer et al., 1982]. The term *Borrelia burgdorferi* sensu lato (*B. burgdorferi* s.l.), collectively refers to the *Borrelia* genospecies found within the same complex.

The helically shaped spirochetes are gram-negative bacteria, 0.2–0.5μm in diameter and 20–30μm long that lives primarily as an extracellular pathogen. The spirochete consists of an outer membrane surrounding the protoplasmic cylinder with the periplasmic endoflagella positioned in-between [Åsbrink & Hovmark, 1990]. The spirochete is highly motile due to the endoflagella [Hayes & Burgdorfer, 1993]. The protoplasmic cylinder contains the cytoplasm and genetic elements composed of linear chromosomes and plasmids. The genes encoding the outer membrane are localised on these linear plasmids, which may enable the spirochete to express protective antigen variation. There are two abundant surface proteins: the outer surface protein A (OspA) and the outer surface protein B (OspB). When various *B. burgdorferi* s.l are compared, the Osps show considerable heterogeneity within and among the different species. Strains isolated from patients in North America seem to be more homogeneous while strains isolated from European patients are more heterogeneous with morphological differences [Hovind-Hougen et al., 1986; Barbour AG et al., 1985].

Three *B. burgdorferi* s.l. genospecies have been isolated from humans with Lyme borreliosis: *B. burgdorferi* sensu stricto (*B. burgdorferi* s.s.), transmitted by the tick species *I. pacificus*, *I. scapularis* in North America and *I. ricinus* in Europe and Asia. *B. afzelii and B. garinii* transmitted by the species *I. ricinus* and *I. persulcatus* in Europe and Asia [Eisen & Lane, 2002].

Table I. The distribution of the Ixodes ticks and the spirochetes causing Lyme borreliosis in humans [Eisen & Lane, 2002].

		Pathogen		
***Ixodes* vector**	**Geographical distribution**	**Bb ss[a]**	**B afz[b]**	**B gar[c]**
I. ricinus	Europe, far western Asia, northern Africa	X	X	X
I. persulcatus	Far-eastern Europe, Asia		X	X
I. pacificus	Western North America	X		
I. scapularis	Eastern North America	X		

[a] *B. burgdorferi sensu stricto,* [b] *B. afzelii,* [c] *B. garinii*

The prevalence of *B. burgdorferi* spirochetes in *I. ricinus* nymphs in Sweden is reported to range from 6 to13% and in adult females from 15 to 36% [Mejlon & Jaenson, 1993].

B. burgdorferi s.l. has also been detected in other species including insects, mosquitoes, flies and fleas. However there are no indications that insects can serve as vectors for LB [Magnarelli & Anderson, 1988; Hubálek & Halouzka, 1998].

Epidemiological aspects of Lyme borreliosis

In Europe, LB has been reported from almost every country. Endemic areas include Scandinavia, the Baltic countries, central and eastern Europe [Stanek et al., 1993]. The tick *I. ricinus* is present in southern Sweden and along the entire coast of the Baltic Sea [Jaenson et al., 1994]. In Sweden most of the LB cases, over 70%, are clinically manifested by EM [Berglund et al., 1995]. Most patients are affected by LB from May through September but infections can occur all year around [Mejlon & Jaenson, 1993; Berglund et al., 1995]. The age distribution is bimodal in Europe and the United States with peaks among children and in adults [Berglund et al., 1995; Orloski et al., 2000; Chow et al., 2003; Dhôte et al., 2001].

Risk factors of being exposed to ticks and tick-borne diseases depend on tick abundance, geographical distribution of ticks in the area and the tick-host relationship [Walker et al., 2001; Robertson et al., 2000; Randolph, 2001; O'Connell et al., 1998; Mejlon et al., 1993; Vassalo et al., 2000; Gray et al., 1998; Lindgren & Gustafson, 2001]. The risk of acquiring LB in south-eastern Sweden is 0.5% per tick bite [Stjernberg & Berglund, 2002b], and darker clothes seem to be associated with a decreased risk of tick bites [Stjernberg & Berglund, 2005a].

In southern Sweden, in 1992, an annual incidence rate of LB of 69 cases per 100,000 inhabitants was reported [Berglund et al., 1995]. In the same study the county of Blekinge was found to be highly endemic for LB with 133 cases per 100,000 inhabitants. In 1997, an incidence rate of 306 cases per 100,000 inhabitants was reported, thus indicating that the LB incidence had increased. Our intentions in **paper III** was thus to study the long-term incidence rate of LB with respect to EM in a population living in a highly endemic area for LB, the county of Blekinge.

Environmental and climate effects on tick abundance and activity

Ticks prefer deciduous woodlands in temperate climates that also harbour large mammals such as deer and elk. Ticks make use of diapause mechanisms to regulate their periods of host seeking activity, which is an approach by ticks to ensure that host seeking activities occur at favourable times of the year, avoiding dry or cold periods [Gray, 1991]. They are generally actively seeking a host during the spring and fall. Ticks that feed in the autumn emerge into the next stage in the autumn the following year, while those feeding in the spring develop directly and moult in the following autumn [Gray, 1982; Randolph et al., 2002b].

In the spring when the mean weekly maximum air temperature rises above 7 ºC the ticks become actively host-seeking [Gray, 1984]. Ticks are reported to rarely survive winter temperatures below –10 ºC [Dautel & Knülle, 1997] or relative humidities (RH) of less than 80%, which restricts them to habitats in which humidity at the base of vegetation rarely falls below 85% RH [Kahl & Knülle, 1988; Randolph & Storey, 1999]. Further, precipitation is reported to have a negative affect on tick attachment rates to humans [O'Connell et al., 1998], and the dampness may also affect the tick host seeking activity by impairing their ability to climb the vegetation.

Studies indicate that climate change is reported to have an impact on tick abundance. Mild winters, early springs, warm summers and warm autumns with a high relative humidity seem to increase tick abundance and the risk of tick-borne encephalitis [Lindgren et al., 2000; Lindgren & Gustafson, 2001; Randolph & Storey, 1999; Randolph, 2001; Randolph, 2002a]. Few reports of climate effect on LB have been produced and therefore we studied the correlations between the seasonal variations in EM incidence and climate factors (**paper III**).

Erythema migrans

As mentioned, the Swedish dermatologist Arvid Afzelius described and named the lesion 'erythema migrans' in 1910, but an Austrian dermatologist noted longstanding erythemas and therefore proposed the name 'erythema chronicum migrans' [Lipschütz, 1914]. Today, the former name is used since it is not considered correct to use the name

'chronic' in both the early EM and late ACA cutaneous manifestation [Åsbrink & Hovmark, 1990].

A *Borrelia*-infected tick is reported to transmit the spirochete to the human skin within 48 hours after tick attachment while feeding [Kahl et al., 1998]. Commonly the infection results in a self-limiting subclinical infection sometimes leaving only specific IgG antibodies, as a sign of infection [Wormser, 2001]. Infected patients may subsequently develop erythema migrans and/or disseminated clinical manifestations of LB.

Lyme borreliosis can be divided into an 'early' or 'late/chronic' infection. Early infections are represented by the localised EM – the most common clinical manifestation representing over 70% of the LB cases [Berglund et al., 1995] – and by disseminated infections such as multiple EM-like lesions, neuroborreliosis, borrelia lymphocytoma (BL), Lyme arthritis (LA), carditis or other organ involvement. Late/chronic infections are usually diagnosed 6 months to years after the primary infection and are represented by ACA, chronic joint/bone involvement, chronic neurological manifestations or other organ involvement. Thus like syphilis, Lyme borreliosis can be divided into different phases acting as another 'imitator' where EM can be compared with the primary chancre in syphilis [Åsbrink & Hovmark, 1990].

Some major differences in the clinical manifestations of LB in the USA and Europe can be seen. In the USA, arthritis and multiple EM are more common than in Europe while BL and ACA rarely occur in the USA [Steere, 2001].

The diagnosis of EM is primarily clinical and the currently used serological tests for antibodies to *B. burgdorferi* are of low diagnostic value, since only 10–40 % of patients are reported to be positive [Åsbrink et al, 1984; Ackerman et al., 1984; Åsbrink et al., 1985b; Steere et al., 1983b]. Due to the fact that cultures of spirochetes from skin biopsies are time-consuming and have a low sensitivity, it is not considered as a routine diagnostic method [Åsbrink & Hovmark, 1990]. The polymerase chain reaction (PCR) technique used in detecting *B. burgdorferi* spirochetes in skin biopsies is currently not a routine method, but may be of diagnostic value.

In earlier studies, EM are reported to appear mostly on the lower limbs [Berglund et al., 1995; Åsbrink & Olsson, 1985], and less than half of the patients have noticed the bite [Steere et al., 1983a; Åsbrink & Olsson, 1985]. The incubation period from tick bite to rash onset is reported to vary from 1–3 weeks [Steere et al., 1983a; Berger, 1984; Weber et al., 1983] and generally the EM begins as a red macula or papule which expands centrifugally. The lesion may be misdiagnosed as for example tinea, erysipelas, fixed drug eruptions, granuloma annulare, lupus erythematosus [Åsbrink & Hovmark, 1990]. EM is typically 'homogeneous' or annular with central clearing. However, atypical lesions also occur with blisters, haemorrhagic or scaling lesions with irregular borders [Åsbrink, 1991; Smith et al., 2002; Müllegger, 2001]. Local symptoms such as itching, burning sensation (dysaesthesia) may occur as well as systemic flu-like symptoms with chills and low grade fever, regional lymphadenopathy, myalgia, arthralgia, headache and

fatigue, that may indicate a disseminating infection [Steere et al., 1983; Berger, 1984; Åsbrink et al., 1986; Åsbrink & Olsson, 1985].

European patients with EM seem to be asymptomatic to a higher extent than North American patients [Strle et al., 1999]. There are few reports in Europe on differences in clinical manifestations caused by the different genospecies of *Borrelia burgdorferi* s.l. Therefore we studied the clinical characteristics of EM infections caused by the *Borrelia* genospecies that are prevalent in the county of Blekinge (**paper IV**).

There has been some debate as to whether the different appearances of the lesions are a result of the duration of the EM at presentation or due to infection by different genospecies [Logar et al., 2004; Åsbrink & Olsson, 1985; Carlsson et al., 2003; Steere et al., 1983a]. We described and categorised the EM into their different clinical appearances and studied factors (i.e. 'gender', 'age', 'genospecies', 'time') influencing this (**paper IV**).

Reinfections of Lyme borreliosis

A new infection of LB i.e. 'reinfection', can occur after successful antibiotic treatment. Even in patients with disseminated LB, reinfections are reported. In a study from south-eastern Sweden, of patients diagnosed with neuroborreliosis in 1992–1993, 4% reported a physician diagnosed reinfection with LB during the 5-year follow-up [Stjernberg & Berglund, 2002a], indicating that disseminated manifestations of LB do not give life-long immunity to LB.

On a small island on the south Swedish coastline, a frequency of reinfection of 9% during a study-period of 3 years was reported [Berglund et al., 1996]. In the USA the annual frequencies reported from endemic parts in the north-east varies; a frequency of reinfection was reported in 18% amongst 215 individuals followed up to 5 years after their primary infection in Westchester County, NY [Asch et al., 1994] and in patients evaluated up to 7 years, a total frequency of reinfections of 9% (5/57) was reported in coastal Massachusetts [Lastavica et al., 1989]. In order to study the frequency of reinfections with LB in a greater perspective, a follow-up study of over 1000 patients initially diagnosed with EM was conducted (**paper I**).

Antibiotic treatment of erythema migrans

If treated with adequate antibiotics the EM lesion heals within 2–4 weeks. However, if the infection leaves untreated, most lesions heal spontaneously within 10 weeks but might last up to a year [Åsbrink & Olsson, 1985]. The risk of a disseminated infection also increases without treatment [Steere, 1987; Åsbrink & Olsson, 1985; Weber & Neubert, 1986].

Because of lack of studies there are no international consensus concerning the optimal dose or duration of antibiotic treatment of LB. Standard-methods for determining the

minimum inhibitory concentration and minimum bactericidal concentration have not been established for *B. burgdorferi* s.l.

The spirochetes are resistant to aminoglycosides, trimethoprim and sulphonamides, quinoline derivates and rifampicin but are inhibited in vitro by several groups of antibiotics such as tetracyclines, amoxicillins, cephalosporines, macrolides and thienamycins. Penicillin is also clinically efficient despite its moderate inhibitory effect on the spirochete [Preac-Mursic & Wilske, 1993; Weber & Phister, 1994]. Few studies have been performed on the long-term outcome of patients with EM treated with antibiotics, and this was our incentive for doing **paper II**.

Table II. Treatment recommendations for erythema migrans in Sweden. [Läkemedelsverket, 1998]

	Adults (reduction of dosage to the elderly)	**Children 8-12 years**	**Children < 8 years**
Uncomplicated Erythema migrans	PcV 1 g x 3, 10 days	PcV 12,5 mg/kg x 3, 10 days	PcV 12,5 mg/kg x 3, 10 days
Penicillin intolerance	Doxycyklin 200 mg x 1, day 1, 100 mg x 1, for 8 days	Cefuroxim axetil 15 mg/kg x 2, for 10 days	Cefuroxim axetil 15 mg/kg x 2, for 10 days
Penicillin allergy (Type I)	Doxycyklin 200 mg x 1, day 1, 100 mg x1, for 8 days	Doxycyklin 4 mg/kg, day 1, 2 mg/kg/daily, for 8 days	Azitromycin 10 mg/kg, day 1, 5 mg/kg/daily for 4 days
Pregnant	PcV 2g x 3, for 10 days		

Aims

The general aim was to study the epidemiology and clinical characteristics of EM, in a primary health care perspective.

Specific aims

- To follow the long-term outcome of patients treated for EM, with respect to the annual mean prevalence of new infections with LB (**paper I**).
- To follow the long-term outcome of patients treated for EM, with respect to the occurrence of disseminated LB infections during and after the treatment with antibiotics (**paper II**).
- To study the incidence rate of EM in a population living in a highly endemic area for LB (**paper III**).
- To study possible correlations between the seasonal variations in EM incidence rates and climate factors (**paper III**).
- To study the clinical characteristics of infections caused by *Borrelia* genospecies in patients with EM, where genospecies were PCR-confimed (**paper IV**).
- To describe and categorize verified EM (**paper IV**)
- To study factors influencing the clinical appearances of verified EM (**paper IV**)

Materials and methods

Study areas and populations

Southern Sweden – papers I & II

The study area comprised the 7 southern counties in Sweden including over 2 million inhabitants, 49% men and 51% women, and an area of 49,000 km².

The area is endemic for LB, with 69 cases per 100,000 inhabitants reported in 1992-1993, but with a considerable variation in incidence rates ranging from 26 per 100,000 to 160 per 100,000 inhabitants, with the higher incidence rates found in the south-eastern parts [Berglund et al., 1995]. Ticks collected from different sites and biotopes contain *B. burgdoferi* s.l. at a frequency varying between 6 and 36% [Berglund & Eitrem, 1993; Mejlon & Jaenson, 1993; Fraenkel et el., 2002].

Agricultural land covers the area in the western and southern parts of the region and forests in the northern and eastern parts. The south eastern parts borders on the Baltic Sea and the south and western parts borders on the Kattegatt and Öresund. The climate is characterised as 'warm temperate,' with mild winters having mean monthly temperatures above –3ºC and summer mean temperatures around 15 ºC.

Patients included in **papers I & II** were all diagnosed and treated for EM in the 'South Swedish borrelia survey' in 1992 [Berglund et al., 1995].

The county of Blekinge – papers III & IV

The study area, the county of Blekinge located in the south-eastern part of Sweden covers an area of 2941 km² and a population of 150,000 inhabitants of which over 123,000 (50% women, 50% men) receive care through the national primary health care system.

The area is highly endemic for LB with 133 cases per 100,000 inhabitants reported in 1992. The county has a rich animal life, especially with respect to tick hosts such as birds, rodent, deer and moose. Deer are assumed to be the main host for ticks in this area. On the island of Aspö, 11% of the nymphal ticks and 26% of the adult ticks have been found to be infested with *B. burgdorferi* s.l. [Berglund & Eitrem, 1993].

In **paper III,** patients diagnosed and treated for EM in primary health care during 1997 – 2003 were included.

In **paper IV,** the study population included patients seeking care for a suspected EM, from May 2001 to December 2003, at seven outpatient clinics.

Study participants and design

Follow-up study on erythema migrans in southern Sweden – papers I & II

In May 1998, a retrospective long-term follow-up study was conducted including patients over 15 years old who all were diagnosed and treated with antibiotics for EM in the 'South Swedish borrelia survey' conducted 1992–1993. The aims were to follow the long-term outcome of in these patients, with respect to the annual incidence rate and total frequency of new infections with LB and to estimate the occurrence of disseminated LB infections during and after the treatment with antibiotics.

After a dropout of 206 people not anwering the questionnaires, 708 individuals participated in the study. The included patients answered a questionnaire concerning the antibiotic treatment and clinical symptoms during and after the treatment of EM.

Patients reporting symptoms or LB that had been seen by a doctor, were contacted to obtain more detailed information and to give their permission to study the medical record. In cases of 'reinfection' we required the following criteria:

- a doctor's visit to confirm the EM diagnosis and the prescribed antibiotic treatment
- in the cases of an EM, a description of the erythema, expanding over a period of days to weeks after the tick bite and reaching a diameter of at least 5 cm
- for other LB manifestations, such as LA, neuroborreliosis, BL and ACA a description of the characteristic clinical manifestation of the disease, a serological confirmation indicating a 4-fold elevation of the titre of *B. burgdorferi* antibodies, or a seroconversion when acute and convalescent sera were analysed simultaneously on the same enzyme-linked immunosorbent assay (ELISA) microplate, were required in the medical records. In the case of BL a skin biopsy with typical histological findings was required.

Epidemiological and climate study on erythema migrans – paper III

In this retrospective study we used the electronic patient record systems 'Swedestar' and 'PAS-ORIGO' to search for all medical records of patients diagnosed with EM attending primary health care and the Department of Infectious Diseases at the county hospital of Blekinge. The study period covered six years 1997–2003, from April 1 through March 31. The seasonal start was the month when most ticks become active in this region [Gray, 1982].

The following inclusion criteria were required:

- information indicating that the EM was preceded by a probable tick bite
- a description in the record of the clinical appearance and size of the lesion reaching at least 5 cm in diameter

- a prescription of antibiotics at the time for diagnosis.

We studied the influences of certain climatic thresholds on EM incidence rates according to earlier published data on climate influence on tick abundance and tick activity [Gray, 1984; Dautel & Knülle, 1997; Kahl & Knülle, 1988; Randolph & Storey, 1999; Knülle & Rudolph, 1982]. Only thresholds applicable to the prevailing climate in the study area were used in the present study. The climate variables are presented in 'Table V'.

To detect other possible correlations, we studied the general influence of the mean temperature, relative humidity and precipitation on EM incidence rates using continuous data from monthly mean temperatures, relative humidity and precipitation.

Climate data were measured from May 15 to September 14 and were correlated with EM incidence rates 14 days later since this is the approximate duration of the incubation period from tick bite to EM. Climate information on temperature, relative humidity, and precipitation were obtained from the Swedish Meteorological and Hydrological Institute. The data were measured 1.5 metres above ground level in Ronneby/Bredåkra, which is located in the central part of the county of Blekinge. The temperature was measured in degrees Celsius, the relative humidity in percent, and the precipitation in millimetres.

Study on clinical characteristics of erythema migrans – paper IV

This prospective study included patients 18 years and older, seeking care for a suspected EM reaching over 5 cm in diameter. Borrelial origin of EM was verified through a positive PCR analysis.

At the doctor's visit, the patients giving their informed consent to participate answered a questionnaire concerning information about the tick bite, the erythema and new clinical symptoms. The erythema was photographed together with an ID number and a plastic ruler using a digital camera. Complete blood counts and liver function tests were performed and 'c-reactive protein' (CRP) levels measured. Serologic testing was done in a routine lab measuring titres of IgM and IgG according to manufacturer's protocol. Skin punch biopsies were taken from the leading edge of the EM after the administration of local anaesthetics. The biopsies were analysed with a PCR method targeting the ospA gene.

All patients were treated with antibiotics. After 14 days the patients were contacted by a nurse asking questions concerning the clinical appearance of the EM and possible clinical symptoms.

Classification of verified erythema migrans lesions

The photos of the lesions were classified by three physicians with extensive experience of treating patients with Lyme disease. The skin lesions were classified into the following predominant patterns:

- *annular erythemas*; round to oval red to bluish red lesions, sharply demarcated with a classic central clearing [Smith et al., 2002; Müllegger, 2001]. The 'bulls eye rash' is a type of annular EM with a darker central bluish-red maculae separated from the peripheral ring by normal skin [EUCALB 1997–2005, Müllegger, 2001].
- *non-annular erythemas*; including 'homogeneous' erythema with homogeneous red sharply demarked lesions [Smith et al., 2002; Müllegger, 2001] and 'central' erythemas; dense central, red to bluish-red lesions surrounded by a paler peripheral ring [Smith et al., 2002].
- *atypical erythemas*; lesions and/or pictures not possible to place in any of the above categories.

PCR and sequence analysis

In order to confirm the EM diagnosis and determine the infecting genospecies, *Borrelia* DNA was amplified using a nested OspA PCR followed by nucleotide sequencing as previously described [Ornstein et al., 2002]. Briefly, DNA extraction was performed using a DNeasy tissue kit (Qiagen, Valencia, Ca) according to manufacturer's protocol using an elution volume of 50µl. The master mix (PCR Core Kit, Roche Diagnostics GmbH, Penzberg, Germany) contained 0.2 µM of each primer, 0.2 µM of each deoxynucleoside triphosphate and 1.25U *Taq* DNA polymerase. A volume of 5 µl and 1 µl DNA template was used in the first and second PCR reaction, respectively. The PCR amplification conditions were: 35 cycles of 94° for 30s, 50° for 60s and 72° for 60s. DNA amplicons were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide. Positive DNA samples were sequenced using the OspA PCR inner primer pair [Ornstein et al., 2002]. ABI PRISM BigDye Terminator v.3.1 Ready Reaction Cycle Sequencing Kit was used (Applied Biosystems) according to manufacturers protocol. Each strand was analysed in an ABI 3100 Genetic Analyzer (Applied Biosystems) by the Biomolecular Resource Facility at Lund University. The BioEdit software (Tom Hall, Department of Microbiology, North Carolina State University, NC) was used for nucleotide sequence analysis.

Statistical methods

Papers I–IV

In all studies *p*-values were two-tailed and *p*-values < 0.05 were considered significant. Students *t*-test was used when comparing normally distributed continuous data. The chi-square (χ^2) test was used when comparing categorical data and the Mann-Whitney U-test was used when comparing non-parametric continuous data.

Analyses were done using the statistical computer software SPSS, version 11.0 (SPSS Corporation, Chicago, Illinois, USA) and Stata, version 8.0 (Stata Corporation, Texas, USA).

Paper III

A multilevel analysis using multiple Poisson regression analysis was performed to evaluate the effect of a particular climate factor after adjustment for other climate factors [Goldstein, 2003]. To examine the extent to which individual and climate characteristics explain the variability in incidence rates of EM, we used a two-level model with a random intercept, with individuals at the first level and municipalities in Blekinge county at the second level. The associations between the variables studied were appraised by incidence rate ratios (IRR) (95 percent confidence intervals (CIs)) in the fixed-effects part of the model. MLwiN software, version 2.0 [Rasbach et al, 2000] was used to perform the analyses. Parameters were estimated by using iterative generalised least squares. Use of restricted iterative generalised least squares gave very similar results.

Paper IV

To evaluate which factors had the greatest influence on the appearances of the lesions, a logistic regression analysis model was used to study the relations between factors influencing the clinical appearance (i.e. annular or non-annular EM) of the EM. The associations between the variables studied were appraised by odds ratios estimating the relative risks for non-annular EM. The variables 'female gender' and '*B. afzelii'*, were set as baseline variables.

Results

Epidemiological aspects of erythema migrans

Paper III

Over 3400 cases were identified and fulfilled the inclusion criteria for EM. This is equivalent to an annual mean incidence rate of 464 cases per 100,000 inhabitants. Women had a significant higher incidence rate than men (506 and 423 cases per 100,000 inhabitants, $p < 0.001$). Patients 15–64 years old had a 47% higher incidence rate ($p < 0.001$) and elderly over 65 years had a 94% higher incidence rate ($p < 0.001$) than youths under 15 years old. The age- and incidence rate distribution was bimodal with a predominant peak in women aged 65–69 of 1174 cases per 100,000 inhabitants, and a less prominent peak amongst boys aged 5–9.

Totally 98.3% of the cases with EM were diagnosed and treated in primary health care.

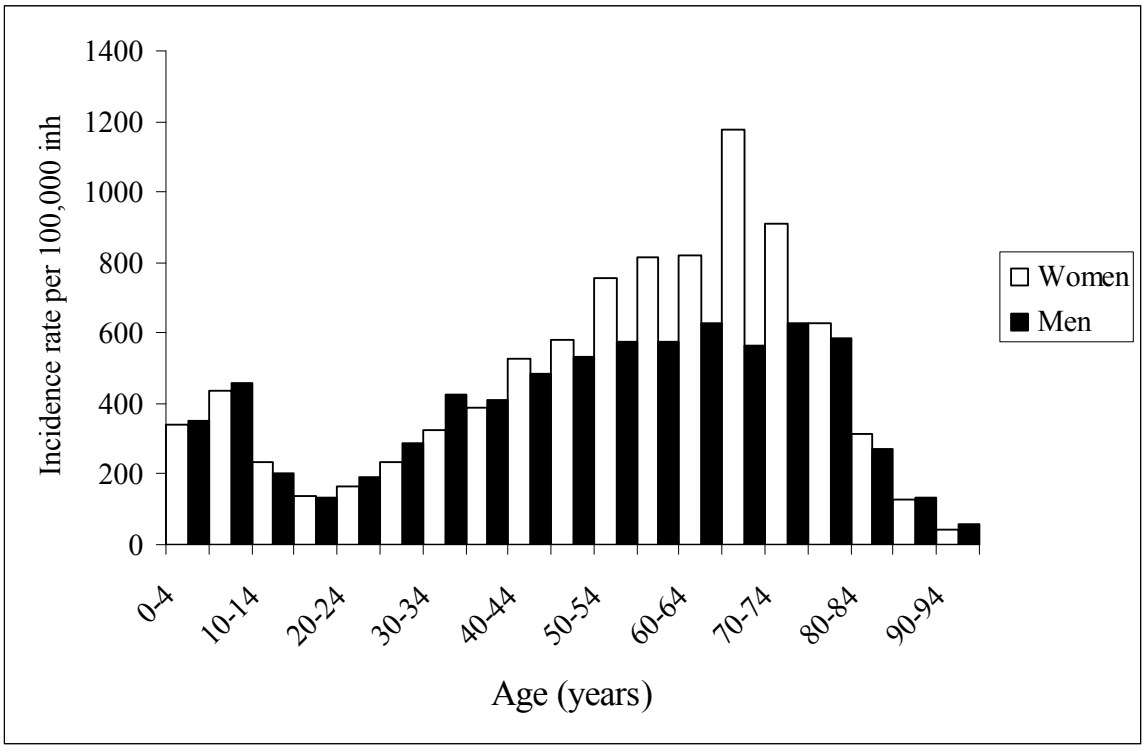


Figure 1. The age and gender distribution of the incidence rates of erythema migrans per 100,000 inhabitants in south-eastern Sweden

Generally almost every second case (47%) occurred in July and August. Youths younger than 15 years of age were to a higher extent affected by EM in July compared with patients 15 years and older who primarily were affected in August.

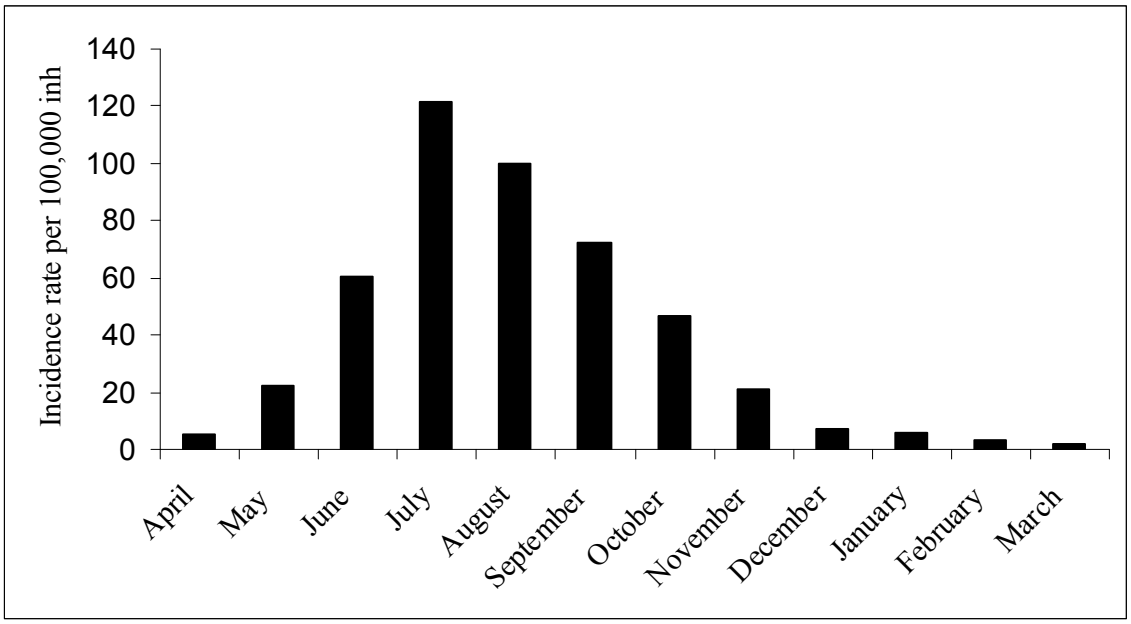


Figure 2. The monthly distribution of the incidence rate of erythema migrans per 100,000 inhabitants in south-eastern Sweden

Paper I

From May 1993 to the end of April 1998 there was an annual mean prevalence of reinfection with LB of 1%, in southern Sweden. The prevalence from May 1993 to the end of April 1994, the first observation year after the EM infection, was significantly lower compared with the overall prevalence 1993-1998 (0.3% vs 1.0%, $p<0.05$).

The number of tick bites influenced the risk of reinfection: those bitten > 10 times during the observation period had significantly more reinfections than those bitten < 5 times (women: 13/43 vs 10/154, $p < 0.001$ and men 2/26 vs 1/104, $p < 0.05$) which is equivalent to relative risks of about 4 and 8 respectively.

When studying the seroreactivity in patients with EM in 1992–1993, no significant differences were found in seropositivity between the reinfected individuals and those without a reinfection, 13/20 and 160/359 respectively. Serological results were not available from all individuals.

Clinical aspects on erythema migrans

Paper IV

One-hundred and eighteen patients (women=54, men=64) fulfilled the inclusion criteria. A total 92% of the patients (109/118) had noticed a tick bite at the location of

a later EM skin lesion. A total 44.7% (38/85) of the EM were annular, 45.8% (39/85) were non-annular and 9.4% (8/85) were 'atypical'.

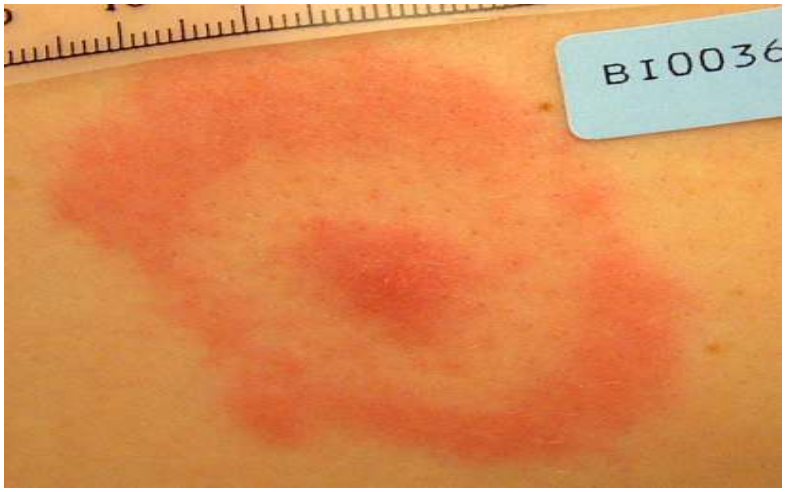


Figure 3. An example of an annular erythema migrans.

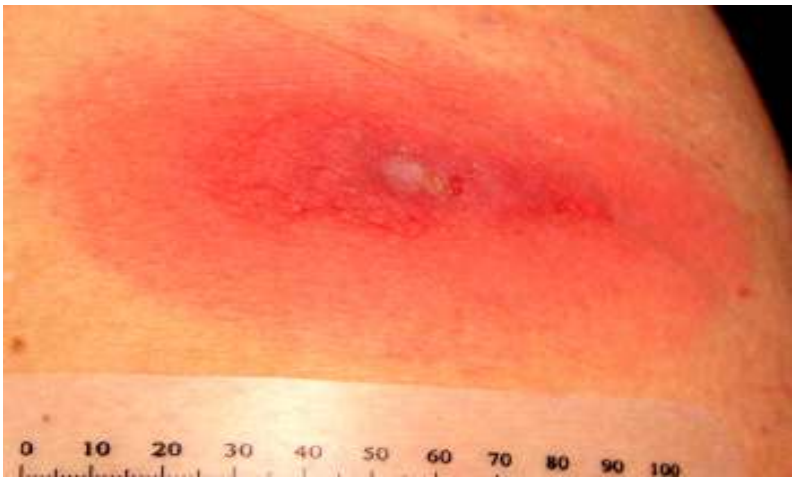


Figure 4. An example of a non-annular erythema migrans.

In order to identify variables with an effect on the clinical appearance of the EM, the different variables were studied in a logistic model in subsequent steps. The variable 'size of the lesion' was not included in the model since the factor was considered to be influenced by 'time' [Nadelman et al., 1996]. We studied the influence of the following variables: 'time', i.e. duration from tick bite to diagnosis, 'genospecies', 'age', 'gender'. The variable 'time' (i.e. duration from tick bite to diagnosis) was then excluded from the model since it was not found to have a significant influence on the appearance. The variables 'female gender' and '*B. afzelii'* were set as baseline variables. In this model, odds ratios to estimate the relative risks of a non-annular EM were studied. An interaction between gender and genospecies was found; the

appearance of the lesion depended both on the gender of the individual and on the genospecies causing the infection. The odds ratio for males infected with *B. afzelii* developing non-annular EM was only 0.09 (95% CI: 0.03–0.33), while the odds ratios were similar for females and males infected with *B. garinii* developing non-annular EM, 1.74 (95% CI: 0.29–10.34) and 1.98 (95% CI: 0.34–11.56) respectively. The odds ratio of the interaction factor between gender and genospecies was 12.46 (95% CI: 0.98–158.80).

Table III. Odds ratio of non-annular erythema migrans according to gender and Borrelia genospecies. The variables 'time', i.e. duration from tick bite to diagnosis, 'genospecies', 'age', 'gender' were included in the logistic model. 'Female gender' and 'B. afzelii', are set as baseline variables. The odds ratio of the interaction factor between gender and genospecies is 12.46 (95% CI: 0.98–158.80). N=77.

	Gender	
	Female (95% CI)	**Male (95% CI)**
Genospecies		
B. afzelii	1.00	0.09 (0.03–0.33)
B. garinii	1.74 (0.29–10.34)	1.98 (0.34–11.56)

CI= Confidence Interval

Significantly more individuals were infected with *B.afzelii* than with *B. garinii,* 73.7% and 26.3%, respectively ($p < 0.001$). The median number of days from tick bite to the initial visit was 17.5 days (d) (range 3.0-97.0 d), in which patients infected with *B. garinii* had a significant shorter duration compared with patients infected with *B. afzelii*; 14.0 d (4.0-78.0 d) and 21.0 d (3.0-97.0 d) ($p = 0.011$), but there were no significant differences in the median size of the lesions at the initial visit (70.0 cm² and 93.0 cm²).

The most common symptoms were headache 27.1% (32/118), muscle/joint pain 14.4% (17/118) and chills 10.1% (12/118). Less common were airway symptoms 8.5% (10/118), neurological symptoms 6.8% (8/118), neck-stiffness 3.4% (4/118) and light sensitivity 1.7% (2/118). Although not a statistical significant finding, two patients (1.7%) infected with *B. garinii* had temperatures above 38.0ºC, compared with none of the patients infected with *B. afzelii* (p=0.06). No significant differences in the frequency of clinical symptoms were found between the genospecies.

Significantly more patients infected with *B. garinii* (12/31) had elevated levels of CRP compared with patients infected with *B. afzelii* (13/87) ($p = 0.006$).

Additionally, 40.4% of all the acute serology results were positive at the initial visit and no differences were seen between the genospecies, *B. afzelii* and *B. garinii.*

It took about a week for the EM to heal after the initiation of treatment, median duration 8.0 d (1.0–35.0 d), with no significant differences between lesions caused by *B. afzelii* or *B. garinii*, median duration 8.0 d (1.0–35.0 d) and 7.0 d (2.0–17.0 d) respectively.

Paper II

In the 5-year follow-up study of patients treated for EM in 1992–1993, a total 98% (556/566) and 94% (100/106) of the patients treated with phenoxymethyl penicillin (pcV) and doxycycline reported complete recovery, respectively. In 17 individuals, the EM did not heal during treatment and seven of these individuals had not completed treatment at the recommended dose. Significantly more individuals treated with doxycycline reported additional symptoms that required a doctors visit during the treatment compared with patients treated with pcV (12/107 vs 12/567, $p < 0.001$). A total 16% developed new symptoms that required a doctor's visit during the 5-year follow-up.

Gender aspects on erythema migrans

Paper III

In this epidemiological study, the incidence rates of EM in women was higher than in men, 506 and 423 cases per 100,000 inhabitants respectively ($p < 0.001$). Especially women over 50 years were affected, with a peak of 1174 cases per 100,000 inhabitants amongst women aged 65–69.

The women were older than the men, mean age women 50.5 years and mean age men 45.5 years ($p < 0.001$)

Paper I

In the 5-year follow-up study, significantly more women than men (6% vs 1%, $p < 0.01$), were reinfected with EM.

When comparing gender according to the number of tick bites, women and men were bitten equally by ticks. All women were older than 44 years.

Table IV. The distribution of reinfections amongst men and women according to the number of tick bites. n/N=percentage of tick-bitten individuals, reinfected.

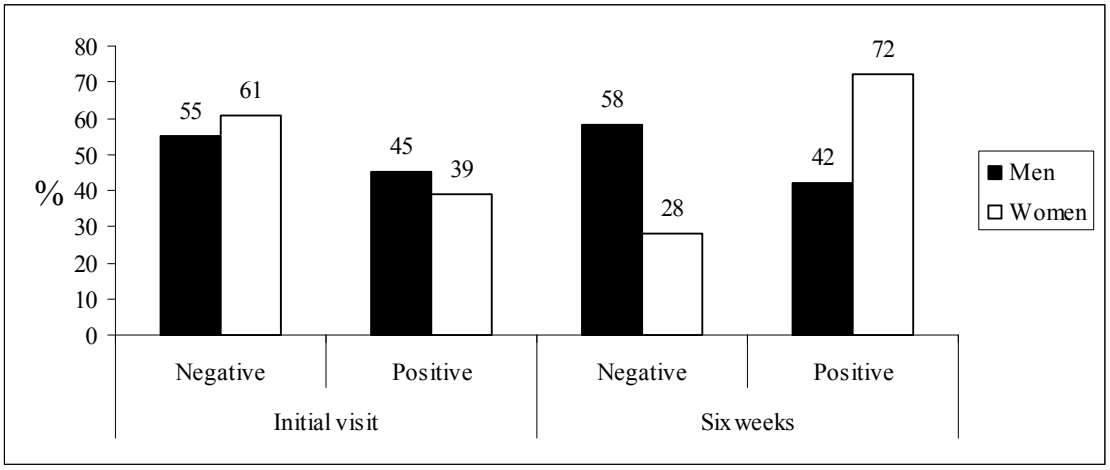
No of tick bites	Women; n/N (%)		Men; n/N (%)		*p*
0	0/194		0/130		
<5	10/154	(6)	1/104	(1)	<0.05
6–10	4/36	(11)	1/21	(5)	ns
>10	13/43	(30)	2/26	(8)	<0.05
Σ n/N	27/233	(12)	4/151	(3)	0.01
Risk ratio	4.7 (13x154/43x10)		8.6 (2x104/1x26)		

Paper IV

Gender was found to have a strong influence on the appearance of the lesion depending on which genospecies caused the infection. The odds ratio for males infected with *B. afzelii* developing non-annular EM was only 0.09 (95% CI: 0.03–0.33), while the odds ratios were similar for females and males infected with *B. garinii* developing non-annular EM, 1.74 (95% CI: 0.29–10.34) and 1.98 (95% CI 0.34–11.56) respectively. The odds ratio of the interaction factor between gender and genospecies was 12.46 (95% CI: 0.98–158.80). 'Time', i.e. the duration from tick bite to diagnosis, was not found to have an influence on the appearance of the lesions.

There were no differences between gender with respect to seropositivity at the initial visit. However after 6 weeks significantly more women than men were seropositive (72% vs 42%).

Figure 5. The percentage of seropositive and seronegative women and men with verified erythema migrans, at the initial visit and after six weeks.



The lesions healed within about a week after the initiation of the treatment; median 8.0 d (1.0-35.0 d). However, the EM disappeared faster in men, median 7.0 d (1.0-21.0 d) than in women, median 11.0 d (2.0–35.0 d) ($p = 0.008$).

Climate aspects on erythema migrans

Paper III

Considerable variation was found in the seasonal EM incidence rates. In a multilevel analysis using the Poisson regression model, the correlations between the EM incidence rates and climate data were studied. The following climate variables were found to correlate with the EM incidence rates during the summer months June through September.

- the monthly mean temperature, measured in ºC (IRR = 1.12; CI 95%, 1.08–1.16; $p < 0.001$),
- the number of days during the winter (from December 1st to the last day of February) with mean temperatures below 0ºC (IRR = 0.97; CI 95%, 0.97–0.98; $p < 0.001$).
- the monthly mean precipitation, measured in mm (IRR = 0.92; CI 95%, 0.84–0.99; $p < 0.05$)
- the number of days during the summer with levels of relative humidity (RH) above 86% (IRR 1.04; CI 95%, 1.00–1.06; $p < 0.05$).

Figure 6. The seasonal incidence rates of erythema migrans in the county of Blekinge 1997–2003.

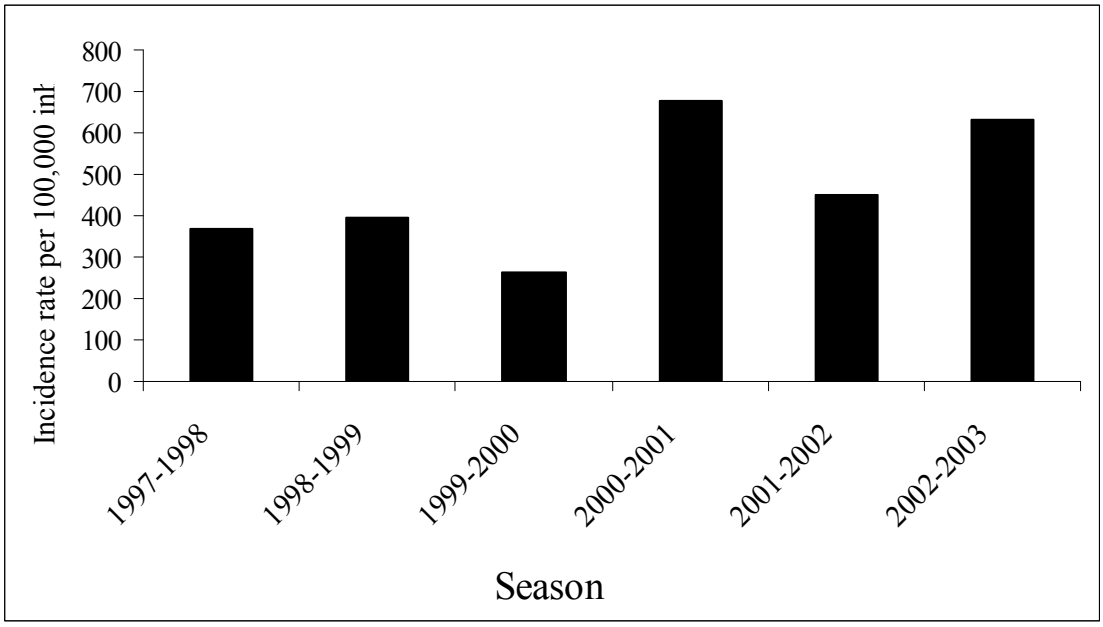


Table V. Climate variables included in the Poisson regression model, influencing Lyme borreliosis incidence from June 1 to September 30 in southern Sweden.

Cimate parameter	**IRR**	**95% CI**		***p***
Monthly mean temperature [A]	1.12	1.08	1.16	<0.001
Monthly mean relative humidity [,A]	0.98	0.94	1.02	ns
Monthly mean precipitation [A]	0.92	0.84	0.99	<0.05
No. of days relative humidity above 86% [A,B]	1.04	1.00	1.06	<0.05
No. of cold winter days below 0 ºC [A,C]	0.97	0.97	0.98	<0.001
No. of cold winter days below 0 ºC, the previous year [A,C]	1.00	0.99	1.01	ns
Variance:	**Variance**	**SE**		
Between municipalities	0.64	0.05		

[A] Climate data were measured from May 15 to September 14 and correlated with EM incidence 14 days later since this is the approximate duration of the incubation period from tick bite to EM diagnosis.

[B]The tick is actively seeking a host if the relative humidity is 86–96%. Below this threshold the tick is dehydrates and cannot continuously seek a host [Knülle & Rudolph, 1982].

[C] Climate factors during winter affecting tick abundance the following seasons, climate data measured from December 1 to the last day of February. Cold winter days with temperatures below 0 ºC can negatively influence tick abundance by reducing the survival of the ticks and their hosts (Jeremy Gray, personal communication). This outcome could affect tick abundance the following 2 years since the tick lives 3 years with larvae and nymphs as host-seeking stages.

Discussion

This thesis has focused on EM from a primary health care perspective including aspects on epidemiology, the clinical picture, gender differences and the climate.

Epidemiological aspects of erythema migrans

The county of Blekinge was found to be highly endemic for LB with a mean annual incidence rate of 464 cases per 100,000 inhabitants (**paper III**). Since 1992, the incidence rates of EM have increased more than 3-fold in the county of Blekinge and the incidence rates are now at the same level as reports from the most endemic areas of LB in the USA [Chow et al., 2003]. Over 98% of patients with EM are treated and diagnosed in primary health care.

The human outdoor exposure seems to be of great importance for the risk of LB. The seasonal activity of the *I. ricinus* nymphs peaks from March to May, and the adult females peak in September–October, thus a peak in the incidence rates of LB would be expected in May–June and in October–November [Gray, 1980; Gray, 1984; Randolph, 2002b]. This is seen in dogs, exposed to outdoor life all year around having the highest incidence rates of tick-borne diseases in spring and autumn (Michael Leschnik, personal communication). Yet in this study, as in an Austrian study [Dennis & Hayes, 2002] every other case had onset of disease in July and August and was consequently, according to the incubation period, exposed to tick bites in June and July. In a recent Swedish study in the county of Blekinge, most individuals were bitten by ticks during the Swedish vacation months June and July [Stjernberg & Berglund, 2002b]. Thus, these vacation months pose the highest risk for acquiring tick bites, indicating that human exposure to ticks has a stronger influence on the seasonal distribution of the EM incidence rates, than the seasonal feeding activity of the nymphal *I. ricinus*. The bimodal age- and incidence rate distribution seen in this study is also reported by others [Berglund et al., 1995; Chow et al., 2003; Orloski et al., 2000; Dhôte et al., 2001]. Our observation that young individuals under age 15 were affected by EM in July rather than in August, and the fact that elderly at the age of retirement had the highest incidence rates of EM may also indicate the strong influence of exposure and behaviour, with adults after retirement possibly less restricted to outdoor life by vacations than non-retired adults.

In **paper I,** the annual mean prevalence of reinfection was 1%, which is significantly higher than the prevalence of LB in southern Sweden in 1992-93 of 0.07% ($p < 0.001$). The risk of reinfection with LB increased with the increasing number of tick bites for both men and women. The increased prevalence rates of EM amongst the reinfected compared with those only infected once, reflects that the reinfected individuals get more tick bites than do single-infected people, probably an effect due to living habits and behaviour that expose them to ticks, rather than increased vulnerability.

There were no significant differences in serological results between patients who were reinfected during follow-up and those who were not, indicating that a positive serology does not give immunity to a new infection with LB. However, the lowest incidence rates of reinfection occurred in 1993, the first year after the EM infection. In tests with mice cured from a previous LB infection it has been observed that all mice were resistant to reinfection when challenged at 1.5 months after cure, but this resistance was lost 10.5 months after cure [Piesman et al., 1997]. Theoretically, then, the initial infection in 1992 might have given a protective effect of antibodies during 1993.

Clinical aspects on erythema migrans

One of the most important findings in this thesis is that in patients with EM where borrelial origin was verified through a positive PCR, the clinical appearance of the EM depends on an interaction between gender and genospecies, which has not been described before (**paper IV**). The clinical appearance of the EM depend whether *B. afzelii* infects a man or a woman; males to a higher extent than women developed annular EM while women more often developed non-annular EM if infected with *B. afzelii*.

In an earlier Swedish study, the appearance of the annular EM is estimated as a function of time, although gender and genospecies were not taken into consideration [Åsbrink & Ohlsson, 1985]. In this study we could not find any correlation with the appearance of the lesions with the median duration of time from tick bite to diagnosis. Other studies indicate that *B. garinii* mostly causes homogeneous EM [Logar et al., 2004; Carlsson et al., 2003] and that different genospecies grow and disseminate with varying intensity, resulting in different clinical symptoms [Strle et al., 1999; Logar et al., 2004; Smith et al., 2002; Nadelman et al., 1996]. The more virulent genospecies *B. burgdorferi* s.s. is reported to cause non-annular EM to a high extent [Strle et al., 1999; Smith et al., 2002; Nadelman et al., 1996] in comparison with *B. afzelii* [Strle et al., 1999]. Thus the genospecies varying virulence and tendency to disseminate could affect the appearances of the lesions. However, as mentioned, we have found that the genospecies by interacting with gender affects the clinical appearance of the lesions. This interaction has likewise not been reported earlier, but this study includes one of the largest series of EM where genospecies are confirmed molecular-biologically, and we consider our data reliable.

Different immunological reactions between gender might explain our findings; this is discussed in the section concerning ‘Gender aspects on erythema migrans’.

In 74% of the patients with confirmed EM, *B. afzelii* was the infecting agent and in 26% of the patients *B. garinii* caused the infection. A considerably higher frequency of patients were infected with *B. garinii* in this study than in a study performed in the same area in 1994–1997 with a frequency of *B. garinii* of 6.1% in patients with EM [Ornstein et al., 2001]. This might be due to an increased awareness amongst physicians of the varying appearances of the EM lesions. In a study in the area in 1999 of

spirochete-infected ticks, 31% contained *B. garinii* spirochetes, indicating the increasing influence of birds as a reservoir for ticks in the area, since birds are the main reservoir for *B. garinii* infested ticks [Olsén et al., 1995; Olsén et al., 1995; Fraenkel et al., 2002]. The increasing frequency of *B. garinii* in the area might also increase the risk of an increasing incidence of neurborreliosis.

Infections caused by *B. garinii* had a significantly shorter duration from tick bite to the initial visit, compared with infections caused by *B. afzelii.* However there were no differences in surface sizes, indicating a faster increase of lesions caused by *B. garinii.* Besides this, we found that individuals infected by *B. garinii* more often had elevated levels of CRP. Altogether more patients infected by *B. garinii* had fever, faster developing and spreading EM lesions, elevated levels of CRP indicating a greater virulence of infections caused by *B. garinii* in comparison with *B. afzelii.* Our results are supported by findings in other studies [Logar et al., 2004; Strle, 1999].

In patients with verified EM, only 40.4% of the serology results were positive at the initial visit which confirms the low sensitivity of the serological testing in the early stages of LB. Erythema migrans is a clinical diagnosis based on the medical history, including data concerning tick bite, clinical symptoms and the clinical appearance of the lesion without any requirements for a serological test since specific antibodies are commonly absent [Hansen & Åsbrink, 1989; Nowakowski et al., 2001].

Almost every patient had noticed a tick bite, in comparison with other studies from the mid 1980s where less than half of the affected patients have noted the tick [Åsbrink & Ohlsson, 1985; Berger, 1984; Steere et al., 1983]. This is probably due to an increased awareness of LB in the population. Since the beginning of the 1990s there has been focus on LB in the area both from the media and from the health service which might have changed peoples´ awareness of ticks and tick borne diseases.

In **paper II** we found satisfactory efficiency of both pcV and doxycycline for the treatment of EM. The symptoms during treatment were unspecific and none of them fulfilled the criteria for late disseminated LB. The symptoms might be caused by the ongoing borrelia infection or by 'co-infections' with other tick-borne bacteria such as human granulocytic ehrlichiosis, although most patients with symptoms during treatment had been prescribed doxycycline and possibly the symptoms could be caused by side effects.

There are few studies comparing the effectiveness of pcV with other antibiotic treatments of EM, but in the conducted studies pcV has been demonstrated to be as effective as azitromycin and cefuroxime [Weber et al., 1993; Arnez et al., 1999]. Even if our study was not designed to compare treatments, it still showed that treatment of EM with pcV is extremely effective with the benefits of a drug with few side effects, low resistance and low costs. Outside Scandinavia pcV treatment is not the tradition, instead antibiotics with a broader spectrum are predominantly used. The Swedish tradition of using pcV when no signs of disseminated infection or coinfection with

other tick-borne pathogens, e.g. ehrlichia, are present is an excellent choice and pcV should be recommended in Europe as the first-line drug.

Gender aspects on erythema migrans

Middle aged and elderly women have been shown in this thesis to be a new risk group for LB. To a higher extent than men they were affected by EM and reinfections of EM (**papers I & III**). Additionally the clinical appearance of the EM was influenced by gender and genospecies (**paper IV**).

In the epidemiological study of EM in Blekinge (**paper III**) we found that women had the highest incidence rates of EM, especially women over 50 years old. Our findings that women to a higher extent than men are affected by EM is supported by earlier studies in Europe [Weber et al., 1983; Åsbrink et al., 1986], but not in the USA [Orloski et al., 2000; Steere et al., 1983a; Berger, 1984]. This might be due to the fact that the genospecies are differing in Europe and the USA. In Europe EM is mostly caused by *B. afelii* and *B. garinii*, both genospecies which do not exist in the USA, where LB is caused only by *B. burgdorferi* s.s. A more speculative factor could be that Swedish women might spend more time outdoors than their American counterparts, which to a higher extent exposes them to ticks and tick-borne infections.

Since women are less exposed to outdoor life [Statistics Sweden, 1982-1999] and take more preventive measures than men [Stjernberg & Berglund, 2005b], women would be expected to be at a lower risk of attracting tick bites and LB than Swedish men. However, in data acquiered from a recent study in the area focusing on studying tick attachment rates [Stjernberg & Berglund, 2002b] women attract 33% more tick bites per hour than men (0.016 vs 0.012 tick bites per hour) while equally exposed to a tick habitat. Ticks recognize their host by several stimuli such as odour, vibrations, visual cues, radiant heat and touch. These stimuli are taken up by sense organs concentrated at the anterior end of the tick. The behaviour response to ticks indicates that ticks can discriminate the odours of different host types and respond more or less sensitively and intensely to the odours [Mehlhorn, 2004]. Perhaps there are differences between women and men according to these stimuli, making us attract ticks differently.

Exposure to ticks may explain some of our data, but other mechanisms might be involved too. In **paper I,** individuals bitten on more than 10 occasions had a higher risk of acquiring a reinfection compared with those bitten on less than 5 occasions, relative risk women of 4.7 and men of 8.6, showing that more tick bites increases the risk for LB. However women bitten on less than 5 occasions and on more than 10 occasions had a significantly higher frequency of reinfections than men indicating women more easily contracted LB than men when tick-bitten (Table IV).

As mentioned, most patients with EM were infected with *B. afzelii.* One of the most interesting findings in this thesis would be the finding of an interaction between gender and genospecies. The clinical appearance of the EM depends on if *B. afzelii* is

infecting a man or a woman; males to a higher extent developed annular EM while women were more likely to develop non-annular EM if infected with *B. afzelii*.

The 'Type 1' cell-mediated immune response is important in eradicating the spirochete in humans [Widhe, 2003]. As mentioned, predominantly postmenopausal women were infected and reinfected with LB. Women in reproductive age are believed to handle infections better than men. In general, oestrogen has a stimulatory effect on the immune system mediated by a stronger tendency to respond to the Type 1 response, whereas testosterone acts as a suppressor. When women enter menopause their levels of oestrogens decrease and thereby the stimulatory effect would diminish, leading to an altered immune status [Olsén & Kovacs, 1996]. A study of reinfected patients with EM in collaboration with the University of Linköping has indicated that this Type 1 immune response is less intense in postmenopausal females than in males [Jarefors, submitted 2005]. This circumstance might result in a higher susceptibility for spirochetal infections and reinfections. Additionally, the less intense Type 1 response in women might cause a more intense local inflammatory skin-reaction, predominantly resulting in non-annular EM. We also found that the lesions healed faster in men also an indication of a faster and more intense Type 1 immune response, and a more efficient spirochetal eradication.

There was no difference between gender and seropositivity at the initial visit. However, after 6 weeks significantly more women than men were seropositive (72% vs 42%). The postmenopausal women also had a more pronounced Type 2 response. Other studies have shown that the Type 2 response might trigger the antibody response and would thus support our theories of postmenopausal women having a less intense Type 1 response and a stronger Type 2 response than men.

One may speculate that non-annular lesions might to a higher extent be misdiagnosed since they do not have the 'classical' ring-shaped or annular appearances. Women infected with *B. afzelii* – the most common infecting agent for EM – develop to a higher extent than men non-annular EM. This might increase women's risk of being misdiagnosed, without treatment and the more susceptible to develop a disseminated disease. This is indicated in our, although not significant, result that more women than men (99 and 84 respectively) were treated for late disseminated LB at the County Hospital in Blekinge, 1997-2003.

Climate aspects on erythema migrans

In **paper III**, the EM incidence rates varied considerably during the six-year observation period. These variations were found to be influenced by climate factors with effect both on tick abundance and activity and on human exposure. Warm, humid summers without rain and mild winters increased the incidence rates of LB during the summer months.

An increase in the mean monthly summer temperature by 1 ºC increased the incidence rates of EM by 12%. Since the host-seeking activity of the ticks is not reported to

increase as temperatures rise above 7 ºC [Gray, 1980], this effect is probably mostly the result of an increase in human exposure to ticks. There are no studies identifying climate factors that influence humans to spend time outdoors, but undoubtedly humans dress more lightly during the summer, thus increasing the risk of attracting tick bites.

An decrease by 1 day of the number of days during winter with mean temperatures below 0 ºC, resulted in a 3% increase of the incidence rates of EM. This is probably a climate effect on ticks and their hosts, as milder winters increase the possibilities of the host animals surviving (Jeremy Gray, personal communication) and thereby increasing the chances of the ticks to survive. In a Swedish study milder winters and an earlier arrival of spring increased the TBE incidence rates [Lindgren & Gustafson, 2001].

An increase of the mean monthly precipitation by 1 mm resulted in a decrease of 8% of the incidence rates of EM. The ticks' ability to climb the lower vegetation is impaired by rain (Jeremy Gray, personal communication) and also a study in England has shown a negative effect of rain on tick attachment rates on humans [O'Connell et al., 1998]. Besides this negative effect of rain on tick attachment rates, human exposure might decrease with the use of a covering rain garment and less time spent outdoors.

An increase of the number of days with relative humidity above 86% by 1 day, increased the incidence rate of EM by 4%. This is in accordance with earlier findings: below the threshold of a relative humidity of 86%, the tick dehydrates and cannot continuously seek a host [Knülle & Rudolph, 1982].

During the twentieth century, world average temperature increased by 0.6 ºC and climatologists forecast further warming with increasing incidence rates of vector-borne diseases [WHO report, 2003]. Further warming may also increase the incidence rates of tick-borne disease in Sweden, especially in the northern parts of the country where it is now too cold for the tick to be actively host seeking and survive.

Methodological considerations

In **paper I** our data could be biased by the retrospective approach, although a study in the area indicates that there is no gender difference in how men and women report tick bites [Stjernberg & Berglund, 2002b]. Thus, there are no indications that women forget previous tick bites to a higher extent than men, and report a lower/higher frequency of bites. Our data might also be biased by the fact that 22.5% of the initial study population were not included since they did not answer the questionnaires. Reviewing our results from paper I & II, significantly more men than women dropped out of the study (107/388 vs 99/526 $p < 0.01$). However we consider our data to be reliable since a greater dropout in men expectedly would underestimate the risk ratios between gender.

In **paper II**, we found satisfactory efficiency of both pcV and doxycycline for the treatment of EM, although it must be stressed that the study was not designed to compare different treatments.

In **paper III** our data are conservative since 14 % of the medical records lacked a definite diagnosis.

In **paper IV** significantly more men than women participated. This is probably due to the inclusion of mainly male patients from the naval- and air base representing two of the seven outpatient clinics engaged in this study.

Implications for clinical practice

EM is a clinical diagnosis for which over 98% of the cases were treated in primary health care. Every other case occurs during the vacation months of July and August. The diagnosis was based on the medical history and the clinical picture. Only 40% of the patients with confirmed EM were seropositive at the initial visit, demonstrating the low sensitivity of serology testing

EM are not predominantly of the 'classical ring-shaped' or annular type. Just as many EM are annular as non-annular. It must be emphasized that women, especially postmenopausal, are at a higher risk of EM and LB in southern Sweden, and that women are identified as a new risk group for LB. Women infected with *B. afzelii* to a higher extent than men develop non-annular EM. Patients with non-annular EM might have an increased risk of being misdiagnosed and without treatment, thus increasing their risk of a disseminated infection. The increasing frequency of LB caused by *B. garinii* also increases the risks of disseminated cases of neuroborreliosis in the area, in both women and men.

EM might seem to be a harmless condition but in fact very important to diagnose and treat adequately because of the risk of dissemination, which might result in severe complications for the affected patient. PcV is an excellent choice of antibiotics as long as no signs of allergic reactions, dissemination, flu-like symptoms or coinfection with other tick-borne pathogens are present. In addition, pcV has few side effects, low resistance and low costs.

Implications for future research

Further research is needed to clarify the biological, immunological and sociological mechanisms explaining why predominantly women and especially postmenopausal women are infected by LB.

Further research on the antibiotic treatment in LB is warranted in Europe and USA to achieve an international consensus for LB treatment.

Conclusions

The county of Blekinge has an extremely high incidence rate of LB especially amongst middle aged and elderly women that are identified as a new risk group for LB. Almost every case of EM is diagnosed and treated in primary health care.

- Reinfections with LB occurred with a mean annual prevalence of 1%. Reinfections occurred more frequently in women than men, 6% and 1% respectively ($p < 0.01$), although women and men were tick-bitten to the same extent (**paper I**)
- Treatment of EM with pcV and doxycycline is efficient. No patients developed disseminated LB during the follow-up (**paper II**).
- The county of Blekinge was found to be highly endemic for LB, with a mean annual incidence rate of over 460 cases per 100,000 inhabitants of EM. Almost every case of EM is diagnosed in primary health care and almost every second case occurred in July and August. Women had a significantly higher incidence rate than men (**paper III**).
- The seasonal variations in incidence of EM were influenced by climate, with effects on human exposure, tick abundance and activity (**paper III**).
- Seventy-four percent of confirmed EM were caused by infections with *B. afzelii* and 26% were caused by infections with *B. garinii*. EM caused by *B. garinii* seems to cause more intense local and disseminated inflammatory reactions than infections with *B. afzelii* (**paper IV**).
- Some 44.7% of the lesions were annular and 45.8% were non-annular. Thus, most EM were not the typical ring-shaped or annular, especially not in women infected by *B. afzelii.* (**paper IV**).
- An interaction between gender and genospecies was found; the clinical appearance of the lesions depends on whether *B. afzelii* infects a man or a woman. Males to a higher extent than women developed annular EM while women more often developed non-annular EM if infected with *B. afzelii*. (**paper IV**)**.**

Populärvetenskaplig sammanfattning på svenska

Lyme borrelios (LB) är den vanligaste vektor-burna infektionen på norra halvklotet. Den lokala borrelia-infektionen i huden, erythema migrans (EM) representerar över 70 % av LB fallen och är således den vanligaste formen av LB. Södra Sverige är högendemiskt för LB och då särskilt de kustnära länen Blekinge och Kalmar.

Redan 1910 beskrev den svenske hudläkaren Arvid Afzelius i en fallrapport, en migrerande ringformad hudrodnad, som debuterat efter ett fästingbett. Han kallade rodnaden för ”erythema migrans”. Under 1940-talet behandlade svenska läkare framgångsrikt både EM och den kroniska hudinfektionen ”akrodermatitis chronica atrophicans” (ACA) med penicillin. Därefter, beskrev svensken Carl Lennhoff en spiroket-liknande struktur som han menade var orsaken till EM. Han publicerade sina teorier 1948 men fick sedan dra tillbaka dem då han blev så kraftigt ifrågasatt. På 1980-talet i Nordamerika, fann man av en händelse, i samband med att man letade efter en annan fästingbakterie, borrelia-spiroketen som nu är uppkallad efter dess upptäckare William Burgdorfer. Man uppkallade också sjukdomen ”Lyme borrelia” efter det område i nordöstra USA – ”Lyme district” - där man i slutet av 70-talet och början av 80-talet hade endemiska utbrott av ledinflammation hos barn som sedermera skulle visa sig vara orsakad av borrelia.

Den här avhandlingen handlar om EM sett ur ett primärvårdsperspektiv med avseende på epidemiologiska-, kliniska-, köns- och klimataspekter.

Under åren 1997–2003 hade Blekinge län en medelincidens på 464 fall per 100,000 invånare och år. Siffrorna visar att insjuknandet (incidensen) i EM har ökat 3-faldigt sedan början på 1990-talet. I över 98 % av fallen ställdes diagnosen EM i primärvården och ungefär vartannat fall inträffade under sommarmånaderna juli och augusti (**studie III**)**.** Av över 700 individer som drabbats av ett EM i början av 90-talet i södra Sverige drabbades 1 % varje år av en ny infektion med EM under en period av 5 år. Frekvensen av borrelia 1992 i södra Sverige var 0.07%, dvs mer än 10 ggr lägre än den årliga frekvensen av nya infektioner. Detta betyder att de som tidigare haft LB har en ökad risk att drabbas av nya infektioner (**studie I**).

I **studie IV** av patienter med EM där man i hudbiopsi från erythemet molekylärbiologiskt verifierat borreliaspiroketen, var de flesta patienterna infekterade med *Borrelia afzelii,* 74 % medan 26 % var infekterade av *Borrelia garinii.* Knappt hälften av erytemen var ’ringformade’ och knappt hälften ’icke ringformade’. Således är det mycket vanligt med icke ringformade erytem vilket kan bidra till att erytemen är svåra att identifiera och känna igen vilket kan leda till en ökad risk för spridning av borreliainfektionen.

Ett av de mest intressanta fynden är att erytemens utseende beror på vilket kön man tillhör och vilken borrelia-stam man smittats av. Däremot spelade tidsfaktorn från fästingbett till diagnos ingen roll. Män utvecklade i högre grad än kvinnor

’ringformade’ erytem medan kvinnor i högre utsträckning utvecklade ’icke ringformade’ erytem om de smittats av stammen *B. afzelii* (**studie IV**).

Infektioner orsakade av *B. garinii* tycktes i högre utsträckning vara benägna att orsaka mer intensiva lokala och systemiska inflammatoriska reaktioner jämfört med infektioner orsakade av *B. afzelii*, dock var dessa fynd inte signifikanta i vår logistiska regressionsmodell (**studie IV**).

Det var en ojämn könsfördelning i antalet diagnostiserade fall av EM. Fler kvinnor än män drabbades av EM, 506 respektive 423 fall per 100,000 invånare, ($p < 0.001$) och framför allt drabbades kvinnor som passerat 50 års ålder dvs. tidpunkten för klimakteriet (**studie III**). Signifikant fler kvinnor än män drabbades också av nya infektioner med borrelia när de följdes under en 5-årsperiod (6 % kvinnor och 1 % män, $p < 0.01$). Alla kvinnor var över 44 år och de var fästingbitna i samma utsträckning som männen (**studie I**).

Både exponering men även immunologiska orsaker kan tänkas förklara de observerade könsskillnaderna. Med tanke på att kvinnor vistas mindre i skog och mark än män och att de vidtar mer förebyggande åtgärder för att inte bli bitna, skulle man kunna förvänta sig att kvinnor hade en lägre risk att utsättas för fästingbett och LB än män. Dock ser vi att det finns indikationer på att kvinnor drabbas av fler fästingbett per tidsenhet när de exponeras för samma utomhusmiljö som männen. Orsaken till detta kanske ligger i att kvinnor attraherar fästingens värdsökande stimuli i högre utsträckning såsom tex genom parfym eller deodorantdoft. Utöver detta tycks också medelålders och äldre kvinnor i högre utsträckning än männen drabbas av en ny borreliainfektion trots att de blivit fästingbitna i samma utsträckning som männen (**studie I**), vilket tyder på att kvinnor lättare blir infekterade med borrelia efter att de blivit fästingbitna.

I samarbete med en forskningsgrupp vid Linköpings universitet har vi studerat immunförsvaret hos individer som drabbades av reinfektion jämfört med en kontrollgrupp som haft EM en gång. Man ser då ingen skillnad mellan reinfekterade och inte reinfekterade medan man däremot ser då att kvinnorna (som nästan samtliga är i klimakteriet) i högre utsträckning än männen har ett mindre uttalat så kallat ”typ 1 svar”. Det immunologiska ”typ 1 svaret” är viktigt för att värden skall kunna avdöda spiroketen och rent teoretiskt kan detta medföra att kvinnor kan vara mer mottagliga för infektion med borreliaspirocheten än männen. Detta skulle också kunna förklara varför kvinnor i högre utsträckning får de icke ringformade erytemen, vilket innebär en mer intensiv lokal inflammatorisk reaktion i huden än hos männen.

Behandling av EM med penicillin (pcV) gav en god utläkning av erytemen, där ingen drabbades av spridd LB. Med tanke på pcV’s smala spektra, låga antibiotika-resistens, låga frekvens av biverkningar och låga kostnad rekommenderas PcV i första hand till patienter med EM (**studie II**).

Säsongsincidensen av EM fall varierade avsevärt mellan åren, och vi fann att klimatfaktorer med inflytande på människans respektive fästingens aktivitet hade stor

inverkan på dessa säsongs-variationer i insjuknandet (**studie III**). Bland annat fann vi att en ökning av månadsmedeltemperaturen under sommarmånaderna med en grad bidrog till en signifikant ökning av EM incidensen med 12 % ($p < 0.001$), och en ökning av antalet kalla vinterdagar under 0ºC med en dag, medförde en signifikant minskning av EM incidensen med 3 % följande sommar ($p < 0.001$)

Sammanfattningsvis är huvudfynden i denna avhandling att framför allt medelålders och äldre kvinnor drabbas av EM och således är de en helt ny riskgrupp för LB. De flesta individer smittas av *B. afzelii*. Vid infektion med denna stam har vi sett att kvinnornas erythem i högre utsträckning än männens inte är klassiskt ringformade. Detta kan öka risken för kvinnorna att få fel diagnos och utebliven behandling, vilket i sin tur kan öka risken för en spridd borreliainfektion.

Orsakerna till könsskillnader i insjuknandet i borrelia och erytemens utseende är inte helt klarlagda men kan teoretiskt förklaras av exponering men även könsskillnader i immunförsvaret.

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Appendix

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healthsciences

Reinfection with Lyme Borreliosis: A Retrospective Follow-up Study in Southern Sweden

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In a 5-y retrospective follow-up study in southern Sweden that initially included >1000 individuals with previously diagnosed erythema migrans, factors that influenced the risk of reinfection with Lyme borreliosis were elucidated. The total frequency of reinfection was 4% and the number of tick bites influenced the risk of reinfection: those bitten >10 times during the observation period had a 4–8-fold increased risk compared with those bitten <5 times. Women manifested to a greater extent than men although both genders were bitten equally by ticks, thus indicating that women may be more susceptible to reinfection. All reinfected women were >44 y old. The county of Kalmar including Öland was found to be highly endemic for reinfection with Lyme borreliosis. Thus the number of tick bites, gender, age and study area influenced the risk of reinfection.

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INTRODUCTION

Lyme borreliosis (LB) is the most common vector-borne disease in Sweden. The most prevalent clinical manifestation of LB is erythema migrans (EM), which occurs with a frequency of 77%. These data were recently presented in a prospective population-based survey by Berglund et al. (1) that involved >2 million inhabitants in southern Sweden. Reinfection with LB can occur and in the present retrospective study of patients treated for, and recovered from, EM in southern Sweden, we aimed to study, in a large-scale setting, the annual incidence and total frequency of reinfection with LB, in comparison with earlier data from local studies in high-risk populations in Scandinavia and the US (2–7). Additionally, we also compared gender and age with respect to the frequency of reinfection and the number of tick bites.

MATERIALS AND METHODS

Study area

The study area consisted of the 7 southern counties of Sweden and covered an area of 49,000 km^2. It initially involved 2,133,068 inhabitants, i.e. 24% of the Swedish population.

Study design

The study was a retrospective, long-term follow-up study based on the epidemiologic population-based prospective study conducted between the beginning of May 1992 and the end of April 1993 by Berglund et al. (1). This follow-up study was conducted in May 1998 and included the 914 patients with EM diagnosed in the former study.

A questionnaire was sent to the participants and the questions of relevance to our study were: "Have you had any new tick bites during the last 5 y, from May 1993 to May 1998?" and "Have you had Lyme borreliosis again during these years?" Those replying affirmatively to either of these questions were asked to provide more details on the back of the form and were contacted by telephone for more information and/or to get permission to study their medical records.

Study population

See Fig. 1 for details of the study population.

Case definitions

The original 1075 patients had EM diagnosed by a physician; the diagnosis also fulfilled the Centers for Disease Control and Prevention criteria, i.e. erythema of ≥ 5 cm in diameter (8). All patients were treated with antibiotics and recovered. In the follow-up study, diagnosis was based on "self-reported" symptoms from the patient and we required the following criteria: (i) a large round erythema expanding over a period of days or weeks after the tick bite and reaching a diameter of ≥ 5 cm; (ii) a doctor's visit to confirm the diagnosis and prescribed treatment with antibiotics; and (iii) for other LB manifestations, such as Lyme arthritis (LA), neuroborreliosis, Borrelia lymphocytoma (BL) and acrodermatitis chronica atrophicans (ACA), a characteristic clinical manifestation of the disease, plus serologic confirmation indicating a 4-fold elevation of the titer of Borrelia burgdorferi antibodies, or a seroconversion when acute- and convalescent-phase serum samples were analyzed simultaneously on the same plate. In the case of BL a skin biopsy with typical histological findings was required. In these cases the patients' medical records were studied in order to assess the accuracy of the information after they had given their informed consent.

Statistical methods

The χ^2 test was used to compare groups. A p-value <0.05 was considered significant.

Ethical considerations

The study was approved by the Ethical Committee at the University of Lund.

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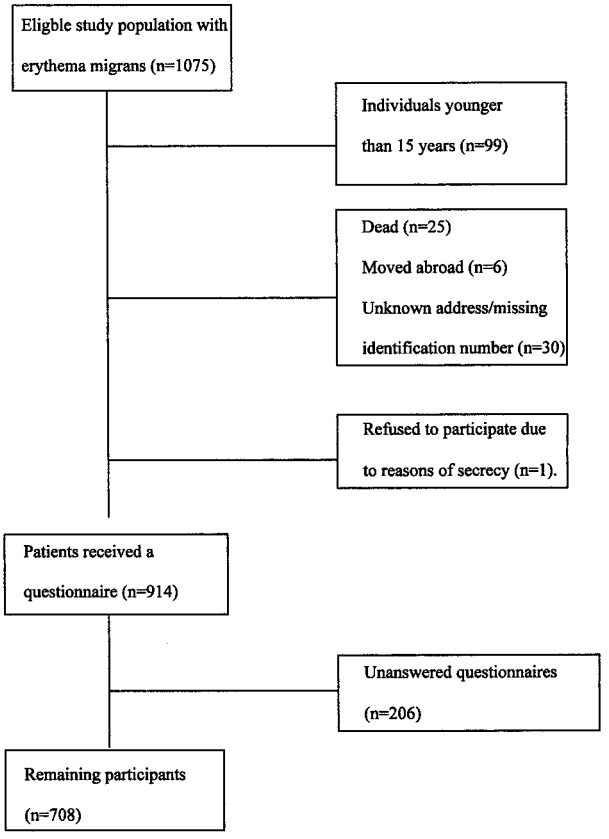

Fig. 1. Schematic illustration of the participant flow in a 5-y follow-up study of patients with EM.

RESULTS

The questionnaire was sent to 914 individuals, of whom 708 replied (77.5%): 427 women (60%) and 281 men (40%), with a mean age of 55.5 y (SD 17.2 y, range 15–91 y; women 56.8 y, SD 16.9 y; men 53.6 y; SD 17.4 y). Excluding the 99 subjects aged < 15 y, a total of 268 individuals [123 women (46%), 145 men (54%); mean age 53.8 y (women 56.8 y, men 51.3 y)] dropped out of the study. There were no significant differences in terms of age or gender between participants and those that dropped out of the study.

Tick bites

A total of 384/708 (54%) of the subjects were bitten by ticks during the observation period, with an even distribution according to sex (Table I).

Reinfections

Reinfections with LB were initially reported in 40 individuals but 9 subjects (6 women, 3 men) did not fulfill the above-mentioned criteria for the diagnosis of EM: in 2 women and 1 man the erythema was < 5 cm in diameter, in 4 women the erythema was discovered outside the observation period, 1 man had died and thus complete information about the reported erythema was lacking and 1 man's reported reinfection with EM was undocumented upon study of his medical records. Other manifestations of LB than EM could not be verified after studying the medical journals and/or laboratory results.

Table I. *Distribution of tick bites according to gender in a 5-y observational study*

No. of tick bites	Women (%)	Men (%)	*p*
0	194 (45)	130 (46)	NS
<5	154 (66)	104 (69)	NS
6–10	36 (15)	21 (14)	NS
>10	43 (18)	26 (17)	NS
$\Sigma\ n/N$ (%)	233/427 (55)	151/281 (54)	NS

N = number of participants (427 women; 281 men).
n/N (%) = percent of the individuals bitten by ticks.

Three individuals (2 women, 1 man) did not answer the question concerning whether they had been reinfected and attempts to contact them by telephone and mail were unsuccessful. During the 5-y observation period, 31 individuals had documented 1 or several reinfections with LB. The total frequency of reinfections was 4% (31/705). Reinfections were observed on 36 separate occasions: 1 woman had 3 different episodes of EM and 3 women were reinfected twice during the observation period. The annual incidence of reinfection during the 5-y observation period was 1% ($36/5 \times 705$). Of the 36 cases of identified reinfection, 44% (16/36) occurred in 1997, the final year of observation. This incidence rate was significantly higher than the overall observed incidence during the study period. The incidence of reinfection in 1993 was significantly lower compared with the overall incidence (Table II).

Of the individuals with observed tick bites, 8% (31/384) manifested EM. None of the 324 individuals who did not report tick bites manifested EM. All of the 31 reinfected individuals reported exposure to tick bites a few weeks before developing EM. As mentioned earlier and in Table I, women and men were bitten to the same extent. Significantly more women than men reported recurrence of EM: 27/31 reinfected individuals were women, or a total of 6% (27/427), compared with 1% (4/281) of the men ($p < 0.01$) (Table III). Middle-aged (45–64 y) women had significantly more reinfections compared with the men in the same age group ($p < 0.01$) (Table III).

Table II. *The annual incidence of reinfections with LB*

Year	No. of reinfections	Incidence (%)	*p* [a]
1993	2	0.28	<0.05
1994	8	1.13	NS
1995	3	0.42	NS
1996	7	0.99	NS
1997	16	2.26	<0.05

[a] Comparing mean incidence of 1% with annual incidence of reinfection.

Table III. *Distribution of reinfection according to age and gender*

Age (y)	Women; n/N (%)	Men; n/N (%)	p
15–44	0/95 (0)	2/84 (2)	
45–64	14/165 (8)	0/115 (0)	
65–84	13/157 (8)	2/73 (3)	
>85	0/10 (0)	0/9 (0)	
Σ n/N (%)	27/427 (6)	4/281 (1)	<0.01

N = number of participants (427 women; 281 men).

Women bitten on > 10 occasions had a significantly higher frequency of reinfection compared with the men ($p < 0.05$) (Table IV). For both genders, those individuals bitten on > 10 occasions had significantly more reinfections compared with those bitten on < 5 occasions (women 13/43 vs. 10/154, $p < 0.001$; men 2/26 vs. 1/104, $p < 0.05$), equivalent to relative risks of about 4 and 8, respectively (Table IV).

Women aged > 44 y had a frequency of reinfection of 15% (27/183), significantly higher compared with women < 44 y [frequency 0% (0/50); $p < 0.01$]. Women aged > 44 y more often reported exposure to tick bites on > 10 occasions ($p < 0.001$). Amongst women aged > 44 y there was a significantly higher frequency of reinfection amongst those bitten very often compared with those bitten seldom ($p < 0.001$), equivalent to a calculated relative risk of 3.6 ($13 \times 110/42 \times 10$).

There were significantly more individuals with reinfection in the county of Kalmar including Öland compared with the rest of the study area (14/198 vs. 17/510; $p < 0.05$). This county also had a significantly higher number of individuals bitten by ticks compared with all the other counties combined (121/198 vs. 263/510; $p < 0.05$).

DISCUSSION

The south-east coast of Sweden, bordering the Baltic Sea, was found to be a highly endemic area in the early 1990s for LB by Berglund et al. (1). As an example, EM was recently reported to occur more frequently than tonsillitis at a general practice in the county of Blekinge (9).

Table IV. *Distribution of reinfections amongst women and men according to the number of tick bites*

No. of tick bites	Women; n/N (%)	Men; n/N (%)	p
0	0/194 (0)	0/130 (0)	
<5	10/154 (6)	1/104 (1)	
6–10	4/36 (11)	1/21 (5)	
>10	13/43 (30)	2/26 (8)	
Σ n/N (%)	27/233 (12)	4/151 (3)	<0.01
Risk ratio	4.7 ($13 \times 154/43 \times 10$)	8.6 ($2 \times 104/1 \times 26$)	

N = number of participants (427 women; 281 men).

In this 5-y follow-up study of the incidence of reinfection with LB in southern Sweden amongst > 700 individuals treated for, and recovered from, EM, we found that reinfection with LB occurred with a frequency of 4% and an annual incidence of 1%. This is significantly higher ($p < 0.001$) than the annual incidence of LB in this area (0.07%; 69/100,000) and thus indicates that individuals with a previous episode of EM are at higher risk for a new infection than individuals without a previous LB infection. This is probably due to living habits or behavior that expose them to ticks, rather than a result of increased vulnerability. Although the data may be biased by the fact that 22.5% of subjects did not fulfill the study criteria, we consider our findings to be reliable. No difference between participants and dropouts was found according to age, gender or area of residence.

In this study the incidence of EM infections in 1997 was significantly higher compared with the overall incidence during 1993–97. This is in agreement with another study conducted in the area, in which EM was 200% more frequent in the county of Blekinge in 1997 than in 1992 (10). The reason for this increase is not yet clear but the indication is that it is caused by the higher frequency of ticks in the area. There was also a significantly lower incidence of reinfections in 1993 compared with the mean annual incidence and this might be explained by the fact that the participants in this study had an EM infection in 1992 and could theoretically therefore have been resistant to a new infection for a while. In naturally infected and cured mice it has been observed that all mice were resistant to reinfection when challenged at 1.5 months after cure but that this resistance was lost 10.5 months after cure (11). One potential source of bias in our results could be that individuals are more likely to remember what happened last year than 5 y ago.

On the island of Aspö located on the Baltic coast of Sweden, Berglund et al. (2) found a frequency of reinfection with LB of 9% (3/32) during a 3-y study period. Gustafson et al. (3), in a 2-y survey of residents of a 4 km^2 area on the island of Lisö, 50 km south of Stockholm on the Baltic coast, found a frequency of EM of 7% (21/303) and 7 of the 21 individuals with EM had a history of previous LB.

The frequency of reinfection with LB reported from endemic areas in the north-eastern parts of the US varies from 9% to 18% (4–7). In addition to the great variation in size of the studied areas and the differences in vegetation, density of ticks and infestation rates with B. burgdorferi, the duration of observation time (1 to 7 y) and the size of the study populations (57–215 individuals) also varied. Asch et al. (4) reported a frequency of reinfection of 18% amongst 215 individuals evaluated up to 5 y after their primary infection in Westchester County, NY, Salazar et al. (5) found a frequency of reinfection of 11% (7/63) amongst children from southern Connecticut up to 4 y after the initial episode. In patients evaluated for up to 7 y,

Lastavica et al. (7) reported a frequency of reinfection of 9% (5/57) in coastal Massachusetts.

In our study women seemed to manifest EM to a greater extent than men, in spite of the fact that they were bitten by ticks to the same extent. Thus factors other than behavioral and exposure factors seem to influence the tendency for reinfection. Other studies have shown that there is no significant difference in primary EM infections according to gender or age but reinfection has not yet been studied in this context (7). Are women more observant than men or is there a difference in the female immune response that causes them to be more susceptible to reinfection? In this study all reinfected women were >44 y of age and possibly postmenopausal. Although this was not a primary analysis in this study these observations are intriguing and further studies need to be carried out in order to clarify and confirm them. Forest workers, orienteers and military recruits are examples of known high-risk groups for tick bites but in this study middle-aged women were at highest risk of reinfection with LB (1). We observed that the risk of reinfection with LB increases with increased number of tick bites for both men and women. This is logical as an increase in the number of tick bites should theoretically give an increased risk of a bite from an infected tick. The relative risk in this study varied between 4 and 8.

The county of Kalmar including Öland has almost perfect conditions for the tick and its hosts because of its humid climate, forests and extensive border with the Baltic Sea. We found this area to be highly endemic for reinfections with LB, and almost half of the cases of reinfection occurred here. This can be explained by the fact that there was a significantly higher frequency of tick bites reported in this county compared with all the other counties.

In conclusion, our results indicate that individuals with a previous episode of EM are at high risk for reinfection with LB compared with individuals without a previous episode of EM. We found that the number of tick bites, gender, age and study area influenced the risk of reinfection. Middle-aged women were most susceptible to reinfection with LB.

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CASE REPORTS

Clinical Outcome of Erythema Migrans After Treatment with Phenoxymethyl Penicillin

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In a 5 y retrospective follow-up study in southern Sweden of 708 adult individuals with erythema migrans as the single manifestation of Lyme borreliosis, the clinical outcome and the antibiotic treatment were studied. 80% were treated with phenoxymethyl penicillin, 15% with doxycycline and 5% with other antibiotics. Phenoxymethyl penicillin and doxycycline were extremely effective: 98 and 94% of the individuals reported complete recovery without complications. Few individuals reported the development of new symptoms following treatment and none developed any late manifestation of Lyme borreliosis during the observation period. Thus, in the area studied the treatment of the early localized manifestation of Lyme borreliosis (erythema migrans) with antibiotics was extremely successful. The current Swedish recommendation to use phenoxymethyl penicillin, when no sign of disseminated infection or coinfection with other tick-borne pathogens is present, seems excellent.

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INTRODUCTION

Erythema migrans (EM) caused by tick bites infested with Borrelia burgdorferi s.l. is the early localized manifestation of the most important vector-borne disease in Sweden, Lyme borreliosis (LB). EM occurs at a frequency of about 80% in LB (1).

Although EM is often a self-limiting condition, untreated cases can lead to late manifestations such as arthritis, neuroborreliosis or cardiac symptoms. Before antibiotic treatment of LB was established, a US study of 55 individuals with untreated EM for a period of 3–8 y showed the development of late manifestations in approximately 80% (2). In Europe, the development of neuroborreliosis was reported in 17% of untreated cases of EM (3).

In Sweden, the use of penicillin in the treatment of EM follows a long tradition of therapy. Successful treatment of EM and meningitis was first reported in 1951 by Hollström, a few years before Binder and co-workers suggested the cause of EM to be an infectious agent susceptible to penicillin (4, 5).

In this investigation, a large, consecutively recruited number of adult LB patients in a prospective surveillance study was subjected to a retrospective follow-up after treatment for EM. The kind of antibiotic treatment prescribed was related to the clinical response and the presence of additional symptoms during and after treatment.

MATERIALS AND METHODS

Study area

The study area consisted of the 7 southern counties of Sweden. It initially involved 2,133,068 inhabitants or 24% of the Swedish population.

Study design

The study was a retrospective long-term follow-up study based on an epidemiological, population-based, prospective study conducted between May 1, 1992 and April 30, 1993 (1). In May 1998, the present follow-up study was conducted, including 914 adult patients with EM as the only manifestation of LB when diagnosed in the former study.

A questionnaire was sent out to the participants. The questions of relevance for this study were: 'Did the EM disappear with the treatment?', 'Did you develop any other symptoms that required a doctor's appointment during the treatment for the EM?' and 'Did you after the treatment develop any new symptoms that you have never had before?' Those replying affirmatively to any of the questions were asked to give more detailed information on the back of the form and were interviewed by telephone.

Case definitions

All included patients had an EM diagnosed by a physician. The diagnosis fulfilled the Centers for Disease Control and Prevention (CDC) criteria for surveillance purpose, i.e. an EM of at least 5 cm in diameter (6). All patients were treated with antibiotics. In the follow-up study, only adult patients were included. For a case to be diagnosed as a late manifestation of LB, such as Lyme arthritis, neuroborreliosis or acrodermatitis chronica atrophicans (ACA), we required a characteristic clinical manifestation of the disease plus serological confirmation indicating a 4-fold elevation of the titre of Borrelia burgdorferi antibodies, or a seroconversion when acute- and convalescent-phase serum samples were analysed simultaneously on the same enzyme-linked immunosorbent assay (ELISA) microplate. In the case of ACA, a skin biopsy with typical histological findings was required. In these cases, after informed consent, the patients' complete medical records were studied to assess accurate information.

Statistical methods

The χ^2-test was used to compare groups. A p-value < 0.05 was considered significant.

Ethics

The study was approved by the ethics committee at the University of Lund.

RESULTS

The prospective surveillance study identified a total of 1075 individuals with EM as the single manifestation of LB. Excluding the 99 patients under the age of 15 y, the questionnaire was sent to 914 individuals and answered by 708 (77.5%), 427 women (60%) and 281 men (40%), with an average age of 55.5 ± 17.2 y (mean $\pm$ SD, range 15–91; women 56.8 ± 16.9 y, men 53.6 ± 17.4 y). Thus, a total of 268 individuals, 123 women (46%) and 145 men (54%), with a mean age of 53.8 y (women 56.8 y, men 51.3 y) had dropped out of the study (Fig. 1). There were no significant differences in age or gender among participants versus dropouts.

Each patient was treated with antibiotics, as decided by his or her physician. The given dose of phenoxymethyl penicillin (PCV) was 2–4 g daily for 10 d, doxycycline 100–200 mg daily for 10–15 d and erythromycin 1 g daily for 10 d. 567/708 (80%) were treated PCV and 107/708 (15%) with doxycycline. The remaining patients were treated with erythromycin ($n = 13$), other antibiotics ($n = 17$) or a combination of 2 different antibiotics ($n = 4$).

In 689/706 (98%) of the individuals, the EM disappeared with treatment. Two individuals did not answer the specific question of whether the EM had healed; 1 was treated with PCV and the other with doxycycline. The EM healed in 556/566 (98%) of those treated with PCV and 100/106 (94%) of those treated with doxycycline.

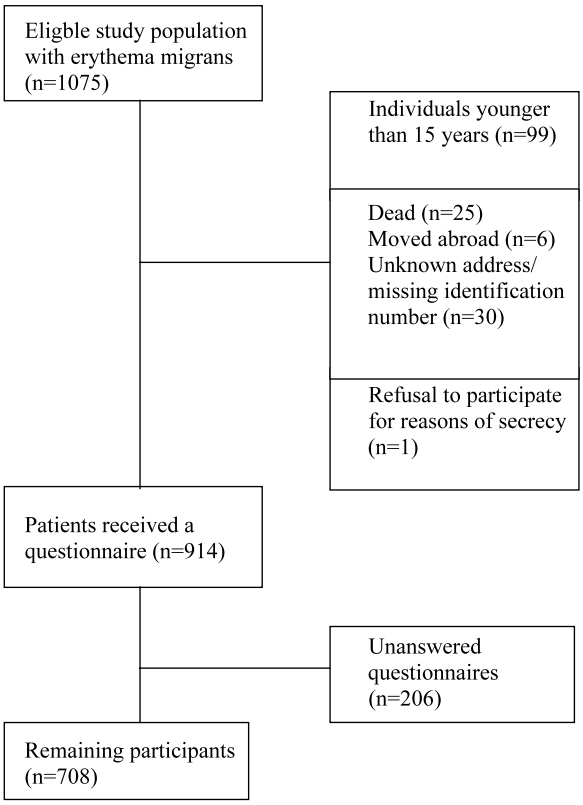

Fig. 1. Schematic illustration of the participant flow in a 5 y follow-up study among patients with erythema migrans.

10 of the 17 individuals where the EM did not heal during treatment were treated with PCV, of whom 4 had received the recommended dose and completed the treatment. Six of the 17 individuals were treated with doxycycline, of whom 3 had completed the treatment at the recommended dose.

In total, 25/708 (3.5%) of the individuals reported additional symptoms that required an extra visit to their physician during the treatment for the EM. All 25 individuals had received the recommended doses of antibiotics. 667/708 (94%) did not report any additional symptoms and 16/708 (2.2%) did not answer this specific question.

There were significantly more individuals reporting additional symptoms in those treated with doxycycline (12/107), compared with PCV (12/567) ($p < 0.001$). Neurological symptoms were reported in 4 cases, joint symptoms in 9, skin symptoms in 8 and other symptoms in 6.

In total, 113/708 (16%) of the individuals reported that during the 5 y following treatment for the EM, they developed symptoms not experienced earlier. 22 of these 113 individuals reported symptoms from several locations. Most of these complaints were unspecific and none of them fulfilled the criteria for late manifestations of Lyme borreliosis. The most common symptoms were arthralgia, reported by 56/135 (41%), and skin symptoms, reported by 39/135 (29%). Cardiac and neurological symptoms were reported in 24/135 (18%) and 16/135 (12%), respectively. New neurological symptoms were reported significantly more frequently by those treated with doxycycline than by those treated with PCV ($p < 0.01$).

DISCUSSION

In this study, treatment of EM with PCV was found to be highly effective, with 98% of the individuals reporting complete recovery. Treatment with PCV was significantly more effective than with doxycycline, but it must be emphasized that 5 times more patients were treated with PCV than with doxycycline and it is possible that the results were biased by a preferential use of doxycycline in cases with more symptoms, i.e. early signs of disseminated infection. The study was not designed to compare different antibiotic treatments according to their efficiency or side-effects.

According to an expert committee at the Swedish Medical Products Agency, 1 g of PCV 2 or 3 times daily for 10 d is currently recommended. If the patient is allergic to penicillin, doxycycline 200 mg for 1 d followed by 100 mg daily for another 8 d is recommended. When multiple erythemas or other early signs of disseminated infection are present, doxycycline 200 mg daily for 14 d is recommended (7).

In other parts of Europe and in the USA, drugs other than PCV are commonly used. Murray and Feder reported that the majority of over 200 physicians prescribed doxycycline or amoxicillin to patients with EM (8). Differences in species distribution may influence the treatment regimen. EM is held to be slower spreading and less intense in Europe than in the USA. This is probably related to the predominant occurrence of B. afzeli and B. garnii in Europe versus B. burgdorferi s.s. in the USA (9). In Sweden, B. afzeli and B. garinii cause most cases EM. (10). However, among Swedish host-seeking adult Ixodes ricinus ticks, the presence of B. burgdorferi s.s. is 13% (11).

In the present study, more individuals treated with doxycycline reported side-effects during the treatment compared with those treated with PCV. However, it is important to stress that each physician independently decided which drug to prescribe to the patient. The doxycycline regimen could have been chosen because the patient was allergic to penicillin, but also because he or she initially presented with mild symptoms indicating a disseminated infection, e.g. headache, fever or muscle pain.

Only 3.5% of the patients reported that, in connection with antibiotic treatment for EM, they developed other symptoms that led them to make a new appointment with a physician. All individuals reporting symptoms from the nervous system, heart, skin or joints were contacted and their records analysed. None of the cases fulfilled the criteria for any late manifestations of LB.

In total, 16% of the individuals in this study reported that during the 5 y following the treatment for the EM they developed other symptoms that they had never had before. The most common symptom was arthralgia, reported by 8% of the participants. A study in southern Sweden reported that 4.9% of 900 randomly selected individuals between the ages of 50 to 70 y had had arthralgia for more than 6 weeks during the past 12 months (12). The findings in the present study do not seem to differ from these figures in the general population, thus indicating no long-term consequences of accurately treated EM.

Few studies have compared the effectiveness of PCV with other antibiotics in the treatment of EM. Arnez et al. showed that cefuroxime and PCV are equally effective in the treatment of children with EM (13). Weber et al., in their study of 32 patients, found that azithromycin seemed to be just as effective as PCV in the treatment of early manifestations of LB, and in another study they pointed out that ceftriaxone ought to be preferred to PCV in patients with 'more severe' early LB (14, 15). The present results show satisfactory efficiency of both PCV and doxycycline for the treatment of EM in Sweden.

The strength of this study was the large size of the study population. It must be emphasized that the study was not designed to compare different antibiotic regimens; however, it still showed that treatment of EM with PCV is extremely successful, bearing in mind the benefits of a drug with low resistance, few side-effects and low costs. Furthermore, the Swedish tradition of using PCV when no signs of disseminated infection or coinfection with other tick-borne pathogens, e.g. ehrlichia, are present, seems an excellent choice and PCV should be recommended as the first-line drug.

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ARTICLE

Increased incidence of Lyme borreliosis in southern Sweden following mild winters and during warm, humid summers

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Abstract The aim of the present study was to investigate the long-term incidence rate of Lyme borreliosis and, additionally, to determine whether a correlation exists between climatic factors and summer-season variations in the incidence of Lyme borreliosis. Climatic variability acts directly on tick population dynamics and indirectly on human exposure to Lyme borreliosis spirochetes. In this study, conducted in primary healthcare clinics in southeastern Sweden, electronic patient records from 1997–2003 were searched for those that fulfilled the criteria for erythema migrans. Using a multilevel Poisson regression model, the influence of various climatic factors on the summer-season variations in the incidence of erythema migrans were studied. The mean annual incidence rate was 464 cases of erythema migrans per 100,000 inhabitants. The incidence was significantly higher in women than in men, 505 and 423 cases per 100,000 inhabitants, respectively ($p<0.001$). The summer-season variations in the erythema migrans incidence rate correlated with the monthly mean summer temperatures (incidence rate ratio 1.12; $p<0.001$), the number of winter days with temperatures below 0°C (incidence rate ratio 0.97; $p<0.001$), the monthly mean summer precipitation (incidence rate ratio 0.92; $p<0.05$), and the number of summer days with relative humidity above 86% (incidence rate ratio 1.04; $p<0.05$). In conclusion, Lyme borreliosis is highly endemic in southeastern Sweden. The climate in this area, which is favourable not only for human tick exposure but also for the abundance of host-seeking ticks, influences the summer-season variations in the incidence of Lyme borreliosis.

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Introduction

In Europe, Lyme borreliosis (LB) is caused by the spirochetes *Borrelia burgdorferi* sensu lato, i.e. *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia burgdorferi* sensu stricto, all of which are transmitted by the tick *Ixodes ricinus*. The prevalence of *B. burgdorferi* sensu lato spirochetes in *I. ricinus* ticks is reported to range from 6 to 13% in nymphs and from 15 to 36% in adult females [1]. The nondisseminated cutaneous form, erythema migrans (EM), is the most frequent clinical manifestation of LB, representing over 70% of the cases diagnosed in southern Sweden [2]. In a study in this area, 94% of EM skin isolates were *B. afzelii* strains and 6% were *B. garinii* strains [3], while in a Finnish study on the island of Åland, 66% of the EM isolates were *B. afzelii* strains and 33% *B. garinii* strains [4].

There are indications that the incidence of LB is increasing in this area of Sweden. In 1992, 164 cases of LB were reported in the county of Blekinge in southern Sweden [2], and in 1997, 459 cases were reported [5].

Studies show that risk factors for human exposure to ticks and tick-borne diseases depend on tick abundance and the prevalence of infected ticks [1, 6–10]. Climate has an influence on tick abundance, and ambient conditions such

as air temperature and humidity affect seasonal tick activity [11–13]. Mild winters, early springs, warm summers, and warm autumns with a high relative humidity seem to increase tick abundance and the risk of tick-borne infections [9, 14–16].

The aim of the present study was to investigate the long-term incidence of EM. In addition, we wanted to study correlations between climatic factors and the summer-season variations in the incidence of EM.

Materials and methods

Study area and population

The study area, Blekinge County, is located in the southeastern part of Sweden and covers an area of 2,941 km^2. It is characterized by long coastlines along the Baltic Sea and an extensive archipelago. Seventy-five percent of the area is covered by deciduous woodlands and pine and mixed forests. The rest of the area is open land and moors with numerous small lakes and rivers. The climate is characterized as "warm temperate," with mild winters. Mean temperatures are above −3°C in winter and around 15°C in summer. Precipitation varies between 500 and 700 mm per year. The county has a rich animal life, especially with respect to tick hosts such as birds, rodents, deer, and elk.

About 150,000 individuals live in Blekinge County, of whom 123,495 (61,712 women, 61,783 men) receive care through the national primary healthcare system. During the study period, the change in population size, at less than 0.2% was considered negligible.

Study design

In this retrospective study, we used the electronic patient record systems Swedestar and PAS-ORIGO to search for all medical records of patients diagnosed with EM attending primary healthcare clinics in Blekinge County and the Department of Infectious Diseases at Blekinge County Hospital. The study period covered 6 years, 1997–2002.

The annual and monthly incidence rates of EM were studied. The influence of climate on the EM incidence rate was studied relative to the summer seasons, since over three-quarters of the cases and the greatest variations in the EM incidence rate occurred during the period 1 June to 30 September. The influences of general climatic factors such as temperature, precipitation, and relative humidity on the EM incidence rate during the summer seasons were studied. Additionally, the influence of certain climatic thresholds on the EM summer incidence rate relative to earlier published data of climatic influence on *I. ricinus* tick abundance and tick activity were studied. Only thresholds applicable to the prevailing climate in the area were used in the present study. The temperature threshold for tick host-seeking activity is a weekly mean maximum temperature of >7°C [17–21]. The tick can actively seek a host at levels of relative humidity above 86%; below this threshold, the tick climbs down to lower vegetation in order to rehydrate [22]. Cold winter days with temperatures below 0°C can negatively influence tick abundance by reducing the survival of the ticks and their hosts (J. Gray, personal communication, 15 September 2005). This outcome could affect tick abundance in the following 2 years, since the tick lives 3 years, with larvae and nymphs as the host-seeking stages.

Climate data were correlated 14 days before the diagnosis of the EM cases, which represents the incubation period from tick bite to diagnosis. Winter temperatures were measured from 1 December to the last of February each year (Table 1).

Case definitions

To identify cases of EM, the electronic patient records were searched by diagnosis. For inclusion in the study, the electronic patient record had to contain the following information indicating that the EM was preceded by a

Table 1 Description of the climatic variables included in the multilevel Poisson regression model

Climate variable	Explanation	Range	Mean	Median	SD
Mean temperature[a]	Mean monthly temperature (°C)	11.2–19.4	15.2		2.2
Mean RH[a]	Mean monthly RH (%)	67.9–90.4	79.4		4.5
Mean precipitation[a]	Mean monthly value of daily precipitation (mm)	0.0–3.9	1.9		0.9
No. of days with RH >86%[a]	No. of days with RH >86%	0–26		8.0	5.6
No. of winter days <0°C[b]	No. of winter days with average temperature <0°C	20–47		35.5	9.4
No. of winter days <0°C (t−1)[b]	No. of winter days with average temperature <0°C, previous year	20–72		41.0	16.6

SD standard deviation, *RH* relative humidity

[a]Climate data were collected 14 days before the EM cases, which represents the approximate mean incubation period from tick bite to diagnosis. EM cases were diagnosed 1 June through 30 September

[b]Winter temperatures were measured from 1 December to the last of February each year

probable tick bite; a description of the clinical appearance and size of the lesion, which had to reach at least 5 cm in diameter; and verification that antibiotics had been prescribed. The cases included fulfilled the surveillance criteria set forth by the Centers for Disease Control and Prevention (CDC) [23].

Climate definitions

Climate information on temperature, relative humidity, and precipitation were obtained from the Swedish Meteorological and Hydrological Institute, Norrköping, Sweden. The data were measured 1.5 m above ground level in Ronneby/Bredåkra, which is located in the middle of Blekinge County. Temperature was measured in degrees Celsius, relative humidity in percent, and precipitation in millimetres.

Statistical methods

In all studies, p values were two-tailed, and a p value <0.05 was considered significant. Student's t test was used when comparing normally distributed continuous data. The chi-squared test was used when comparing categorical data and the Mann–Whitney U test when comparing nonparametric continuous data. Analyses were performed using the SPSS statistical computer software, version 11.0 (SPSS, Chicago, IL, USA).

To analyse "count data", multilevel analysis using multiple Poisson regression analysis was done to evaluate the effect of a particular climatic factor after adjusting for other climatic factors [24]. To examine the extent to which individual and climatic characteristics explain the variability in the summer incidence rate of EM, we used a two-level model with a random intercept, with individuals at the first level and municipalities in Blekinge County at the second level. The associations between the variables studied were expressed as incidence rate ratios (IRRs) (95% confidence intervals [CIs]) in the fixed-effects part of the model. MLwiN software, version 2.0 [25], was used to perform the analyses. Parameters were estimated by using iterative generalized least squares. The use of restricted iterative generalized least squares gave very similar results.

Ethical considerations

The study was approved by the Ethics Committee at Lund University. These experiments complied with the current laws in Sweden.

Results

We identified 3,437 cases (1,869 women, 1,567 men) that met the criteria for EM. The annual mean incidence rate of EM was 464 cases per 100,000 inhabitants. There were considerable variations in incidence during the study period: 1999 had the lowest incidence, at 264/100,000, while 2000 had the highest incidence, at 664/100,000 (Fig. 1).

The mean age of women was 51.7 years (standard deviation [SD]=19.8 years, range 0.7–92.0 years) and of men, 46.9 years (SD=20.4 years, range 1.0–90.0 years) (p<0.001).

Significantly more women than men were affected (505 and 423 cases per 100,000 inhabitants; p<0.001). Among women, the incidence of EM was highest in those aged 65–69 years, with a prominent peak of 1,174/100,000 seen in the incidence curve. The incidence was higher in women over 40 years of age than in men of the same age (Fig. 2).

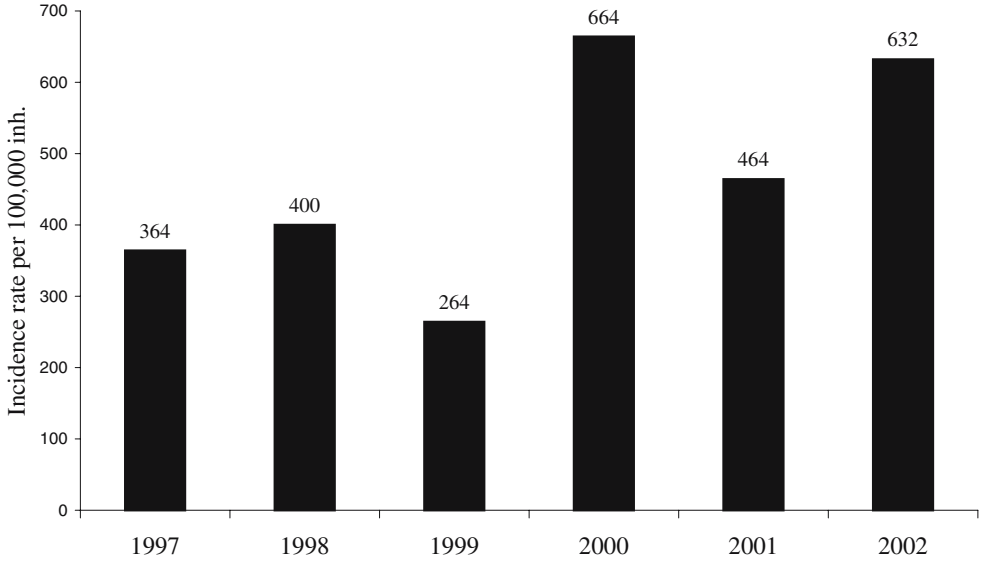

Fig. 1 Annual incidence of erythema migrans per 100,000 inhabitants in Blekinge County

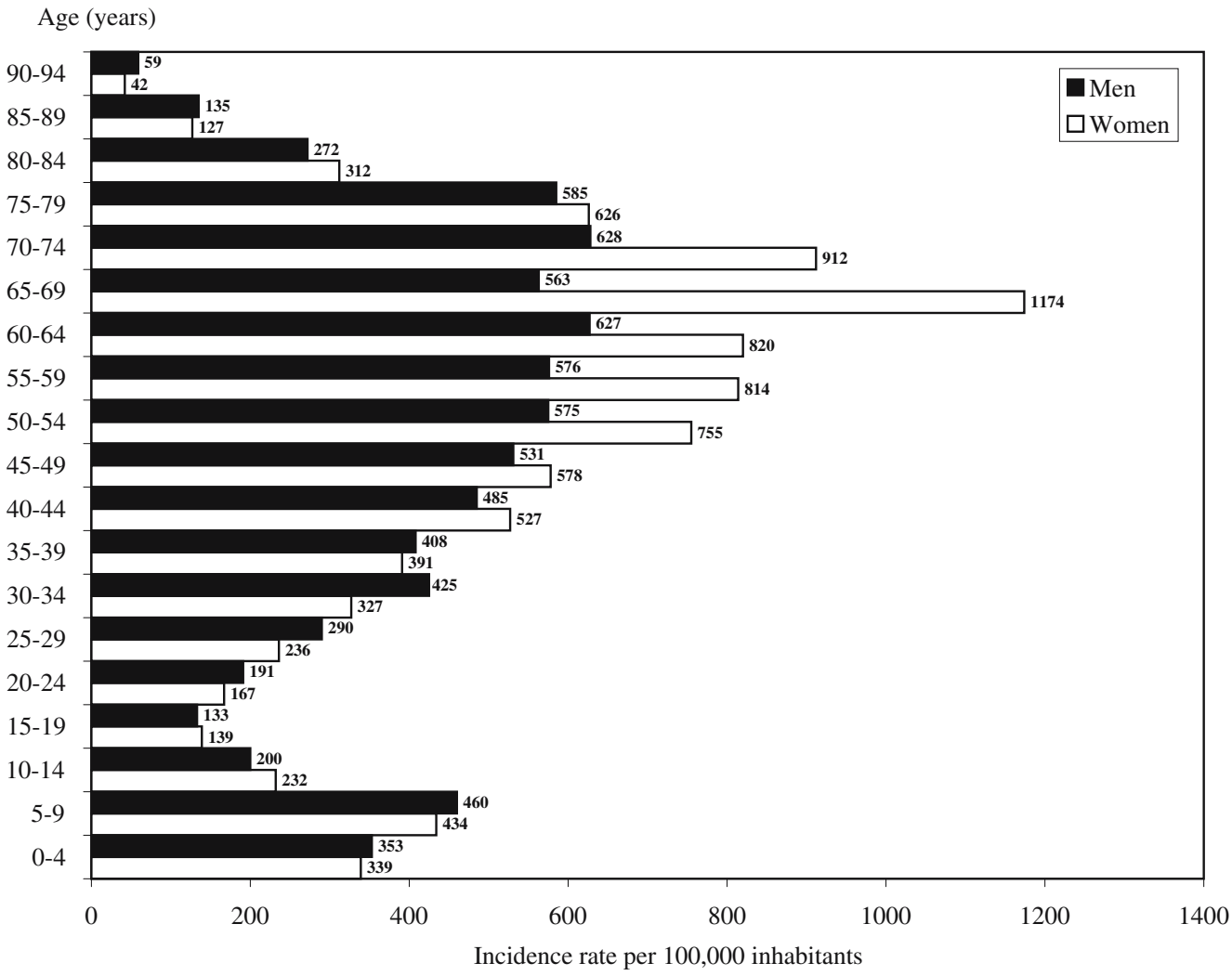

Fig. 2 Incidence of erythema migrans in women and men in different age groups, 1997–2002

Cases occurred throughout the year. However, youths under 15 years of age were affected by EM earlier in the year, i.e. in July, compared with individuals 15 years and older, who were affected primarily in August (p=0.021) (Fig. 3). Both women and men were affected by EM primarily in August (p=0.61).

Overall, 47% of the cases appeared in July and August. The greatest variations in the EM incidence rate were also observed during these months, which in large part explained the seasonal variations in EM incidence rate.

In a multilevel analysis using the Poisson regression model, the correlations between the summer-season EM incidence rate and climatic data were studied. The summer seasons, 1 June to 30 September 1997–2002, included 76% of the cases and the greatest variations in EM incidence rate. The monthly mean temperatures (IRR 1.12, 95% CI 1.08–1.16; p<0.001) and the number of days during the winter with mean temperatures below 0°C (IRR 0.97, 95% CI 0.97–0.98; p<0.001) correlated with the EM incidence rate during the period studied. In addition, the EM incidence rate correlated negatively with mean monthly precipitation (IRR 0.92, 95% CI 0.84–0.99; p<0.05) and positively with the number of days with relative humidity levels above 86% (IRR 1.04, 95% CI 1.00–1.06; p<0.05). Additionally, in this model the incidence rate was 14% lower in men than in women (IRR 0.86, 95% CI 0.76–0.96; p<0.05). The incidence rate was 47% higher in individuals aged 15–64 years (IRR 1.47, 95% CI 1.25–1.72; p<0.001) and 94% higher in individuals over 65 years (IRR 1.94, 95% CI 1.64–2.30; p<0.001) compared with youths under 15 years of age (Table 2). The variance between municipalities was 0.64, with a standard error of 0.05.

Discussion

LB is highly endemic in Blekinge County, Sweden, with an annual mean EM incidence rate of 464 cases per 100,000 inhabitants. This figure, however, is conservative, since 14% of the electronic patient records from the primary healthcare

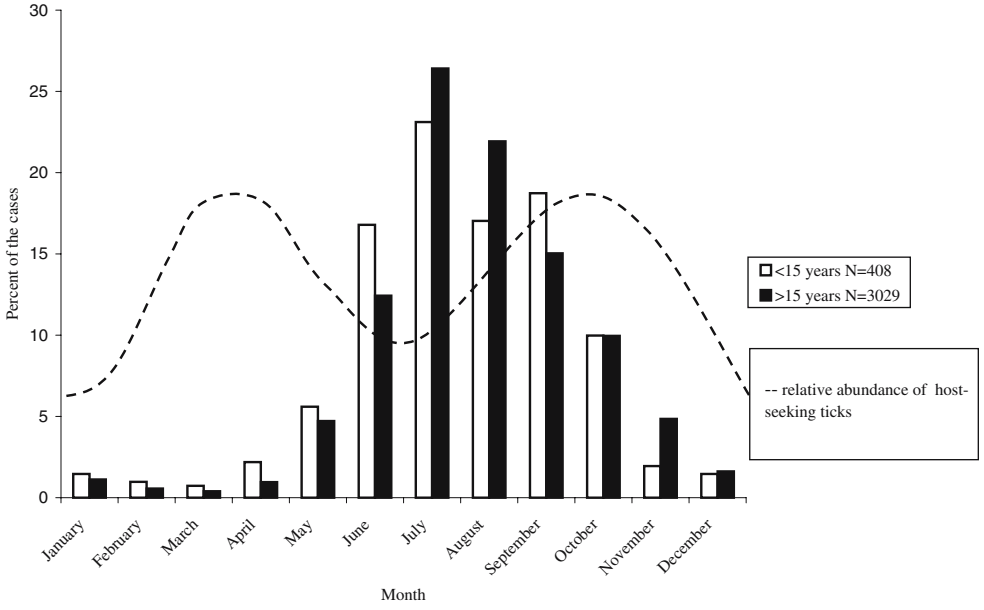

Fig. 3 Month of affliction with erythema migrans for individuals <15 years compared with individuals ≥15 years, and the relative abundance of host-seeking ticks

system lacked a definitive diagnosis and thus some cases of EM could have been missed. A total of 98.3% of the EM cases were treated in primary healthcare clinics, which confirms that EM cases in Sweden are addressed primarily by the national primary healthcare system.

There was great variability and a general increase in the seasonal incidence rate of EM during the study period. The population has been stable during the entire study period, and therefore the great variations in the EM incidence rate cannot be attributed to population changes. The highest incidence rates were observed in 2000 and 2002, and the incidence rate more than doubled between 1999 and 2000 (Fig. 1). Interestingly, in the UK the number of LB cases also doubled during the same period, from 150 to 300 cases per 100,000 population per year [26, 27].

We studied climatic factors that may have influenced the summer-season variations in the EM incidence rate. The number of EM cases depends on many factors, including human exposure to ticks, tick–host relationships, and tick abundance. To directly measure the tick or host abundance on a large scale is difficult; instead, we correlated climatic data with the incidence of EM. In the literature, certain climatic thresholds have been described as influencing tick activity and the abundance of ticks and hosts. We also studied the influence of climate from a more general perspective, not categorizing data according to thresholds, but instead using continuous data from monthly mean temperatures, relative humidity, and precipitation. We found a strong positive correlation between the monthly mean incidence rate of EM and the monthly mean temperatures during June, July, August, and September. An increase in the mean monthly summer temperature by 1°C increased the incidence rate of EM by 12%. Thus, the warmer the weather, the higher the probability of an increased number of cases. This effect is probably mostly the result of an increase in human exposure to ticks, since no positive correlations between summer air temperature and host-seeking activity for nymphs and adults have been observed [17–21]. In a German study, an increase in the incidence of tick-borne encephalitis was observed during

Table 2 Variables included in the multilevel Poisson regression model that influenced fluctuations in the LB incidence rate from 1 June through 30 September 1997–2002 in southern Sweden

Parameter	IRR	95% CI	*p* value
Gender			
Female	Reference		
Male	0.86	0.76–0.96	<0.05
Age			
0–14 years	Reference		
15–64 years	1.47	1.25–1.72	<0.001
65+ years	1.94	1.64–2.3	<0.001
Climate			
Mean temperature	1.12	1.08–1.16	<0.001
Mean RH	0.98	0.94–1.02	ns
Mean precipitation	0.92	0.84–0.99	<0.05
No. of days with RH >86%	1.04	1.00–1.06	<0.05
No. of winter days <0°C	0.97	0.97–0.98	<0.001
No. of winter days <0°C (*t*−1)	1.00	0.99–1.01	ns

ns not significant, *RH* relative humidity

warmer summers, an effect attributed to increased human exposure to outdoor activities [28]. We found no studies that correlated climatic factors with the amount of time humans spend outdoors, but undoubtedly humans dress more lightly during the summer, thus increasing the risk of tick bites.

In this study, there was also a strong positive correlation between milder climate during the winter and the EM incidence rate: a decrease by 1 day of the number of winter days with temperature below 0°C increased the incidence rate of EM by 3% the following summer season. This result is interpreted as an effect on the survival of ticks and their hosts. The findings are in accordance with studies performed by Lindgren et al. [14, 15], who concluded that a warmer climate with milder winters and earlier arrival of spring was related to increased tick density and an increased number of tick-borne encephalitis cases in Sweden.

We also found a significant negative correlation between the mean monthly precipitation and the EM incidence rate; an increase in the mean monthly summer precipitation by 1 mm decreased the EM summer incidence rate by 8%. This finding might be explained by two factors: decreased human tick exposure because of less time spent outdoors, and the use of rain garments, which provide more coverage. A study in the UK identified a negative effect of rain on the rate of attachment of ticks to humans [7]. The result could also be explained by an impaired host-seeking ability in ticks; the dampness may inhibit their ability to climb vegetation when seeking a host (Jeremy Gray, personal communication, 15 September 2005).

Furthermore, the number of days with levels of relative humidity above 86% also correlated positively with the incidence rate of EM. An increase by 1 day in the number of days with relative humidity above 86% increased the EM incidence rate by 4%. This is interpreted as having a direct effect on ticks, as earlier studies have shown that the host-seeking activity of *I. ricinus* depends on the relative humidity [12], and others have shown that below a relative humidity threshold of 86%, the tick dehydrates and cannot continuously seek a host [22].

The lowest incidence rate occurred in 1999, a year that was preceded by a cold winter with many days below 0°C (n=42 days) and that had few summer days with relative humidity above 86% (n=7.0 days). The highest incidence rates occurred in 2000 and 2002. In 2000 the preceding winter was mild, with only a few days below 0°C (n=27 days), and the summer had many days with relative humidity above 86% (n=11.3 days). In 2002 there were low amounts of summer precipitation, a mean daily precipitation of 1.5 mm, a mild winter with few days below 0°C (n=31 days), and a high mean temperature of 16.9°C. Cases occurred all year round. This may have been due not only to patient delay in seeking treatment but also to the mild winters. For example, during the year with the mildest winter, 2000, the highest numbers of cases were observed from January to March.

The seasonal activity of the *I. ricinus* nymphs and adults peaks from March to May and from August to October, respectively [1, 17, 18, 29]; thus, peaks in the incidence rate of LB would be expected in April/June and in September/November. Yet in this study, in almost half of the cases the onset of disease occurred in July and August; consequently, on the basis of the incubation period, subjects were exposed to tick bites in June and July. This is supported by a recent study in Blekinge County, where most individuals were bitten by ticks during the Swedish vacation months of June and July [30]. The fact that the majority of EM cases occurs after a period when the numbers of host-seeking ticks drops strongly points to the influence of increased human outdoor activity (Fig. 3). Thus, human exposure to ticks has a stronger influence on the EM incidence rate than the seasonal feeding activity of the nymphal *I. ricinus*.

The bimodal distribution seen in this study has been reported in other work from the USA and France [31–33]. In this study, as in a former study from southern Sweden [2], the incidence rate was highest in middle-aged and elderly women, with incidence peaking among women aged 65–69 years. Our finding that women are affected by EM to a higher extent than men is supported by earlier studies in Europe [34, 35] but not in the USA [31, 36, 37].

Further studies are needed to explain the gender differences between EM incidence rates in Europe and the USA. Exposure to outdoor activities may explain the differences between Swedish women and their American counterparts, although differences in genospecies, as well as differences in immunological characteristics, may also affect gender differently in Europe and the USA. In collaboration with the University of Linköping, a study of reinfected Swedish patients with EM has indicated that the type 1 immune response is less intense in postmenopausal females than in males (unpublished data, September 2005). The type 1 cell-mediated immune response is important in eradicating the spirochete in humans [38]. Thus, postmenopausal women may be more susceptible to spirochetal infections.

In conclusion, LB is highly endemic in Blekinge County, and almost every patient with EM seeks assistance through the national primary healthcare system. Most cases of EM appear during the summer vacation period, when people have the opportunity to spend more time outdoors. The EM incidence rate was influenced by a climate favourable not only for human tick exposure but also for an abundance of host-seeking ticks.

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Clinical appearance of erythema migrans caused by *Borrelia afzelii* and *Borrelia garinii* – effect of the patient's sex

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Erythema migrans nach Infektion durch *Borrelia afzelii* oder *Borrelia garinii:* Abhängigkeit des klinischen Erscheinungsbildes vom Geschlecht der Patienten

Zusammenfassung. *Ziel der Studie:* Untersuchung der klinischen Charakteristika von durch *Borrelia* Genospezies bedingten Infektionen bei Patienten mit Erythema migrans, bei denen die Diagnose der Borreliose durch PCR gesichert war. Zusätzlich sollten Faktoren untersucht werden, die das klinische Erscheinungsbild des Erythema migrans beeinflussen könnten.

Methoden: Diese Studie wurde in Südschweden von Mai 2001 bis Dezember 2003 an Patienten, die älter als 18 Jahre waren und die die Ambulanz wegen Erythema migrans aufgesucht hatten, durchgeführt. Alle Erythema migrans wurden durch PCR bestätigt, photographiert und als „annulär" oder „nicht-annulär" kategorisiert. Ein logistisches Regressionsmodell wurde zur Analyse von möglichen Zusammenhängen zwischen der Erscheinungsform des Erythema migrans (annullär oder nicht-annulär) und verschiedenen Faktoren, die die klinische Erscheinungsform beeinflussen könnten, angewandt.

Ergebnisse: 118 Patienten, 54 Frauen (45,8%) und 64 Männer (54,2%), erfüllten die Einschlusskriterien. 74% der Patienten war mit *B. afzelli* und 26% mit *B. garinii* ($p < 0{,}001$) infiziert. Insgesamt waren 45% (38/85) der Erythemata annulär, 46% waren nicht-annulär und 9,4% wurden als atypisch eingestuft. Die Odds Ratios für Männer die mit *B. afzelii* infiziert waren, ein nicht-annuläres Erythema migrans zu entwickeln, lagen im Vergleich zu Frauen mit der gleichen Infektion bei 0,09 (95%; CI: 0,03–0,33).

Schlussfolgerungen: In dieser prospektiven Studie an einer großen Zahl von Patienten mit Erythema migrans, deren Genospezies durch PCR gesichert war, hatte das Geschlecht einen starken Einfluss auf die klinische Erscheinungsform des Erythema migrans bei *B. afzelii* Infektion. Die Patienten mit einer Infektion durch *B. garinii* hatten öfter nicht-annuläre Erythemata, die Infektion verlief virulenter mit häufigerem Fieber, häufiger erhöhten CRP Spiegel und positiver Seroreaktivität in der Genesungsphase.

Summary. *Aim:* The aim in this survey was to study the clinical characteristics of infections caused by *Borrelia* genospecies in patients with erythema migrans where borrelial origin was confirmed by polymerase chain reaction. The aim was also to study factors influencing the clinical appearance of erythema migrans.

Methods: The study was conducted in southern Sweden from May 2001 to December 2003 on patients 18 years and older attending with erythema migrans at outpatient clinics. All erythema migrans were verified by polymerase chain reaction, photographed and categorized as "annular" or "non-annular" lesions. A logistic regression model was used to analyze relations between the appearance of the erythema migrans (i.e. annular or non-annular) and factors that influenced its clinical appearance.

Results: A total of 118 patients, 54 women (45.8%) and 64 men (54.2%), fulfilled the inclusion criteria. Of these patients, 74% were infected by *B. afzelii* and 26% by *B. garinii* ($p < 0.001$). A total of 45% (38/85) of the erythema migrans were annular, 46% (39/85) were non-annular and 9.4% (8/85) were atypical. For men infected by *B. afzelii*, the odds ratio of developing non-annular erythema migrans was 0.09 (95% CI: 0.03–0.33) in comparison with women with the same infection.

Conclusions: In this prospective study of a large series of erythema migrans, where infecting genospecies were confirmed by polymerase chain reaction, the sex of

patients infected with *B. afzelii* had a strong influence on the appearance of the rash. Patients infected by *B. garinii* more often had non-annular erythema migrans and a more virulent infection with more individuals presenting with fever, raised levels of C-reactive protein and seroreactivity in the convalescence sera.

Key words: Lyme borreliosis, erythema chronicum migrans, *Borrelia garinii*, *Borrelia afzelii,* polymerase chain reaction.

Introduction

Lyme borreliosis is caused by tick-borne spirochetes belonging to the genospecies complex *Borrelia burgdorferi* sensu lato (s.l.). The most common clinical manifestation is the non-disseminated cutaneous form erythema migrans (EM) occurring at the site of inoculation [1–3]. The geographical distribution of *Borrelia* genospecies differs in the USA and Europe: in the USA all isolates have been members of the genomic group *B. burgdorferi* sensu stricto (s.s.), whereas in Europe EM is mostly caused by the spirochetes *B. afzelii* and *B. garinii* [4–6]. In culture-confirmed EM, patients bitten in the USA develop systemic symptoms and seroreactivity more often than European patients with EM caused by *B. afzelii* [7]. Also, European patients infected by *B. garinii* more frequently present with flu-like symptoms than those infected with *B. afzelii* [8, 9].

EM is typically "annular" with a central clearing or "homogeneous", but atypical lesions may occur [3, 10–12]. There has been some debate on whether the different predominant patterns reflect different genospecies or the duration of EM at presentation [8, 9, 13].

There are few reports in Europe on differences in clinical manifestations caused by the various genospecies of *B. burgdorferi* s.l. The aim of this study was to investigate the clinical characteristics of infections caused by *Borrelia* genospecies in patients with EM where genospecies were confirmed by polymerase chain reaction (PCR). An additional aim was to describe and categorize EM patterns and the factors influencing their clinical appearance.

Methods

Study area and population

This study was part of the multi-center "EM biopsy study" conducted in southern Sweden from May 2001 to December 2003. The study population included patients attending at seven outpatient clinics in the county of Blekinge.

Study design

This prospective study included patients 18 years and older seeking care for EM > 5 cm in diameter where a borrelial origin of the EM was verified through a positive PCR analysis.

The patients gave their written informed consent to participate in the study. During their visit to the doctor, each participant answered a questionnaire about the tick bite, the erythema and clinical symptoms. The erythema, together with an ID number and a plastic ruler measuring the area of the EM, was photographed using a digital camera. Complete blood counts and liver function tests were performed and C-reactive protein (CRP) levels were measured. Titers of IgM and IgG were measured according to the manufacturer's protocol (kit 6029, Dako; antigen, *B. afzelii*; strain DK-1). Skin-punch biopsies were taken from the leading edge of the EM after the administration of local anesthetics, and the biopsies were analyzed with PCR targeting the ospA gene.

All patients were treated with penicillin V (pcV) 1 g three times daily for 10 days or doxycycline 200 mg daily for 10 days, according to the recommendations of the Swedish Medical Product Agency [14]. After 14–21 days, patients were contacted by a nurse and asked about their clinical symptoms and the clinical appearance of the EM.

Classification of EM

The pictures of the lesions were classified by three physicians with extensive experience of treating patients with Lyme borreliosis. The lesions were classified into the following predominant patterns:

- *Annular erythemas*: round to oval, sharply demarcated, red to bluish-red lesions with a classic central clearing [10, 11]. The "bull's eye rash" is a type of annular EM with a darker, central, bluish-red macula, separated from the peripheral ring by normal skin [11, 12] (Fig. 1).
- *Non-annular erythemas*, including *homogeneous erythemas*: homogeneous red sharply demarcated lesions [10, 11] and *central erythemas*: dense central, red to bluish-red lesions surrounded by a paler-red peripheral ring [10] (Fig. 2).
- *Atypical erythemas*: lesions and/or pictures of lesions not assignable to any of the above categories, mainly because of artefacts such as the influence of local anesthetics or photos not possible to interpret.

PCR and sequence analysis

In order to confirm the EM diagnosis and determine the infecting genospecies, *Borrelia* DNA was amplified using a nested OspA PCR followed by nucleotide sequencing as previously described [15]. Briefly, DNA was extracted using a DNeasy tissue kit (Qiagen, Valencia, Ca, USA) and an elution volume of 50 µl according to the manufacturer's protocol. The master mix (PCR Core Kit, Roche Diagnostics GmbH, Penzberg, Germany) contained 0.2 µM of each primer, 0.2 µM of each deoxynucleoside triphosphate and 1.25U *Taq* DNA polymerase. Volumes of 5 µl and 1 µl DNA template were used in the first and second PCR reactions respectively. The PCR

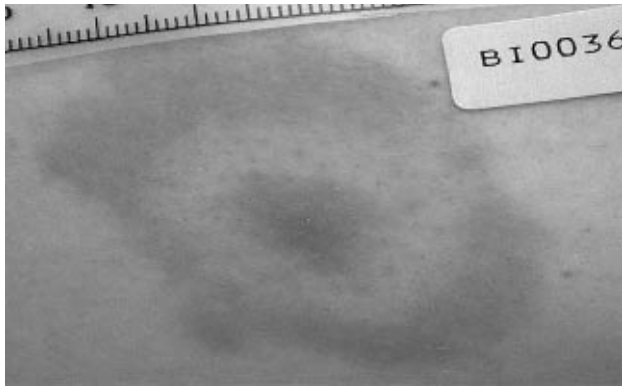

Fig. 1. An example of an annular erythema migrans

amplification conditions were: 35 cycles of 94 °C for 30 s, 50 °C for 60 s and 72 °C for 60 s. DNA amplicons were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide. Positive DNA samples were sequenced using the OspA PCR inner primer pair [15] and an ABI PRISM BigDye Terminator v.3.1 Ready Reaction Cycle Sequencing Kit (ABI, www.appliedbiosystems.com) according to the manufacturer's protocol. Each strand was analyzed in an ABI 3100 Genetic Analyzer (Applied Biosystems) by the Biomolecular Resource Facility at Lund University. The BioEdit software (Tom Hall, Department of Microbiology, North Carolina State University, NC, USA) was used for nucleotide sequence analysis.

Statistics

Student's *t*-test was used when comparing normally distributed continuous data. The Pearson chi-squared test was used when comparing categorical data. The Mann-Whitney U-test or the Kruskal–Wallis test was used when comparing non-parametric continous data. All *p*-values were two-tailed and p-values < 0.05 were considered statistically significant.

To evaluate which factors influenced lesion appearance, we used a logistic regression model to analyze the relations between clinical appearance (i.e. annular or non-annular EM) and age, sex, genospecies and time (i.e. time from tick bite to diagnosis). "Female sex" and "*B. afzelii"* were set as baseline variables. The associations between the variables studied were appraised by odds ratios estimating the relative risks for non-annular EM.

SPSS version 11.0 (SPSS Corp., Chicago, IL, USA) or Stata version 8.0 (Stata Corp., TX, USA) were used for statistical analyses.

Ethical considerations

The study was approved by the ethics committee at Lund University.

Results

Distribution of patients by age and sex

In this study, 118 patients, 54 women (45.8%) and 64 men (54.2%), fulfilled the inclusion criteria. The median age was 56.0 years (range 19.0–94.0), where the median age of the women was 59.0 years (27.0–94.0) and that of the men 53.5 years (19.0–80.0; $p = 0.020$).

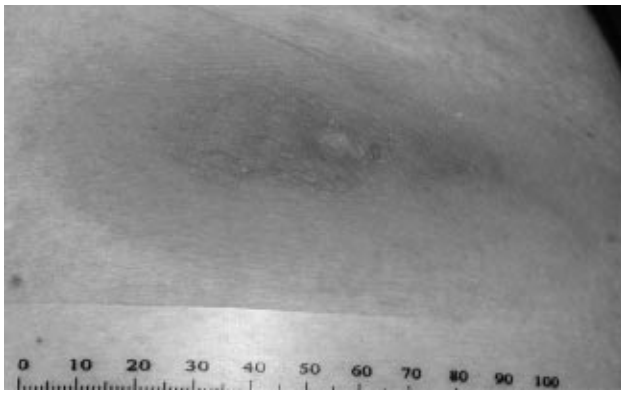

Fig. 2. An example of a non-annular erythema migrans

Table 1a. Characteristics of patients with erythema migrans infected with *B. afzelii* and *B. garinii* respectively

Characteristic	Patients with *B. afzelii* ($N = 87$)	Patients with *B. garinii* ($N = 31$)	*p* Value
Male, *N* (%)	47 (54)	17 (54)	n.s.[2]
Age, median, years (range)	55.0 (19–78)	61 (30–94)	0.005[1]
Size of lesion at presentation, median, cm^2 *N* (range)	70.0 85 (7.0–1593.0)	93.0 30 (24.0–414.0)	n.s.[1]
Annular/Non-annular EM, *B. afzelii* $N = 58$; *B. garinii* $N = 19$ (%)	34/24 (58.6/41.4)	4/15 (21.0/78.9)	0.004[2]
Duration from tick bite to diagnosis, median, days *N* (range)	21.0 72 (3–97)	14.0 24 (4–78)	0.011[1]
Duration from start of treatment to disappearance of EM, median, days *N* (range)	8.0 64 (1–35)	7.0 26 (2–17)	n.s.[1]
Treatment with pcV, *N* (%) doxycycline, *N* (%)	72 (82.7) 15 (17.2)	28 (90.3) 3 (9.7)	n.s[2]
Geographic area at the time of the tick bite coastal area, *N* (%) garden, *N* (%) forest, *N* (%)	18 (25.4) 31 (43.7) 19 (26.8)	6 (23.1) 13 (50.0) 4 (15.4)	n.s[3]

[1] Mann-Whitney test; [2] two-sided Pearson chi-squared test; [3] Kruskal-Wallis test.

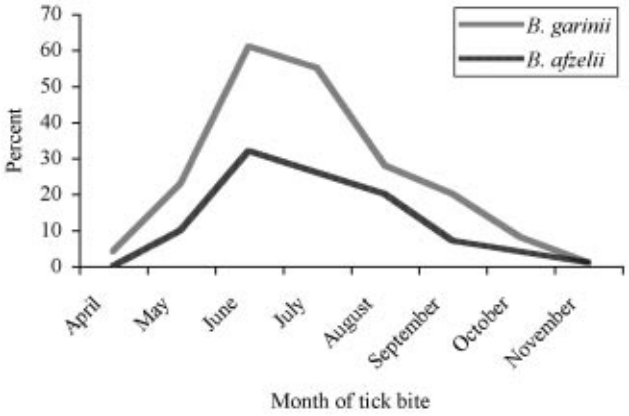

Fig. 3. The monthly distribution of tick bites for patients with erythema migrans caused by *B. afzelii* and *B. garinii,* respectively

Tick bites

In total, 92.4% (109/118) of the patients had noticed a tick bite at the location of a later EM skin lesion. The most common localizations of the erythemas were legs and feet (63.6%; 75/118) followed by chest and back (24.6%; 29/118), arms (10.2%; 12/118) and genitals (1.7%; 2/118). Most individuals were bitten when gardening or visiting meadows (48.4%; 44/91). In addition, 26.4% (24/91) were bitten by the seashore and 25.3% (23/91) in forest. There were no significant differences in the distribution of *B. afzelii* and *B. garinii* infections according to tick habitat (Table 1a). Most individuals were bitten in June (63.5%; 61/96) (Fig. 3).

Clinical appearances of EM

In 72.0% of patients (85/118), photographs were taken and the lesions categorized. The median size for the involved lesions was 80.0 cm^2. Just as many lesions were annular (44.7%; 38/85) as non-annular (45.8%; 39/85). Of the non-annular lesions, 27.0% (23/85) were homogeneous and 18.8% (16/85) were central erythemas. A total of 9.4% (8/85) were classified as atypical erythemas.

Most lesions caused by *B. afzelii* were annular, whereas most caused by *B. garinii* were non-annular [Table 1a]. There was a male preponderance of annular EM (27/38) and a female preponderance of non-annular EM (26/39; $p = 0.001$). The EMs on the abdominal region and back were not bigger than those on the legs, arms or neck ($p = 0.607$) and they did not appear faster than EMs on the extremities and neck ($p = 0.134$). The annular erythemas were significantly bigger than the non-annular erythemas ($p = 0.011$) and the duration from bite to diagnosis was significantly longer for patients with annular EM than for patients with non-annular EM ($p = 0.044$) (Table 1a).

The variables were studied in a logistic model in sequential steps to identify variables affecting the clinical appearance of the EM. The variable "size of the lesion" was not included in the model since this was considered as being influenced by time [16]. We studied the influence of age, sex, genospecies and time (i.e. time from tick bite to diagnosis) on the clinical appearance of the EM. The variable "time" was excluded from the model after it was found to have no significant influence on appearance. The variables "female sex" and "*B. afzelii"* were set as baseline variables. For men infected by *B. afzelii,* the odds ratio of developing non-annular EM was only 0.09 (95% CI: 0.03–0.33), whereas for women and men infected by *B. garinii*, the odds ratios of developing non-annular EM were similar, i.e. 1.74 (95% CI: 0.29–10.34) and 1.98 (95% CI: 0.34–11.56) respectively. The odds ratio of the interaction factor between patients' sex and the infecting genospecies was 12.46 (95% CI: 0.98–158.80) (Table 2).

Clinical aspects and laboratory results according to infecting genospecies and sex of patient

Characteristics of EMs caused by *B. afzelii* and *B. garinii* are summarized in Table 1. Significantly more individuals had EM caused by *B. afzelii* than by *B. garinii,* i.e. 73.7% vs. 26.3% ($p < 0.001$). There were no sex differences with respect to which *Borrelia* species had caused the EM. Individuals infected by *B. afzelii* were younger than those infected by *B. garinii* ($p = 0.005$). The median number of days from tick bite to initial visit to the doctor was 17.5 (3.0–97.0). Patients with lesions caused by *B. garinii* had a shorter duration from tick bite to diagnosis than patients with lesions caused by *B. afzelii* ($p = 0.011$) (Table 1a).

Table 1b. Clinical symptoms presenting in patients with erythema migrans caused by *B. afzelii* and *B. garinii*

Clinical symptoms	Patients with *B. afzelii* ($N = 87$)	Patients with *B. garinii* ($N = 31$)	p Value[1]
Headache, *N* (%)	24 (27.5)	8 (25.8)	NS
Muscle/joint pain, *N* (%)	12 (13.8)	5 (16.2)	NS
Chills, *N* (%)	8 (9.2)	4 (12.9)	NS
Airway symptoms, *N* (%)	6 (6.9)	4 (12.9)	NS
Neurological symptoms, *N* (%)	8 (9.2)	3 (9.7)	NS
Fever, *N* (%)	0 (0.0)	4 (12.9)	0.003
Neck stiffness, *N* (%)	3 (3.4)	1 (3.2)	NS
Photosensitivity, *N* (%)	2 (2.3)	0 (0.0)	NS

[1] Two-sided Pearson chi-squared test.

Table 1c. Laboratory findings in patients infected with *B. afzelii* and *B. garinii,* respectively

Laboratory test	Patients with *B. afzelii* ($N = 87$)	Patients with *B. garinii* ($N = 31$)	*p* Value[1]
Positive serological results			
at presentation, *N* (%)	24 (35.8)	12 (54.5)	n.s.
after 6 weeks, *N* (%)	27 (45.0)	16 (72.7)	0.017
CRP > 8 mg/l, *N* (%)	13 (14.9)	12 (38.7)	0.006
WBC < 3.5×10^9/l, *N* (%)	2 (2.3)	0 (0.0)	NS
WBC > 8.8×10^9/l, *N* (%)	5 (5.7)	3 (9.7)	NS
Platelets > 350×10^9/l, *N* (%)	6 (6.9)	1 (3.2)	NS
Platelets < 150×10^9/l, *N* (%)	3 (3.4)	0 (0.0)	NS
ALT > 0.75 µkat/l (w), *N* (%) > 1.10 µkat/l (m), *N* (%)	9 (10.3)	3 (9.7)	NS
AST > 0.60 µkat/l (w), *N* (%) > 0.75 µkat/l (m), *N* (%)	5 (5.7)	3 (9.7)	NS
LD > 7.0 µkat/l, *N* (%)	30 (34.5)	16 (51.6)	NS

[1] Two-sided Pearson chi-squared test; *CRP* C-reactive protein; *WBC* white blood count; *ALT* alanine aminotransferase; *AST* aspartate aminotransferase; *LD* lactate dehydrogenase; *w* women; *m* men.

All lesions healed within about a week after starting treatment, but there were no differences in this respect between lesions caused by *B. afzelii* and those caused by *B. garinii* or between annular and non-annular lesions [Table 1a]. However, the EM disappeared faster in men than in women; median 7.0 days (1.0–21.0) compared with median 11.0 days (2.0–35.0) ($p = 0.008$).

Local symptoms were reported by 24.6% (29/118) of the patients. The most common systemic symptoms were headache (27.1%; 32/118) followed by muscle/joint pain (14.4%; 17/118) and chills (10.1%; 12/118). Less common were airway symptoms (8.5%; 10/118), neurological symptoms (6.8%; 8/118), neck stiffness (3.4%; 4/118) and photosensitivity (1.7%; 2/118). Four patients (12.9%; 4/31) infected with *B. garinii* had temperatures above 38.0 °C, compared with none of the patients infected with *B. afzelii* ($p = 0.003$). No other significant differences in the frequency of clinical symptoms were found in relation to the sex of the patient or the infecting genospecies (Table 1b).

Significantly more patients with EM caused by *B. garinii* had elevated levels of CRP than did patients with EM caused by *B. afzelii* ($p = 0.006$). There were no differences according to sex or infecting genospecies in complete blood counts or liver tests (Table 1c).

A total of 40.4% of the acute serology results were positive but there were no significant differences between *B. afzelii* and *B. garinii* infections or according to patients' sex. However, after six weeks significantly more women than men were seropositive (women 71.8%, men 41.9%; $p = 0.013$) and more individuals infected by *B. garinii* were seropositive compared with those infected by *B. afzelii* (*B. garinii* 72.7%, *B. afzelii* 45.0%; $p = 0.017$) (Table 1c).

In total, 100/118 patients were treated with pcV and 18/118 with doxycycline, with no significant differences between *B. afzelii* and *B. garinii* infections (*B. afzelii* 72/87, *B. garinii* 28/31); however, significantly more men than women were treated with pcV (92.2%, 59/64 versus 75.9%, 41/54; $p = 0.014$).

Discussion

In this prospective study of patients from southern Sweden with EM verified by PCR, 74% of patients were infected by *B. afzelii* and 26% by *B. garinii*. We found an interesting interaction between the sex of the patient and the infecting genospecies, namely that the clinical appearance of the EM depended on whether *B. afzelii* was infecting a man or a woman. When infected by *B. afzelii,* men developed annular EM more often and women developed non-annular EM more often.

In an earlier Swedish study [13], the appearance of annular EM was estimated as a function of time, and the influence of a patient's sex and the particular genospecies was not considered in that study. In the present study, we found no correlation between lesion appearance and median time from tick bite to diagnosis. Other studies have indicated that *B. garinii* mostly causes homogeneous EM [8, 9] and that different genospecies disseminate with varying intensity, resulting in different symptoms [7, 8, 10, 13, 16]. In the USA, EM is caused solely by *B. burgdorferi* s.s. and, in comparison with European EM caused by *B. afzelii*, patients in the USA more often have non-annular EMs that develop faster [7, 8, 10, 13, 16]. In addition, infections with *B. burgdorferi* s.s are reported to be more virulent than infections with *B. afzelii* [7]. In our study we found that EM caused by *B. garinii* developed faster and was more likely to be non-annular. Thus, genospecies' varying virulence and tendency to disseminate could affect lesion appearance. However, we also found that the sex of the patient, by interacting with the infecting genospecies, was of importance. More women than men

Table 2. Odds ratio of non-annular erythema migrans according to sex of patient and *Borrelia* genospecies. "Female sex" and "*B. afzelii*" are baseline variables. The odds ratio of the interaction factor between sex and genospecies is 12.46 (95%CI: 0.98–158.80). N = 77

	Sex of patient	
	female (95% CI) N = 26	male (95% CI) N = 13
Genospecies		
B. afzelii	1.00 (68%)	0.09 (0.03–0.33) (16%)
B. garinii	1.73 (0.29–10.34) (78%)	1.98 (0.34–11.56) (80%)

CI confidence interval.

had detectable antibodies against *B. burgdorferi* s.l. six weeks after diagnosis and we also found that men's lesions disappeared faster than women's. Altogether different immunological reactions in the two sexes might explain our findings. Most women in our study were postmenopausal with a median age of 59 years, and after menopause the levels of estrogen decrease, leading to an altered immune status [17]. The "type-1" cell-mediated immune response is important in eradicating the spirochete in humans and the "type-2" antibody-mediated immune response is important in down-regulating the "type-1" response and the anti-inflammatory process [18]. A study of Swedish patients reinfected with Lyme borreliosis has indicated that this "type-1" immune response is less intense in postmenopausal women than it is in men [19].

Patients in the USA are reported as having local and systemic symptoms that disseminate to other organ systems more often than European patients [7, 10, 16]. In the present study the frequency of systemic symptoms was less than reported in the USA but was similar to other European studies [8, 24]. Concerning genospecies, four individuals with *B. garinii* infections had fever, compared with none of the patients infected by *B. afzelii*. Fever has seldom been reported in individuals with lesions caused by *B. afzelii*, whereas in individuals with lesions caused by *B. burgdorferi* s.s. or *B. garinii* fever and other systemic symptoms are much more common [7, 8]. Our results also indicate a faster increase in size of *B. garinii* lesions and that individuals infected by *B. garinii* more often had raised levels of CRP. After six weeks, significantly more individuals infected by *B. garinii* were seropositive. Overall, patients infected by *B. garinii* were more likely to have fever, faster-developing and faster-spreading EM lesions and raised levels of CRP, and were more likely to be seropositive, all indicating that infections caused by *B. garinii* are more virulent than those caused by *B. afzelii*. Our findings are supported by those of others [7–9].

In this study individuals infected by *B. garinii* were significantly older than individuals infected by *B. afzelii*. This interesting finding has also been reported in a Slovenian study [8]. We do not have an explanation for this observation. Nevertheless, there is a tendency for *B. garinii* infections to occur later in the season than *B. afzelii* infections (Fig. 3), and in an epidemiological study conducted in the county of Blekinge, older persons were infected later in the season than younger ones [28]. Perhaps spring-fed ticks mainly harbor *B. afzelii* and autumn-fed ticks *B. garinii*, and the main reservoir hosts in the spring are rodents infected with *B. afzelii* and in the autumn birds mainly infected with *B. garinii* [21, 22]?

Over a quarter of the EMs in this study were caused by *B. garinii*. In a previous study in southern Sweden in 1994–1997, only 6.1% of EMs were caused by *B. garinii* [20]. The increased frequency of EM caused by *B. garinii* in the present study might be explained by increasing knowledge and recognition of the varying appearances of EM by physicians in the area, but might also indicate an increasing importance of birds as a tick reservoir in the area. Birds are the main reservoir for *B. garinii*-infested ticks [21, 22] and in 1999 31% of spirochete-infected host-seeking ticks in the area contained *B. garinii* spirochetes [23].

As reported by others, only a minority of the patients with EM had detectable antibodies against *B. burgdorferi* s.l. at the initial visit, showing that the sensitivity of serology testing is too low to use as a diagnostic tool in clinical practice [25, 26].

In our study, almost every patient had noticed a tick bite, in comparison with other studies in which about every second patient noted the vector [13, 24, 27–29]. This is possibly due to increased awareness of Lyme borreliosis in our study population as the result of extensive research on Lyme borreliosis conducted in the area since 1990, research often followed and reported by the media.

Most of the individuals in our study sustained their tick bites in June, corresponding well with the vacation months and with earlier European data [24, 30]. Distribution of the tick bites predominantly on the lower limbs agrees with earlier studies in Sweden [2, 13].

Ticks prefer deciduous woodlands [31] harboring hosts such as deer and rodents. However, most tick bites occurred in gardens or meadows, areas more visited by humans than the forest [32], which indicates that human exposure is of greater importance than tick abundance when estimating the risk of a tick bite.

In conclusion, in this prospective study of a large set of EMs where borrelial origin was confirmed by PCR, an interaction between the sex of the patient and the infecting genospecies was found, where the clinical appearance of the EM caused by *B. afzelii* infection was sex-dependent. Further, patients infected by *B. garinii* had more extensive symptoms, indicating a more intense local and systemic inflammatory reaction than in patients infected by *B. afzelii*.

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