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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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ABSTRACT

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: This 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 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, elevated levels of C-reactive protein and seroreactivity in the convalescence sera.

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

INTRODUCTION

Lyme borreliosis is caused by tick-borne spirochetes belonging to the genospecies *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 *Borrelia 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 *Borrelia 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-centre “EM biopsy study” conducted in southern Sweden from May 2001 to December 2003. The study population included patients seeking care 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 EM was verified through a positive PCR analysis.

The patients gave their written informed consent to participate in this study. During their visit to the doctor, each participant answered a questionnaire asking 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 anaesthetics, and the biopsies were analysed with PCR targeting the *ospA* gene.

All patients were treated with V penicillin (pcV) 1 gram, 3 times daily for 10 days or doxycycline 200 mg daily for 10 days, according to the recommendations by 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 “bulls 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.I]
- *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. II].
- *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 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 μ M of each deoxynucleoside triphosphate and 1.25U *Taq* DNA polymerase. A volume of 5 μ l and 1 μ l 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 (15) and an ABI PRISM BigDye Terminator v.3.1 Ready Reaction Cycle Sequencing Kit (ABI, www.appliedbiosystems.com) 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, USA) was used for nucleotide sequence analysis.

Statistics

Student's *t*-test was used when comparing normally distributed continuous data. The Pearson chi-square test was used when comparing categorical data. The Mann-Whitney U-test or the Kruskal-Wallis test, was used when comparing non-parametric continuous 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 Ethical 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; $p= 0.020$).

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 localisations 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 tick bitten in June (63.5 %; 61/96) [Fig. III]

Clinical appearances of EM

In 72.0% (85/118), photographs were taken and the lesions categorized. The median size for the involved lesions was 80.0 cm². 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, while 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 EM on the abdominal region and back were not bigger than the EM on the legs, arms or neck ($p=0.607$) and they did not appear faster than the EM 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 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 EMs. 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 genospecies was 12.46 (95% CI: 0.98 - 158.80) [Table 2].

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

Characteristics of EM 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 days (d) (3.0–97.0 d). 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].

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 d (1.0–21.0 d) compared with median 11.0 d (2.0–35.0 d) ($p = 0.008$).

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 were treated with penicillin V (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, however the influence of a patients' sex and the particular genospecies was not considered in that study. In the present study, we found no correlation between 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 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 old. After menopause the levels of oestrogen in women 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 LB has indicated that this "type-1" immune response is less intense in postmenopausal women than 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 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 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. III] 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 harbour *B. afzelii*, and autumn fed ticks *B. garinii*, and the main reservoir hosts in the spring are rodents infected by *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 1994–1997, only 6.1% of EM 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 among 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 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 the population as a 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) harbouring 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 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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Table 1a. Characteristics of patients with erythema migrans infected with *B. afzelii* and *B. garinii* respectively.

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

¹ Mann-Whitney test, ² Two-sided Pearson chi square test, ³ Kruskall-Wallis test.

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

Clinical symptoms	Patients with <i>B.afzelii</i> (<i>N</i> = 87)	Patients with <i>B. garinii</i> (<i>N</i> = 31)	<i>p</i> Value ¹
Headache, <i>N</i> (%)	24 (27.5)	8 (25.8)	NS
Muscle/Jointpain, <i>N</i> (%)	12 (13.8)	5 (16.2)	NS
Chills, <i>N</i> (%)	8 (9.2)	4 (12.9)	NS
Airway symptoms, <i>N</i> (%)	6 (6.9)	4 (12.9)	NS
Neurological symptoms, <i>N</i> (%)	8 (9.2)	3 (9.7)	NS
Fever, <i>N</i> (%)	0 (0.0)	4 (12.9)	0.003
Neckstifness, <i>N</i> (%)	3 (3.4)	1 (3.2)	NS
Photosensitivity, <i>N</i> (%)	2 (2.3)	0 (0.0)	NS

¹Two-sided Pearson chi square test

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

Laboratory test	Patients with <i>B.afzelii</i> (<i>N</i> = 87)	Patients with <i>B. garinii</i> (<i>N</i> = 31)	<i>p</i> Value ¹
<hr/>			
Positive serological results			
at presentation, <i>N</i> (%)	24 (35.8)	12 (54.5)	n.s.
after 6 weeks, <i>N</i> (%)	27 (45.0)	16 (72.7)	0.017
CRP >8 mg/L, <i>N</i> (%)	13 (14.9)	12 (38.7)	0.006
WBC < 3.5x10 ⁹ /L, <i>N</i> (%)	2 (2.3)	0 (0.0)	NS
WBC > 8.8x10 ⁹ /L, <i>N</i> (%)	5 (5.7)	3 (9.7)	NS
Platelets > 350x10 ⁹ /L, <i>N</i> (%)	6 (6.9)	1 (3.2)	NS
Platelets < 150x10 ⁹ /L, <i>N</i> (%)	3 (3.4)	0 (0.0)	NS
ALT > 0.75 µkat/L (w), <i>N</i> (%)			
> 1.10 µkat/L (m), <i>N</i> (%)	9 (10.3)	3 (9.7)	NS
AST > 0.60 µkat/L (w), <i>N</i> (%)			
> 0.75 µkat/L (m), <i>N</i> (%)	5 (5.7)	3 (9.7)	NS
LD > 7.0 µkat/L, <i>N</i> (%)	30 (34.5)	16 (51.6)	NS
<hr/>			

¹Two-sided Pearson chi square test

CRP= c-reactive protein

WBC= white blood count

ALT= alanine aminotransferase

AST= aspartate aminotransferase

LD= lactate dehydrogenase

w = women

m = 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			
<i>B. afzelii</i>	1.00 (68 %)	0.09 (0.03–0.33) (16 %)	
<i>B. garinii</i>	1.73 (0.29–10.34) (78 %)	1.98 (0.34–11.56) (80 %)	

CI= Confidence interval

Figure I. An example of an annular erythema migrans.

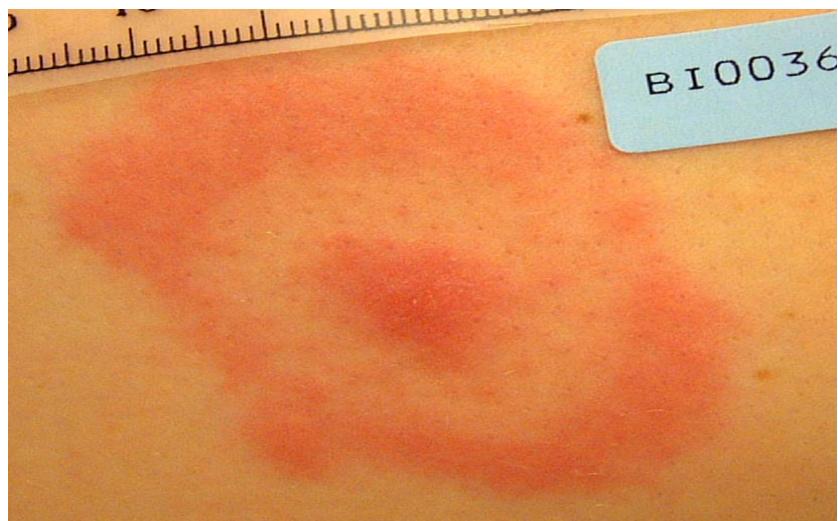


Figure II. An example of a non-annular erythema migrans.



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

