

## Transmission risk of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks to humans in southwest Germany

M. MAIWALD<sup>1</sup>\*, R. OEHME<sup>2</sup>, O. MARCH<sup>1</sup>, T. N. PETNEY<sup>3</sup>, P. KIMMIG<sup>2</sup>,  
K. NASER<sup>2</sup>, H. A. ZAPPE<sup>4</sup>, D. HASSLER<sup>5</sup>, AND M. VON KNEBEL DOEBERITZ<sup>6</sup>

<sup>1</sup>Hygiene-Institut der Universität, Abt. Hygiene und Med. Mikrobiologie, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany

<sup>2</sup>Landesgesundheitsamt Baden-Württemberg, Wiederholdstrasse 15, 70174 Stuttgart, Germany

<sup>3</sup>Hygiene-Institut der Universität, Abt. Parasitologie, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany

<sup>4</sup>Medizinische Klinik der Universität, Sektion Allgemeinmedizin, Bergheimer Strasse 147, 69115 Heidelberg, Germany

<sup>5</sup>Allgemeinarztpraxis, Untere Hofstatt 3, 76703 Kraichtal, Germany

<sup>6</sup>Chirurgische Klinik der Universität, Sektion Molekulare Diagnostik und Therapie, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany

(Accepted 8 January 1998)

### SUMMARY

The risk of *Borrelia burgdorferi* infection and the value of antibiotic prophylaxis after tick bite are controversial. In this study, performed in two areas of southwestern Germany, ticks were collected from 730 patients and examined by the polymerase chain reaction (PCR) for *B. burgdorferi*. To assess whether transmission of *B. burgdorferi* occurred, the patients were clinically and serologically examined after tick removal and during follow-up examinations. Data from all tick bites gave a total transmission rate of 2.6% (19 patients). Eighty-four ticks (11.3%) were PCR positive. Transmission occurred to 16 (26.7%) of 60 patients who were initially seronegative and could be followed up after the bite of an infected tick. These results indicate that the transmission rate from infected ticks in Europe is higher than previously assumed. Examination of ticks and antibiotic prophylaxis in the case of positivity appears to be indicated.

### INTRODUCTION

The geographical range of Lyme disease, caused by *Borrelia burgdorferi* sensu lato, extends through much of North America, Europe and Asia [1, 2]. It is transmitted by ticks of the genus *Ixodes* and is the

most common vector-borne disease in Central Europe and in the USA [1, 2]. In the USA, Lyme disease is notifiable with 11 603 cases reported in 1995 [3], however, data from Maryland indicate that the majority of cases probably go unreported [4]. Rough estimates for Germany (population approximately 80 million) indicate that 40 000–80 000 cases of Lyme disease occur per year [5]. The rate of new infections in endemic regions can reach up to 0.6% of the population per year [6]. It is, therefore, one of the most significant diseases caused by a microbial pathogen in both the USA and Germany.

\* Author for correspondence. Present affiliation: Department of Microbiology and Immunology, Stanford University, CA, USA. Mailing address: VA Palo Alto Health Care System 154T, Building 101, Room B4-185, 3801 Miranda Avenue, Palo Alto CA 94304, USA. Phone: +1 650 493 5000, ext 6-3193 or 6-3163. Fax: +1 650 852 3291.  
E-mail: un69mm@genius.embnet.dkfz-heidelberg.de

Although Lyme disease has been intensively studied, there is still little information available on the individual risk of infection after tick bite. In particular, information on the rate of transmission of the pathogen from ticks to humans, which could be used as the basis for a prophylactic strategy, is limited. This has led to a controversy over several issues, including the tick attachment time which allows transmission to occur and the value of antibiotic prophylaxis after a tick bite [2, 7–9].

From American studies [10–12] it is estimated that Lyme disease is transmitted in approximately 1–3% of all tick bites. Magid and colleagues [11] concluded that it is cost-effective to treat patients with antibiotics after each tick bite only in areas in which the risk of infection is 3·6% or greater. Shapiro and colleagues [12] examined ticks removed from patients for infection with *B. burgdorferi* using the polymerase chain reaction (PCR). They then compared the outcome of patients given antibiotics (205 subjects) with a group receiving a placebo (182 subjects). In a subgroup of 23 patients bitten by infected ticks and not receiving antibiotic treatment, only one (4%) became infected. The authors concluded that antibiotic prophylaxis after tick bite is not routinely indicated.

Several features of Lyme disease in Europe are different from those in the USA. The tick species transmitting the pathogen are different. The main European vector is *Ixodes ricinus* although the closely related *Ixodes persulcatus* becomes dominant in parts of European Russia [1]. In the USA, ticks attaching to humans and transmitting *B. burgdorferi* are predominantly nymphal *I. scapularis* [13], whereas in Europe it appears that both nymphal and adult *I. ricinus* are commonly found on humans [14]. In contrast to America, Lyme disease in Europe is caused by several different genospecies of the pathogen, namely *Borrelia burgdorferi sensu stricto*, *Borrelia garinii*, and *Borrelia afzelii* [15, 16]. In addition, the spectrum of clinical manifestations in Europe is different from that in the USA [17].

Previous German studies [18, 19] found a risk of 4·0–5·6% for seroconversion and 0·3–1·4% of acquiring manifest disease after a tick bite. On the basis of such studies it is assumed that the individual risk of acquiring Lyme disease after a tick bite in Germany is sufficiently low not to require prophylactic treatment [20]. However, the information provided by these studies is limited in terms of the number of subjects studied, the fact that the subjects of both studies were

predominantly children and the follow-up protocol used.

In contrast to the American and German studies, a Russian study involving 1181 subjects bitten by *I. persulcatus* found a 12·3% transmission rate in a group of 97 individuals bitten by *B. burgdorferi* positive ticks who did not receive antibiotic prophylaxis [21]. Almost all of these individuals were bitten by adult *I. persulcatus*. The risk of infection in the untreated group was significantly greater than that in another group treated prophylactically with antibiotics. These authors, therefore, recommend prophylaxis after a tick bite in case the tick is infected with *B. burgdorferi*.

The differences between the European and American situations, the limited data on transmission of *B. burgdorferi* and the contrast between the Russian study and those from Germany and America all stress the need for additional data on the rate of transmission from *I. ricinus* to susceptible humans in Europe.

## MATERIALS AND METHODS

### Patients and ticks

Ticks which had bitten patients were collected by general practitioners in the Heidelberg and Stuttgart regions of Germany and submitted either to the Hygiene Institute at the University of Heidelberg or to the Landesgesundheitsamt in Stuttgart for examination. All patients from whom ticks were removed and who were willing to come to follow-up examinations were included. Serum was taken from each patient at the time of tick removal. Venipuncture and serology were optional for children younger than 10 years. The practitioners had to fill out a questionnaire for each patient at the time of tick removal and at each subsequent examination. The questions queried the site of tick attachment, where the tick was encountered and an estimated time of tick attachment. Additional questions were asked for possible symptoms of previous or current Lyme borreliosis. The procedure included an inspection of the site of the tick bite and a general physical examination in order to detect possible symptoms of Lyme disease. The practitioners from the Heidelberg area were requested to follow up the patients clinically and serologically at 2 weeks and 6 weeks after tick bite. Practitioners from the Stuttgart area were requested to follow up their patients between 8 and 13 weeks after tick removal,

provided the tick gave a positive result. Each institute worked independently.

### Determination of infection

On removal, each tick was placed in an individual vial containing 70% ethanol. No information on the clinical outcome of the tick bite was available at the time of PCR examination. The ticks were examined by PCR using primers which yield a 259 bp amplification product from the 23S rRNA gene of all genospecies of *Borrelia burgdorferi* sensu lato [22]. Sample preparation of ticks, amplification conditions and the detection of PCR products have been described previously [23, 24]. Serology was performed by testing for IgG and IgM antibodies against *B. burgdorferi*. At Heidelberg, an in-house indirect fluorescent antibody test [25] was used to detect IgM antibodies (cut-off titre 32) and a commercially available enzyme immunoassay (Progen, Heidelberg, Germany) to detect IgG antibodies (cut-off 100 units). At Stuttgart, enzyme immunoassays (Behring, Marburg, Germany) were used to detect IgM (qualitative results) as well as IgG (cut-off 4 units) antibodies. The specificity of indirect fluorescent antibody tests has been estimated to range between 84 and 93% [26], that of the Progen enzyme immunoassay is 79% according to the manufacturer, and that of the Behring IgG/IgM enzyme immunoassays is 87.5/98.8% according to the manufacturer and 93.5/89.2% according to a published series [27]. The sensitivity of serological tests is dependent on the clinical stage of Lyme disease and has been estimated to be approximately 50% for IgM indirect fluorescent antibody tests in the diagnosis of erythema migrans [28]. As an average for all stages, the sensitivity of the Progen IgG enzyme immunoassay has been calculated to be 92% (manufacturer) and that of the Behring IgG/IgM test to be 75.2/61.7% (manufacturer) and 96.4/87.9% [27], respectively.

Seroconversion was diagnosed if a fourfold or more titre rise to above the cut-off titre occurred in the immunofluorescence assay, if a conversion from negative to positive occurred in the IgM enzyme immunoassay or if the unit values of the IgG enzyme immunoassays increased two fold or more to a value above the cut-off. All positive sera received a *Treponema pallidum* haemagglutination assay (Mast, Reinfeld, Germany) to exclude for positive syphilis serology. In each case of seroconversion the tests were repeated to include both sera in the same assay. In

addition, the clinical criteria applied by the practitioners and provided on the questionnaire were used to establish the diagnosis. Erythema migrans was diagnosed by the attending physicians when an annular, spreading lesion developed around the site of the tick bite which had been recorded on the first questionnaire.

### RESULTS

A total of 355 ticks were collected from 345 patients in the Heidelberg area (Table 1). There were 170 female and 175 male patients aged 1–81 years with a mean age of 34.7 years ( $\pm 21.7$  s.d.) and a median of 35 years. Of the ticks collected 168 were not determined to species and life cycle stage, and of the other 187 ticks, 76 were adult female *I. ricinus*, 104 *I. ricinus* nymphs, 3 *I. ricinus* larvae, 2 *Ixodes hexagonus* and 2 *Dermacentor marginatus*. Follow-up after tick bite was possible for 334 patients. Of the 355 ticks, 39 (11.0%) were positive for *B. burgdorferi*. These ticks were collected from 38 patients of whom 35 could be followed up and were seronegative on their initial examination. Eight (22.9%) of these patients acquired *B. burgdorferi* infection. The combinations of symptoms and serological results of these patients are listed in Table 2. In each case of erythema migrans the rash was localized around the site of the tick bite. Two patients whose ticks gave a negative PCR result also developed erythema migrans. Thus, a total of 10 patients, 3.0% of those who were followed up, became infected with *B. burgdorferi*. One additional patient bitten by a PCR positive tick developed non-specific symptoms suggestive of early Lyme borreliosis, was immediately treated with an antibiotic and did not seroconvert. None of the patients with transmission reported an additional tick bite within the follow-up period.

In the Stuttgart region, 388 ticks were collected from 385 patients (Table 1). There were 173 female and 212 male patients aged 1–82 years with a mean age of 29.2 years ( $\pm 23.7$  s.d.) and a median of 27.5 years. Of the ticks collected, 168 were adult *I. ricinus*, 205 *I. ricinus* nymphs, 13 *I. ricinus* larvae, and 2 ticks belonged to species other than *I. ricinus*. Forty-five ticks (11.5%) were positive for *B. burgdorferi*. Follow-up examinations were carried out on 27 patients from whom an infected tick was removed. Of these 27 patients, 25 were seronegative on their initial examination. Eight (32.0%) of these patients became infected. The combinations of symptoms and sero-

Table 1. *The prevalence of Borrelia burgdorferi infection in ticks and the rate of transmission to humans in the Heidelberg and Stuttgart areas*

Area	No. of ticks collected	No. of patients examined	No. of ticks infected with <i>B. burgdorferi</i> %	No. of susceptible follow-up patients with infected ticks	No. of susceptible patients infected (%)
Heidelberg	355	345	39 (11.0)	35	8 (22.9)
Stuttgart	388	385	45 (11.5)	25	8 (32.0)
Total	743	730	84 (11.3)	60	16 (26.7)

Table 2. *Combination of symptoms and serological results of 16 patients from the Heidelberg and Stuttgart areas who acquired Borrelia burgdorferi infection after being bitten by a positive tick*

	No. of patients	
	From the Heidelberg area	From the Stuttgart area
Seroconversion	1	3
Nonspecific symptoms and seroconversion	1	2
Erythema migrans	4	1
Erythema migrans and seroconversion	0	1
Erythema migrans and seroconversion followed by lymphocytoma	1	0
Neuroborreliosis and seroconversion	1	1
Total	8	8

logical results of these patients are listed in Table 2. Again, each erythema migrans occurred at the site of the tick bite. One additional patient bitten by a PCR negative tick and coming for a follow-up examination seroconverted. Altogether, nine patients from the Stuttgart area (2.5% of those coming for the initial examination) became infected with *B. burgdorferi*. Again, none of the patients with transmission reported an additional tick bite within the follow-up period.

There were no statistical differences between the prevalence of infection in ticks or the rate of transmission from infected ticks to humans between the Heidelberg and Stuttgart samples ( $\chi^2_{(1)} = 0.24$  and 0.05 respectively).

## DISCUSSION

The question of prophylaxis after tick bite is of basic medical importance in all areas in which Lyme disease is endemic. However, the development of a well

grounded prophylactic strategy is dependent on the availability of data indicating the individual risk of infection after tick bite, taking regional differences into account.

This study, performed in Europe with *I. ricinus* as the vector of *B. burgdorferi* sensu lato, provides information on two aspects of Lyme disease epidemiology. First, the 26.7% likelihood of transmission of *B. burgdorferi* from infected *I. ricinus* to humans is substantially higher than previously assumed. Second, the infection rate, taking all ticks into consideration, is within the range of previous reports. These conclusions are strengthened by the fact that two independent institutions from separate but ecologically similar areas produced very similar results.

The assessment of whether transmission has taken place depends on the criteria used for the diagnosis of Lyme borreliosis. Of the 16 patients who acquired infection, 9 would fulfil the Centers for Disease Control and Prevention criteria [29]. These criteria were designed for epidemiological purposes and require either an erythema migrans or a late manifestation in combination with positive serology for diagnosis. The other seven patients developed seroconversion as determined by a significant antibody titre rise. However, using the follow-up protocol described, seroconversion is an unequivocal sign that transmission of *B. burgdorferi* has taken place. None of the patients with seroconversion recalled being bitten by another tick between tick removal and seroconversion.

The fact that two patients, on whom no other ticks were found, developed Lyme disease after being bitten by PCR negative ticks indicates that false negative PCRs can occur. This implies that a negative PCR does not completely rule out the possibility of contracting the disease, a fact of which the attending physician should be aware.

The relatively high rate of asymptomatic seroconversion (4 of 16 patients, 25%) is one of the

differences between this and previous American studies [10, 12]. The study of Shapiro and colleagues [12] found only two erythema migrans cases in a group of 182 patients not treated with antibiotics and seroconversion only in one of these cases. These authors assume that almost all individuals infected with *B. burgdorferi* will develop erythema migrans before progressing to later stages and that the risk of late sequelae for infected persons without erythema migrans is low.

European surveys demonstrate, however, that persons exposed to tick bites have a relatively high rate of asymptomatic seroconversions [30, 31]. There are presently too few data available to determine what proportion of these seroconverters will develop symptomatic Lyme disease in the long term although such a clinical course has been observed [32]. The view that the infection may persist in asymptomatic seropositive individuals is supported by the detection of *B. burgdorferi* DNA in the urine of such persons [33].

The fact that the overall rate of transmission is within the range of previous studies does not contradict the high transmission rate from infected ticks. Our overall rate of transmission is a little over twice that found by Shapiro and colleagues [12] but less than that found in the other German studies by Paul and colleagues [18] as well as by Heininger and colleagues [19]. The transmission rate from infected ticks is coupled to the high overall transmission rate in Germany as well as to the slightly lower prevalence of *B. burgdorferi* in *I. ricinus* as compared to *I. scapularis*. It seems therefore likely that the transmission rates from infected ticks in the other German studies would have been high if they had been calculated.

Our data confirm and extend the Russian results of Korenberg and colleagues [21] who also found a relatively high rate of transmission from positive ticks to humans. The results of the European and Russian studies [18, 19, 21], therefore, appear to differ from previous American studies [10, 12] in the transmission rate. The reasons for these differences are not known but may involve a variety of factors, such as the different genospecies of *B. burgdorferi* sensu lato occurring in Europe and the different tick vectors transmitting the pathogen.

The usefulness of antibiotic treatment in eradicating *B. burgdorferi* in the early stages of Lyme disease is well documented [34]. However, the efficacy of antibiotic prophylaxis against Lyme disease after tick bite was questioned by Nadelman and colleagues [7] because up until this time no study had yielded

sufficient data for the difference between treated and untreated groups of patients to show significance. More recently the value of prophylaxis has been demonstrated in a Russian study [21]. Nevertheless, at present, prophylaxis is not recommended after a tick bite unless the patient came from a high risk area [11]. These recommendations were made without reference to the infective status of the tick although the possibility of giving a prophylaxis to patients bitten by a tick carrying *B. burgdorferi* has been suggested [9].

Our data and those of Korenberg and colleagues [21] from Russia suggest that a reconsideration of the diagnostic and prophylactic strategies after tick bite should take place in at least some European endemic areas. The fact that a quarter of patients from the Heidelberg and Stuttgart areas who were bitten by an infected tick seroconverted or developed overt symptoms of Lyme disease supports the strategy of testing all ticks removed from patients in these areas and administering antibiotic prophylaxis when the tick has been shown to carry *B. burgdorferi*. The concern that antibiotic prophylaxis would be given to a large number of patients not likely to contract the disease [11] would not apply for patients bitten by infected ticks. The general applicability of our conclusions will depend on future work, such as studies describing the distribution and epidemiology of the different genospecies of *B. burgdorferi* sensu lato.

## ACKNOWLEDGEMENTS

This work was supported in part by grants from the Forschungskommission des Universitätsklinikums Heidelberg, the Sozialministerium Baden-Württemberg and the Grimminger-Stiftung für Zoonosenforschung Stuttgart, Germany. The authors wish to thank all practising physicians who participated in the study and submitted ticks and sera from their patients.

## REFERENCES

1. Stanek G, Satz N, Strle F, Wilske B. Epidemiology of Lyme borreliosis. In: Weber K, Burgdorfer W, eds. Aspects of Lyme borreliosis. Berlin: Springer-Verlag, 1993: 358–70.
2. Steere AC. Lyme disease: a growing threat to urban populations. Proc Natl Acad Sci USA 1994; **91**: 2378–83.
3. Centers for Disease Control. Lyme disease – United States, 1995. MMWR 1996; **45**: 481–4.

4. Coyle BS, Strickland GT, Liang YY, Peña C, McCarter R, Israel E. The public health impact of Lyme disease in Maryland. *J Infect Dis* 1996; **173**: 1260–2.
5. Horst H, Epidemiologie. In: Horst H, ed. *Einheimische Zeckenborreliose (Lyme-Krankheit) bei Mensch und Tier*, 2nd ed. Nürnberg: Perimed Fachbuchgesellschaft, 1993: 48–54.
6. Hassler D, Zöller L, Haude M, Hufnagel HD, Sonntag HG. Lyme-Borreliose in einem europäischen Endemiegebiet. Antikörperprävalenz und klinisches Spektrum. *Dtsch Med Wochenschr* 1992; **117**: 767–74.
7. Nadelman RD, Nowakowski J, Wormser GP. Can Lyme borreliosis be prevented after tick bite? *Lancet* 1993; **342**: 1052.
8. Matuschka F-R, Spielman A. Risk of infection from and treatment of tick bite. *Lancet* 1995; **342**: 529–30.
9. Godfroid E, Driesschaert P, Bollen A. Early detection of *Borrelia burgdorferi* infection: to treat or not? *Lancet* 1995; **346**: 321.
10. Costello CM, Steere AC, Pinkerton RE, Feder HM Jr. A prospective study of tick bites in an endemic area for Lyme disease. *J Infect Dis* 1989; **159**: 136–9.
11. Magid D, Schwartz B, Craft J, Schwartz JS. Prevention of Lyme disease after tick bites. A cost-effectiveness analysis. *N Engl J Med* 1992; **327**: 534–41.
12. Shapiro ED, Gerber MA, Holabird NB, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. *N Engl J Med* 1992; **327**: 1769–73.
13. Fish D. Environmental risk and prevention of Lyme disease. *Am J Med* 1995; **98** (Suppl 4A): 2S–9S.
14. Cornely M, Schultz U. Zur Zeckenfauna Ostdeutschlands. *Angew Parasitol* 1992; **33**: 173–83.
15. Baranton G, Postic D, Saint-Girons I, Boerlin P, Piffaretti JC, Assous M, Grimont PAD. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* 1992; **42**: 378–83.
16. Canica MM, Nato F, du Merle L, Mazie JC, Baranton G, Postic D. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. *Scand J Infect Dis* 1993; **25**: 441–8.
17. Weber K. Clinical differences between European and North American Lyme borreliosis – a review. *Zentralbl Bakteriol* 1989; Suppl **18**: 146–55.
18. Paul H, Gerth HJ, Ackermann, R. Infectiousness for humans of *Ixodes ricinus* containing *Borrelia burgdorferi*. *Zentralbl Bakteriol Hyg A* 1986; **263**: 473–6.
19. Heininger U, Zimmermann T, Schoerner C, Brade V, Stehr K. Zeckenstich und Lyme-Borreliose. Eine epidemiologische Untersuchung im Raum Erlangen. *Monatsschr Kinderheilkd* 1993; **141**: 874–7.
20. Bundesgesundheitsamt. Lyme-Borreliose – Erkennung und Verhütung. *Bundesgesundheitsblatt* 1991; **34**: 187–8.
21. Korenberg EI, Vovoybyeva NN, Moskvitina HG, Gorban LY. Prevention of borreliosis in persons bitten by infected ticks. *Infection* 1996; **24**: 187–9.
22. Schwartz I, Wormser GP, Schwartz JJ, et al. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans lesions. *J Clin Microbiol* 1992; **30**: 3082–8.
23. Maiwald M, Petney TN, Brückner M, et al. Untersuchungen zur natürlichen Epidemiologie der Lyme-Borreliose anlässlich des gehäufteten Auftretens von Erkrankungen in einem Vorort einer nordbadischen Gemeinde. *Gesundheitswesen* 1995; **57**: 419–25.
24. Maiwald M, Stockinger C, Hassler D, von Knebel Doeberitz M, Sonntag HG. Evaluation of the detection of *Borrelia burgdorferi* DNA in urine samples by polymerase chain reaction. *Infection* 1995; **23**: 173–9.
25. Zöller L, Haude M, Hassler D, Burkard S, Sonntag HG. Spontaneous and post-treatment antibody kinetics in late Lyme borreliosis. *Serodiag Immunother Infect Dis* 1989; **3**: 345–53.
26. Raoult D, Hechemy KE, Baranton G. Cross-reaction with *Borrelia burgdorferi* antigen of sera from patients with human immunodeficiency virus infection, syphilis, and leptospirosis. *J Clin Microbiol* 1989; **27**: 2152–5.
27. Putzker M, Zöller L. Vergleichende Bewertung kommerzieller Hämagglutinations-, Enzymimmun- und Immunblotttests in der Serodiagnostik der Lyme-Borreliose. *Clin Lab* 1995; **41**: 655–66.
28. Wilske B, Preac-Mursic V. Microbiological diagnosis of Lyme borreliosis. In: Weber K, Burgdorfer W, eds. *Aspects of Lyme borreliosis*. Berlin: Springer-Verlag, 1993: 267–99.
29. Centers for Disease Control. Case definitions for public health surveillance. *MMWR* 1990; **39** (No. RR-13): 19–21.
30. Schmutzhard E, Stanek G, Pletschette M, et al. Infections following tickbites. Tick-borne encephalitis and Lyme borreliosis – a prospective epidemiological study from Tyrol. *Infection* 1988; **16**: 269–72.
31. Fahrer H, van der Linden SM, Sauvain MJ, Gern L, Zhioua E, Aeschlimann A. The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. *J Infect Dis* 1991; **163**: 305–10.
32. Steere AC, Taylor E, Wilson ML, Levine JF, Spielman A. Longitudinal assessment of the clinical and epidemiological features of Lyme disease in a defined population. *J Infect Dis* 1986; **154**: 295–300.
33. Karch H, Huppertz HI, Böhme M, Schmidt H, Wiebecke D, Schwarzkopf A. Demonstration of *Borrelia burgdorferi* DNA in urine samples from healthy humans whose sera contain *B. burgdorferi* specific antibodies. *J Clin Microbiol* 1994; **32**: 2312–4.
34. Rahn DW, Malawista SE. Lyme disease: recommendations for diagnosis and treatment. *Ann Intern Med* 1991; **114**: 472–81.