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Article

Incidence of Lyme Borreliosis in the Würzburg Region of Germany

H.I. Huppertz, M. Böhme, S.M. Standaert, H. Karch, S.A. Plotkin

Abstract To assess the incidence of Lyme borreliosis in Central Europe, a 12-month, prospective, population-based surveillance study of Lyme borreliosis was conducted in the Würzburg region of central Germany, following an aggressive awareness campaign. The diagnosis of Lyme borreliosis required the presence of (i) erythema migrans (diameter ≥ 5 cm); (ii) lymphocytoma; or (iii) another specific manifestation including Lyme arthritis, neuroborreliosis, carditis or acrodermatitis chronica atrophicans in conjunction with serological confirmation. A total of 313 cases of Lyme borreliosis was diagnosed, giving an incidence of 111 cases/100 000 inhabitants, the highest rates occurring in children and elderly adults living in wooded as opposed to agricultural areas. The incidence in city dwellers and inhabitants of rural areas was not significantly different. Erythema migrans was the only manifestation in 279 (89%) patients. Of the 34 patients with manifestations other than erythema migrans alone, 15 had arthritis, nine neuroborreliosis, six lymphocytoma, four acrodermatitis chronica atrophicans and one carditis. Children were more likely than adults to have manifestations other than erythema migrans alone. Lyme borreliosis was very common in central Germany, and one of the most frequent bacterial infections. The observation of more cases of arthritis than neuroborreliosis was similar to that in the USA. These results may be representative for many parts of central Europe and suggest the need for development of a vaccine against borreliosis caused by European strains of *Borrelia* species.

Introduction

Lyme borreliosis is a bacterial zoonosis caused by infection with the spirochete *Borrelia burgdorferi*, which is transmitted by infected *Ixodes* ticks. It has been described as the most frequent vector-borne disease in the USA and several other countries of the northern hemisphere [1, 2]. Due to the limitations of most surveillance systems, there is an ongoing debate about the incidence and impact of the disease. Underreporting is always a problem with voluntary reporting systems; on the other hand there is concern, particularly in the USA, that many reported cases may not represent true *Borrelia burgdorferi* infection [3].

While in North America only *Borrelia burgdorferi* sensu stricto has been found to be responsible for human disease, in Eurasia human isolates are heterogeneous and have been identified as the three distinct genospecies *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*. A vaccine developed for

H.I. Huppertz (✉), M. Böhme
Children's Hospital, University of Würzburg, Germany

S.M. Standaert
Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

S.M. Standaert
Association pour l'Aide à la Médecine Préventive, Marnes-la-Coquette, France

H. Karch
Institute of Hygiene and Microbiology of the University of Würzburg, Germany

S.A. Plotkin
Medical and Scientific Consultant, Pasteur Mérieux Connaught, Marnes-la-Coquette, France

Present address: H.I. Huppertz, Zentralkrankenhaus, Sankt-Jürgen-Strasse, Professor-Hess-Kinderklinik, D-28205 Bremen, Germany

prevention of borreliosis in North America has been licensed which is based on an outer surface protein expressed by *Borrelia burgdorferi* sensu stricto, whereas no comparable vaccine is available in Europe. It was therefore of interest to determine whether the incidence of borreliosis in Europe would justify a vaccine development programme.

Because Lyme borreliosis is not a notifiable disease in most European countries, accurate data on the incidence is limited [2–6]. In order to obtain accurate incidence data for a European population we conducted a 12-month, prospective, population-based surveillance study of Lyme borreliosis in a well-defined area in central Germany.

Patients and Methods

Study Design, Population and Area. The study was conducted in the city and rural district of Würzburg in Lower Franconia, Germany, in the period from 1 May 1996 to 30 April 1997. The rural district consists of wine-growing villages and small settlements in the Main and Tauber river valleys, large forests in the north and west, and agricultural villages with crop cultivation on the plateaus in the south. The city of Würzburg serves as the reference centre for tertiary medical services for the entire study area and beyond. The study area is 1055 km^2 in size with a population of 279000 (128000 in the city and 151000 in the rural district). The area is well demarcated, the nearest major cities, Frankfurt and Nürnberg, being more than 100 km away.

During the study period, all physicians in the study area were asked to report all cases of suspected Lyme borreliosis (see case definition below) to the study administration. Prior to and during the study period, an extensive awareness campaign was conducted in the area by local television, radio and newspapers to inform the public of the study. All physicians in the study area were notified by personal letter, telephone call and notices from their local medical organisation and medical insurance companies. In addition, two conferences on Lyme borreliosis were organised for the physicians in the study area, and we offered continuous assistance with diagnosis and treatment. All hospitals in the area agreed to participate in the study. All laboratories in the area performing serological tests for Lyme borreliosis reported any positive results to the study administration and encouraged the attending physician to report such suspected cases.

The study was approved by the research ethics committee of the faculty of medicine of the University of Würzburg, and informed consent was obtained from all patients.

When a patient with suspected Lyme borreliosis was identified, either the reporting physician or one of two study investigators completed a standardised questionnaire with the patient's details, medical history and clinical manifestations, including any history of tick bite or tick exposure. Whenever requested, one of these two investigators would examine a patient with suspected Lyme borreliosis to confirm the clinical findings. Information regarding the response to treatment and any sequelae was obtained 3 months after identification of the patient.

Case Definition. Only patients with active Lyme borreliosis that was newly diagnosed during the study period were included in the analysis. The case definition was based on that of the Centers for Disease Control and Prevention (CDC) with minor modifications to account for dermatological signs that occur more commonly in Europe [7]. The diagnosis of Lyme borreliosis required the presence of either of the pathognomonic lesions erythema migrans ($\geq$5 cm in diameter) or lymphocytoma, or a positive serological test in conjunction with the presence of another specific manifestation of Lyme borreliosis. Other manifestations included neuroborreliosis, Lyme arthritis, carditis and acrodermatitis chronica atrophicans (ACA) (Table 1).

Table 1 Case definitions for Lyme borreliosis. Criteria included any of the following clinical manifestations observed by the physicians. Serological confirmation was required for manifestations other than erythema migrans or lymphocytoma

Clinical manifestations:
Erythema migrans ($\geq$5 cm in diameter)
Lymphocytoma
Neuroborreliosis: presence of lymphocytic meningitis, cranial neuritis, or radiculoneuritis
Carditis: presence of an acquired conduction abnormality confirmed by a cardiologist
Acrodermatitis chronica atrophicans (ACA): presence of the skin lesion on an extremity with subsequent atrophy, to be confirmed by a dermatologist
Lyme arthritis: objective joint swelling
Serological confirmation:
Either EIA or haemagglutination assay with confirmation of all positive results on immunoblot demonstrating at least 5 (2) specific bands for IgG (IgM) antibodies against *Borrelia burgdorferi*.
A positive serological result was defined as: presence of IgM or IgG antibodies at the time of presentation; fourfold or greater rise in IgG titre at 3 to 4 weeks for early disseminated cases; presence of IgG antibodies for late cases

Climate Analysis. The daily climatic conditions of the study area, including soil temperature, precipitation and relative humidity, as recorded by the local government weather station since 1977, were obtained.

Statistical Methods. Testing for significance of results between groups was performed using Fisher's exact test. All *P* values were two-tailed and a value below 0.05 was considered significant. The 95 percent confidence interval (95% CI) for incidence rates was calculated using the Poisson distribution.

Results

During the 12-month study period, a total of 313 cases of Lyme borreliosis, for which adequate data was available for analysis, were identified within the study area. Of these cases, 176 (56%) were female and 137 (44%) male. The age of the subjects ranged from 1.3 years to 85 years (median 41 years). The overall incidence was 111 cases/100000 (95% CI: 100 to 125/100000). The rate of infection varied with age, the highest rates occurring in children and elderly adults (peak of 265/100000 among adults 60 to 65 years old) (Figure 1). The overall rate of infection was higher among females than males (120 vs 101) and higher in people living in the rural district than those living in the city (123 vs 96), although these differences were not statistically significant. This general pattern was found in nearly all age groups, with the notable exception of children below 10 years of age. Among these children, the rates were highest for males and city residents.

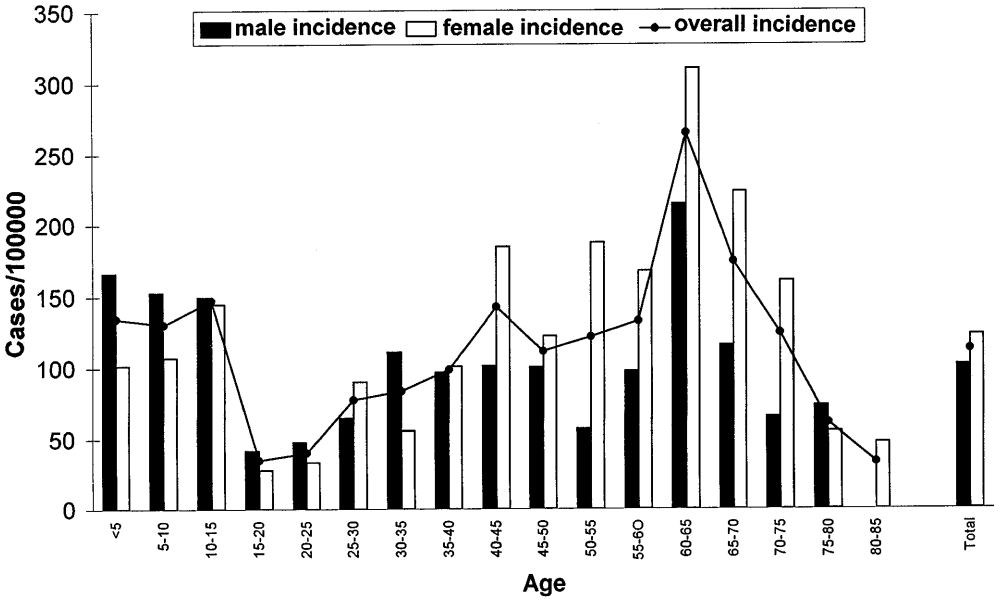

Figure 1 Incidence of Lyme borreliosis by age and sex in the Würzburg area, Germany, diagnosed in the period from 1 May 1996 to 30 April 1997

The majority of cases were found in the Main river valley, including the city of Würzburg, where the population density is highest. In addition, there was a striking difference in the distribution of cases within the rural district, many cases being reported in the north-western wooded area but extremely few cases in the southern agricultural plateau (Figure 2).

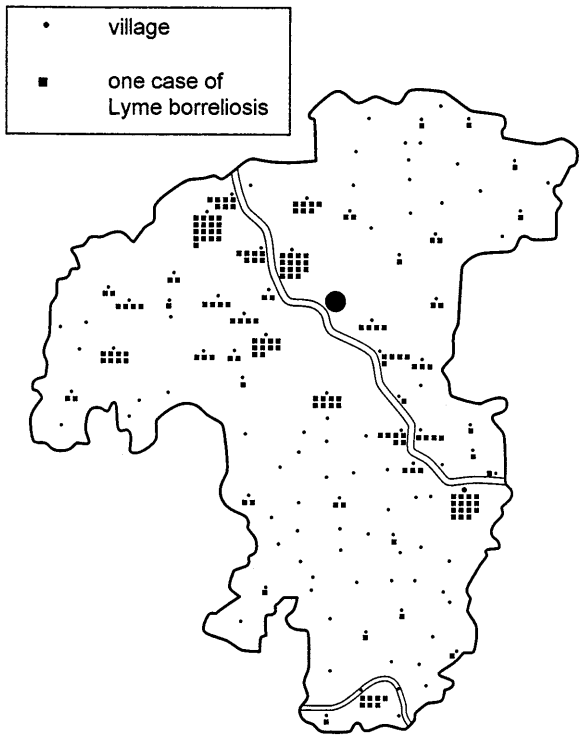

Figure 2 Site of residence for 179 patients with Lyme borreliosis in the rural area of Würzburg, Germany. The river Main is shown running south-east to north-west, passing through the city of Würzburg (large dot). In the south, the contributary river Tauber is shown. Cases found in the city of Würzburg are not shown

The most common specific clinical manifestation of Lyme borreliosis was erythema migrans, occurring in 288 (92%) patients (Table 2). Erythema migrans was the only manifestation observed in 279 (89%) patients and 26 patients had multiple erythema migrans lesions (9% of all patients with erythema migrans). Other specific manifestations of Lyme borreliosis with or without erythema migrans occurred in 34 (11%) patients. Fever was noted in only 26 (8%) patients.

A total of 15 (5%) patients were hospitalised, 14 of whom had clinical manifestations in addition to erythema migrans (7 with neuroborreliosis, 3 with arthritis, 2 with ACA, 1 with carditis, and 1 with arthritis and ACA). The age of the hospitalised patients ranged from 6 to 76 years with no significant difference in hospitalisation rates between children and adults.

Overall, children (age <18 years) were more likely than adults to have manifestations other than erythema migrans alone; conversely, adults were more likely to have erythema migrans as their only manifestation (Table 3). Of 62 children, 14 (22%) had manifestations other than erythema migrans alone, compared with 20 (8%) of 251 adults ($P<0.001$). Lyme arthritis, neuroborreliosis and lymphocytoma occurred more frequently in children than adults, although the between-group difference only achieved statistical significance for arthritis. There were no significant differences in the frequencies of clinical manifestations between males and females.

In the 15 patients with Lyme arthritis, the large joints were most frequently involved, most commonly the knee (11/15, 73%). Most patients (10/15, 67%) had symptoms in more than one joint, usually the shoulders, elbows or contralateral knee.

Table 2 Distribution of clinical manifestations in patients with Lyme borreliosis identified in the city and rural district of Würzburg, Germany in the period from 1 May 1996 to 30 April 1997

Manifestation	No. of patients		Total no. (%) of patients
	With erythema migrans	Without erythema migrans	
Erythema migrans alone	279	0	279 (89)
Arthritis	4	11	15 (5)
Neuroborreliosis	3	6	9 (3)
Lymphocytoma	1	5	6 (2)
ACA	1	3	4 (1)
Carditis	1	0	1 (<1)
Total no. (%) of patients	288[a] (92)	25 (8)	313[a] (100)

[a] One patient had two other manifestations (arthritis and ACA) in addition to erythema migrans

Table 3 Frequency of clinical manifestations of Lyme borreliosis among children and adults as identified in the city and rural districts of Würzburg, Germany, in the period from 1 May 1996 to 30 April 1997

Manifestation	No. (%) of children ($n=62$)	No. (%) of adults ($n=251$)	P value
Erythema migrans (EM) alone	48 (77)	231 (92)	<0.001
Other than EM alone	14 (23)	20[a](8)	<0.001
Arthritis	7 (11)	8[a](3)	0.015
Neuroborreliosis	4 (7)	5 (2)	0.08
Lymphocytoma	3 (5)	3 (1)	0.09
ACA	0 (0)	4[a](2)	n.d.
Carditis	0 (0)	1 (<1)	n.d.

[a] One patient had two manifestations (arthritis and ACA other than EM alone)
n.d., not done

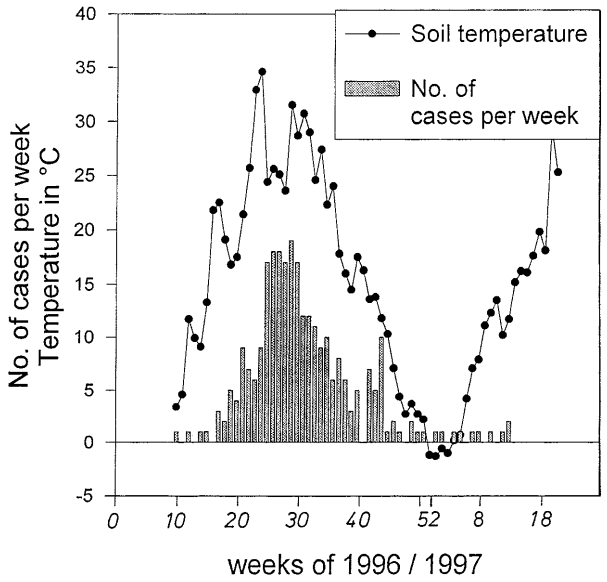

Figure 3 Daily soil temperature and distribution of cases of Lyme borreliosis per week (disease onset) in the city and rural districts of Würzburg, Germany, 1996–1997. Data for 276 patients was available; disease onset was not known or prior to January 1996 in 37 cases

Of the nine patients with neuroborreliosis, five had symptoms of meningitis; one of these patients also had a facial nerve palsy. One patient had uveitis and oedema of the optic disk, and the remaining three patients had symptoms of radiculoneuritis, such as paresthesia or palsy of the lower extremities. Lumbar puncture was performed in six patients, three of whom had symptoms of meningitis and three radiculoneuritis. In all cerebrospinal fluid samples, either elevated protein or pleocytosis was found alone or in combination, and in four samples, intrathecal antibody was detected (in 2 by immunoblot, in 1 by enzyme immunoassay and in 1 by haemagglutination assay).

Of the 34 patients with manifestations other than erythema migrans alone, all patients experienced improvement or resolution of their symptoms after antibiotic therapy, except for one patient with ACA who refused therapy.

A total of 185 (60%) patients reported a recent tick bite. The anatomical distribution of tick bites varied significantly between children and adults. Children were more frequently bitten in the head and neck region compared with adults (23% of all bites vs 1%, $P=0.000006$). The most frequent location of tick bites in adults was the legs (60% of all bites), whereas children reported only 20% of bites on the legs ($P=0.00001$). The most frequent location of tick bites in children was the trunk, but the frequency of bites at this location did not differ significantly from that in adults (34% vs 20%, $P=0.07$). Although information on the circumstances of the tick bite was not available from all patients, several patients reported that they had acquired tick bites in public parks within city limits or in their own gardens.

Cases were reported throughout the year; however, the peak incidence occurred during the months of June to August, being closely related to a rise in the soil temperature (Figure 3). Less than 15% of the total number of cases occurred in the period from November to April.

Serological testing was performed in 295 (94%) patients. Of 235 patients with erythema migrans alone, 72 (31%) were positive for either IgM or IgG in an enzyme immunoassay (EIA), 16 of whom were also positive for IgG in an immunoblot assay. Of the patients with manifestations of infection other than erythema migrans alone, all of those with arthritis, ACA or carditis were positive for IgG in both EIA and immunoblot assays, except for one patient who was negative for IgG in an EIA but positive in an haemagglutination assay. Four of the nine patients with neuroborreliosis were negative for IgG in immunoblot assays, but three of these also had erythema migrans and the other had evidence of intrathecal antibody production. Four of the five patients with lymphocytoma were negative in an immunoblot assay, but all showed improvement on appropriate antibiotic treatment.

The average monthly temperature, precipitation and relative humidity during the study period were within the range of values reported during the preceding 20 years.

Discussion

We found an overall annual incidence of Lyme borreliosis of 111 cases per 100000 inhabitants in the Würzburg area in central Germany. This is the highest incidence reported to date for an area and a population of this size. As in previous studies, we found a bimodal age distribution, most cases occurring in children up to 15 years of age and older adults. The peak incidence in our population occurred in older adults (age 60–65 years) and is virtually identical to that found in a similar study in Sweden [2]. Despite the higher overall rate of infection in adults, the more serious manifestations of infection occurred predominantly in children.

In the USA the reported national annual incidence in 1996 was 6.2 cases per 100000, with most cases being concentrated in eight states. Of all of the states Connecticut had the highest incidence (95 cases per 100000), but in several counties in other northeastern states the rates reported were over 100 cases per 100000 [1]. In the USA, in contrast to European countries, Lyme borreliosis has been a nationally notifiable disease with a uniform case definition since 1991. Although Lyme borreliosis has been reported in every country in Europe, comparable incidence data for the general population is limited. The only other study using surveillance methods similar to ours documented an overall yearly incidence of 69 cases per 100000 in 12 counties in southern Sweden, with considerable geographic variation (range 26–160 cases per 100000) [2]. Although methodological differences make direct comparisons of the data difficult, several countries in Europe should be considered endemic for Lyme borreliosis, particularly in light of the very high incidence rates being reported from central European countries [8].

The incidence of Lyme borreliosis has been steadily increasing in the USA over the last decade. Undoubtedly, this apparent increase represents in part enhanced awareness and reporting, but we believe it also reflects a true increase in disease frequency due to increased tick density and subsequent exposure of susceptible humans. Whether the incidence is increasing in Europe and other parts of the world is not known and can only be determined by improving current surveillance techniques. As part of a concerted action within the European Union, standardised surveillance methods are currently being developed for investigating the problem [9, 10].

Although the incidence in the study area was high, we suspect that it nevertheless represents an underestimate of the actual rate of infection. Although the majority of physicians in the area agreed to participate, a few physicians refused due to lack of time or interest. Moreover, there were several cases reported which would have been accepted as Lyme borreliosis in a clinical investigation, but which did not meet the strict case definition used in our study, such as those with erythema migrans of less than 5 cm in diameter. Although Gerber et al. [11] included such cases in their analysis of Lyme borreliosis in children, we excluded them in order to be able to compare results with those of other studies using the CDC criteria [2]. Patients presenting with only a pain amplification syndrome, despite having a history and serological results compatible with Lyme borreliosis, were likewise excluded since they did not have objective findings. We do not have information on patients residing in the study area who consulted physicians practising outside the study area. However, since the medical institutions in Würzburg are the reference centres for a large region extending beyond the study area, this is unlikely to have been a substantial limitation of the study.

The frequencies of various clinical manifestations differed somewhat in our study population from those noted in the USA. As was the case in Sweden [2], the proportion of patients whose primary complaint was erythema migrans was higher in our study area than in the USA. This probably reflects a high degree of awareness of Lyme borreliosis among both the public and physicians, particularly after the extensive media campaign conducted before and during our study. Although erythema migrans was the most common

manifestation of infection, later complications contributed overwhelmingly to morbidity, and children suffered disproportionately from these complications.

The incidence in the Würzburg area was remarkably similar to that in southern Sweden [2]. Although the population density is much higher in the Würzburg area (265 inhabitants per square meter versus 43 in Sweden) and the study area is nearly 900 km further south, the age distribution of cases, with peaks in the subgroups children and elderly people, was nearly identical. The somewhat lower overall incidence in southern Sweden (69 versus 111 cases per 100000 inhabitants in Würzburg) and the lower percentage of cases with erythema migrans in southern Sweden (77% versus 92% in Würzburg) might be due to differences in study design. We suspect that apparently mild cases of erythema migrans are more readily reported in a setting with high public and professional awareness of the study, as in our study, as opposed to a setting in which Lyme borreliosis is made a notifiable disease for the study period. This comparison suggests that the data that we and the group from southern Sweden found might be representative of many parts of Europe.

There appears to be considerable geographic variation in the clinical presentation of Lyme borreliosis. Lymphocytoma and ACA are rarely reported in North America, whereas they have been reported in Europe for decades. Some evidence suggests that the wider spectrum of signs and symptoms in Europe may be due to differences in strains of *Borrelia burgdorferi*. There are at least three different genospecies of *Borrelia burgdorferi* sensu lato in Europe, *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*, compared with one genospecies, *Borrelia burgdorferi* sensu stricto, in the USA. While all three genospecies may cause erythema migrans, *Borrelia afzelii* has been predominantly associated with ACA, *Borrelia garinii* with neurological disease and *Borrelia burgdorferi* sensu stricto with arthritis. It is possible that these strain differences contributed to the variation in clinical presentation seen in our population. We have isolated *Borrelia garinii* from cerebrospinal fluid of children from the Würzburg area and obtained serological evidence of infection with *Borrelia garinii* [12, 13]. Although there is no direct evidence of other strains being involved, the frequent occurrence of ACA and arthritis in the study area suggests that both *Borrelia afzelii* and *Borrelia burgdorferi* sensu stricto are present in the environment.

Tick populations are known to increase during warm humid weather conditions. The weather conditions during the study period were similar to those of the previous 20 years; therefore, our results were not likely to be the consequence of climatic changes causing an unusually large tick population. In fact, during February and March 1996, just prior to the onset of surveillance, there was a 4-week period of low temperatures below freezing-point, which could have reduced the number of ticks during the study period.

The incidence figures for Lyme borreliosis in this area of Germany are comparable with the highest figures for the infection in other parts of the world. Reports of tick infestation and of Lyme borreliosis from all parts of Germany, including urban centres such as Berlin and Munich, indicate that the disease probably occurs with a similar frequency throughout Germany [5, 14, 15].

The recent development of vaccines capable of preventing infection with *Borrelia burgdorferi* sensu stricto [16, 17] has rejuvenated interest in obtaining accurate epidemiological data on Lyme borreliosis to help evaluate the efficacy and cost-effectiveness of immunisation strategies. Several questions remain open with respect to the newly available vaccines, including their effectiveness in children and in persons over the age of 60 years, their ability to prevent manifestations other than erythema migrans alone, and the degree of cross-protection they provide against European strains. Even after these questions are answered, determining who should receive the vaccine will be problematic. Targeting only persons with occupational risk, such as foresters, may be insufficient in highly endemic areas, similar to our study area, where people may be at risk in their own backyards. Studies to address these questions as well as continued accurate surveillance will be necessary to judge the full impact of Lyme borreliosis in Europe and the efficacy of prevention efforts with vaccines.

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