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Asymptomatic Infection of *Borrelia burgdorferi* in Xinjiang, China

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Abstract

The natural history of asymptomatic infection of *Borrelia burgdorferi* (*B. burgdorferi*) and genotype infected population of Xinjiang has been unclear. We report here, on the basis of molecular epidemiological studies on *B. burgdorferi* in population of Tacheng, the endemic area in Xinjiang China, the outcome of asymptomatic infection of *B. burgdorferi* and PCR result to *B. burgdorferi* in participants of a follow-up visit in 2006, based on the sero-epidemiological investigation of *B. burgdorferi* infection in 2002, and detected the serum anti-*B. burgdorferi* antibody levels in participants using western blot assay. In addition, urine samples were collected from participants at the time of follow-up and tested by nested PCR for the presence of *B. burgdoferi* DNA. Eight positive products were sequenced by the Beijing Genomics Institute (BGI). We show that asymptomatic infection with *B. burgdorferi* occurred more frequently than clinical cases of Lyme disease. Sequencing analysis of the 5s-23SrRNA spacer region of eight PCR-positive samples revealed that 7 were of *B. garinii* and 1 of *B. afzelii*, these genospecies are identical with those infecting local vectors.

Keywords: Lyme disease; *Borrelia burgdorferi*; Western-blot; PCR

Introduction

Lyme Disease (LD), also known as Lyme borreliosis, is a zoonotic disease caused by infection with several different genospecies of *B. burgdorferi*. LD is transmitted by the bite of a tick that is infected with *B. burgdorferi*. At present, more than 30 countries have reported the existence of the disease. It is one of the most common zoonosis in the United States and Europe. LD was found in the local population of 29 provinces (municipalities and autonomous regions) in China, where multiple natural foci of LD were found in the 19 provinces. The Xinjiang Uyghur Autonomous Region has favorable conditions for the formation and development of natural epidemic foci[1]. It has complex geographical, topographical and climatic conditions with a variety of vegetation types and wildlife groups. Natural foci of disease are widely distributed, with more than 10 types. Since 1987, more than 30 strains of *B. burgdorferi* were detected in the TianShan, the Altai and the Ala Mountain counties. Natural infection rate of *Ixodes persulcatus*, which is the main media, is 23% to 40%. Apodemus, common voles, and gray Oakland mice are the main hosts. The sero epidemiological investigations have confirmed that cattle, horses, sheep, dogs, rats and other animals have Lyme disease infection. An overall average positive rate of 35.49% was found in the residents within the survey point, which was significantly higher than the national average infection rate of 5.06% in China. Restriction Fragment Length Polymorphism (RFLP) analysis of ticks infected with *B. burgdorferi* has indicated that ticks carried *B. garinii* and *B. afzelii* genospecies in Xinjiang. To investigate the natural history of asymptomatic infection of *B. burgdorferi* and its gene typing in the endemic areas, we selected the Tacheng area as the investigation site in Xinjiang (Figure 1).

[1]Xinjiang is located at longitude 73032 ~ 96021, and between north latitude 43032 ~ 49031. Most of Xinjiang region has been formed from the collision of the Indian plate with the Eurasian plate, forming the TianShan, Kunlun Shan, and Pamir mountain ranges. Xinjiang has within its borders the point of land remotest from the sea, the so-called Eurasian pole of inaccessibility (46°16.8′N 86°40.2′E) in the Dzoosotoyn Elisen Desert, 1,645 miles (2648 km) from the nearest coastline. It is split by the TianShan mountain which divides it into two large basins (from Wikipedia).

Materials and Methods

Research participants

In the spring of 2002, 1,406 individuals (forestry workers, farmers and herders, middle school students, elementary school students, faculty, and members of the police force) of the native population within the natural foci of LD in the Tacheng area were selected for this epidemiological investigation into the prevalence of Lyme borreliosis infection. Based on the study of 2002, a follow-up examination was performed in the late fall of 2006 on the 16 cases that were antibody positive in 2002 as well as 119 randomly selected cases that were antibody negative in 2002. The general characteristics of the research participants are displayed in Table 1.

Anti - *B. burgdorferi* antibody detection

From each individual in the study, 5 ml of venous blood was collected. A Western blot approach [1-6] was used to detect antibodies against *B. burgdorferi*. The bacteria were lysed, and proteins in the lysate were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to their relative molecular weights. The separated proteins were then transferred to nitrocellulose membranes and used as antigens that would be recognized by the antibodies. Serum samples were then added to initiate the antigen-antibody reaction. The results were determined by the color of the protein band. Acrylamide, N,N'-methylenebisacrylamide, TEMED,

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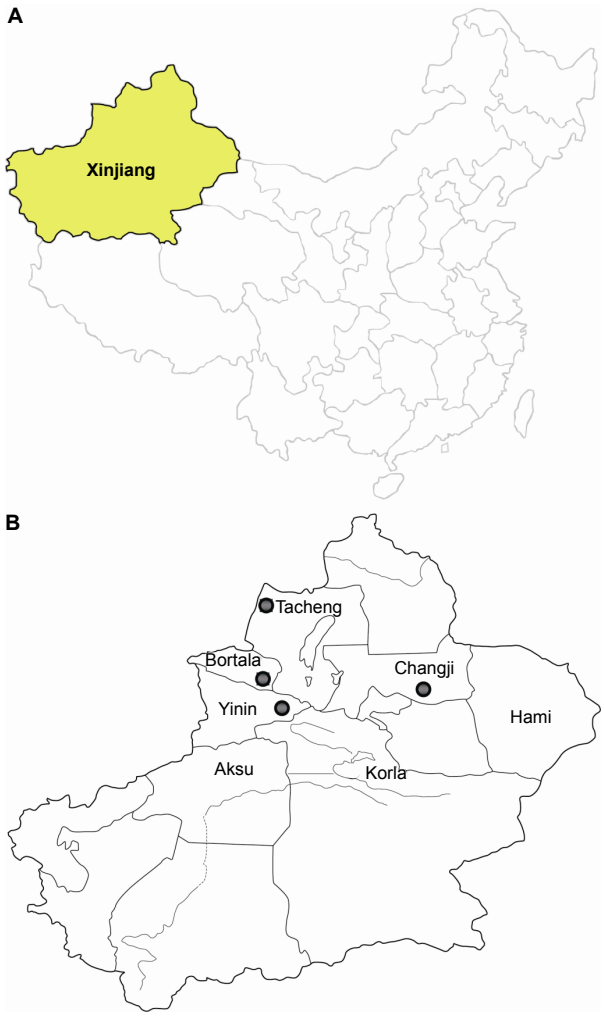

Figure 1: Distribution of *Borrelia* infection in Xinjiang, China. Xinjiang Uygur Autonomous Region is highlighted in yellow (in Panel A), and the enlarged version of the areas with ticks identified are indicated with black dots (in Panel B).

protein molecular weight standards, and 4-Cl-1 naphthol were purchased from Sino-American Biotechnology Company. SORVALL RC5C Plus Refrigerated Centrifuge was purchased from the company DuPont (U.S.A).

Bacterial protein electrophoretic separation

The various proteins of the PD_{91} bacterial strain were separated using SDS-PAGE, for which a Laemmli discontinuous electrophoresis system was used, with a 5% loading gel (4 cm in length) and a 12% separation gel (12 cm in length). During electrophoresis, voltages of 60 V and 80 V were used through the loading gel and the separation gel respectively. The representative Chinese *B. burgdoferi* strain PD_{91} *B. garinii* was provided by the National Institute of Communicable Disease Control and Prevention of the Chinese Center for Disease Control and Prevention. Constant temperature and pressure electrophoresis DF-C was purchased from Beijing Orient Instrument Factory (Beijing, China).

Preparation of the membrane

After electrophoresis, the separated protein antigens were transferred to a 0.2 μm nitrocellulose membrane using a current of 100 mA for 5 h. After using Ponceau S on the nitrocellulose membrane to check the quality of the nitrocellulose membrane and labeled marker, the membrane was blocked with 5% skim milk for 24 h. Ponceau(Tonceau S) was purchased from Shanghai Biological Engineering Company (Shanghai, China).

Antibody detection

After leaving a 1:25 dilution of serum in PBST (PBS (0.01 mol/L, pH 7.4): Tween20=2000:1) to react at 4°C for 4 h, the membrane was washed with PBST five times, for at least 10 min each time. This was followed by the addition of goat anti-human IgM-HRP and IgG secondary antibody. After allowing the SPA-HRP reaction to progress for 4 h, the membrane was washed in PBST five times, for at least 10 min each time, and developing solution was added for 15-30 min before the results were observed. Healthy New Zealand rabbits that were approximately 2.0 kg in weight were selected, and each rabbit was injected twice with *B. burgdorferi* PD_{91} in a 1 ml inguinal subcutaneous injection (containing > 10^5/ml bacteria); each injection was spaced apart by 15 days intervals. One month after the challenge, the animals showed symptoms such as erythema, swelling of the lymph nodes, and lethargy. Blood was collected from the carotid artery in a sterile environment, and the serum was isolated and stored at -20°C.

Horseradish peroxidase (HRP), *Staphylococcus aureus* protein A (SPA), and goat anti-human IgM-HRP were purchased from Sigma. The HRP-labeled anti-IgG antibody SPA was marked in our laboratory. Shaker TS2100 equipment was purchased from Beijing Science and Technology Corporation (Beijing, China). Nitrocellulose membranes were purchased from a subsidiary factory of Beijing Chemical (Beijing, China).

Analysis of the Western blotting results

The Gel-Pro Analyzer gel analysis software, provided by the Chinese Center for Disease Control and Prevention, was used to determine the colored bands. Serum from rabbits immunized against PD_{91} was used as the positive control.

Etiological determination

PCR analysis of urine specimens: Urine samples were collected from 135 individuals at the time of follow up and tested by nested polymerase chain reaction (PCR) for the presence of *B. burgdorferi* DNA. Urine specimens (5 ml) were centrifuged at 14000 rpm for 30 min, and the precipitant was dissolved in 50 μl of Phosphate Buffer Saline. Next, 50 μl of 10% chelex-100 was added. The sample was then boiled at 100°C for 5 min and immediately

Gender	Cases	Distribution of age (years)					Occupational group			
		10-19	20-29	30-39	40-49	50-60	Police Force	Forestry	Farmer or Herder	Student and Faculty
Male	69	27	12	13	9	8	13	9	16	31
Female	66	31	9	14	10	2	4	16	8	38
Total	135	58	21	27	19	10	17	25	24	69

Table 1: General information regarding the 135 research participants (2006).

chilled at 4°C. Next, the sample was centrifuged at 3000 r/min for 2 min, and the supernatant was used as the PCR template. The outer primers were 5'-CGACCTTCTTCGCCTTAAAGC-3' and 5'-TAAGCTGACTAATACTAATTACCC-3'. The inner primers were 5'-TCCTAGGCATTCACCATA-3' and 5'-GAGTTCGCGGGAGA-3'. The reaction was performed in a final volume of 50 μl, comprising 2xTaq PCR Master Mix (TIANGEN Biotech, Beijing), 50 μM of each primer of a primer pair, and 4 μl of temperate DNA. PCR reactions were performed as: 1 min at 94°C; 35 cycles (45 s at 95°C, 45s at 55°C/59°C, 45s at 75°C) and a final extension at 72°C for 5 min. Positive products were analyzed using a 5S-23S rRNA intergenic spacer and restriction fragment length polymorphism (RFLP) to determine the genospecies of infecting *B. burgdorferi*. The PCR product was digested by MseI and then loaded on a 1.6% agarose gel for electrophoresis. The positive samples were sequenced by the BGI.

Statistical analysis

We used ratios and compositions to describe gender, age distribution, occupational groups, and LD infection status. We also used ratios to calculate the negative seroconversion rate (antibody positive to antibody negative), the positive seroconversion rate (antibody negative to antibody positive), the total positive rate, and the asymptomatic IgG conversion rate (asymptomatic IgG conversion was defined when anti-*B. burgdorferi* antibody detection revealed IgG-positive cases in 2006 among individuals who did not have LD symptoms during the period between 2002 and 2006).

Results

Conversion of anti - *B. burgdorferi* antibodies in serum

In 2002, out of the 1406 research participants, there were 16 *B. burgdorferi* antibody-positive cases (12 cases positive for IgM, 2 cases positive for IgG, and 2 cases positive for IgM and IgG). The positive antibody ratio was 1.14% (16/1406 cases), and there were 1390 *B. burgdorferi* antibody-negative cases. In the 16 *B. burgdorferi* antibody-positive cases in 2002, 7 cases (3 cases positive for IgM+, 2 cases positive for IgG+, and 2 cases positive for IgM+ and IgG+) became seronegative in 2006. The negative conversion rate was 43.75% (7/16 cases). Moreover, out of the 9 IgM-positive cases in 2002, 5 cases remained IgM positive in 2006, 1 developed LD, and 4 changed from positive for IgM(+) to IgG(+) in 2006. A follow-up examination was performed on 135 people from the 2006 study, including the 16 antibody-positive cases in 2002 as well as 119 randomly selected individuals from the original group that was shown to be antibody negative in 2002. Among these 119 cases, there were 58 *B. burgdorferi* antibody-positive cases found in the follow-up examination (14 cases positive for IgM+, 25 cases positive for IgG+, and 19 cases positive for IgM+ and IgG+). The positive conversion rate was 48.73% (58/119 cases), and 2 cases were diagnosed with LD. The total anti-*B. burgdorferi* antibody-positive ratio was 49.62% (67/135 cases) in 2006. The asymptomatic serum IgG conversion rate was 34.07% (46/135 cases), including 4 cases that changed from positive for IgM+ in 2002 to positive for IgG+ in 2006, 25 cases that were positive for IgG+ in 2006, and 19 cases that were positive for IgM+ and IgG+ in 2006 (Table 2 and Table 3).

LD development

Among the 135 cases in the 2006 follow-up investigation, a total of 3 were diagnosed with LD (2.22%). The comparison of the serological and etiological examination (PCR vs urine sample analysis) is displayed in Table 4.

Etiological investigation by 5S-23S rRNA intergenic spacer RFLP analysis

PCR-positive products from 8 cases were analyzed by RFLP (by the BGI).The results indicated that 7 cases of infection were caused by *B. garinii* (the second genospecies), whereas 1 case was caused by infection with *B. afzelii* (the third genospecies). However, each of these 8 cases did not display clinical symptoms. The sequence alignment is presented in Figure 2.

Discussion

The results of the study conducted in the Tacheng area of Xinjiang demonstrate that the *B. burgdorferi* infection can be asymptomatic, and the spontaneous progression (natural history) was most often benign. These results provide the basis for the prevention and control of LD. In this study, antibody positive conversion rate was higher than other regions. It may be related to the following factors. First, winter in Xinjiang is long. Summer starts in June when the ticks are active. During the 4 year period from the spring of 2002 to the fall of 2006,

A

Spring (2002)						Fall (2006)					
No. with antibody serology						No. with antibody serology					
Positive (%)			Total (%)	Negative (%)	Total	Positive (%)			Total (%)	Negative (%)	Total
IgM	IgG	IgM/IgG				IgM	IgG	IgM/IgG			
12	2	2	16(1.14)	1390(98.8)	1406	19	29	19	67(49.6)	68(50.3)	135

B

Spring (2002)				Fall(2006)					
No. with antibody serology				No. with antibody serology					
Positive (%)			Total	Positive (%)			Total (%)	Negative (%)	Total
IgM	IgG	IgM/IgG		IgM	IgG	IgM/IgG			
12	2	2	16(1.14)	5	4	0	9(56.2)	7(43.8)	16

C

Spring (2002)					Fall (2006)					
No. with antibody serology					No. with antibody serology					
Positive (%)			Negative (%)	Total	Positive (%)			Total(%)	Negative (%)	Total
IgM	IgG	IgM/IgG			IgM	IgG	IgM/IgG			
0	0	0	119	119	14	25	19	58(48.7)	61(51.3)	119

Table 2: Western blot IgM and IgG anti-*Borrelia burgdorferi* antibodies in the fall of 2006 compared with presence of antibodies in the spring of 2002.

there were no significant natural disasters. Trees and animals were in a rich growing environment. The environmental condition was favorable for *Ixodes persulcatus* being active. The second explanation consists of subjective factor, such as misjudged color development for the protein band, which may have led to the high false-positive rate. This type of error can be prevented or reduced by the use of standardized methods. The strain PD_{91} used in the study was the representative strain attributed to predominant genospecies *B. garinii* of *B. burgdorferi sensu lato*. Strain PD_{91} has a stable passage and low variation, and its protein maps are comparatively clear and complete. It has a higher specificity with 99.4% for IgG and 93.1% for IgM, which is better than the European and the U.S. standards. However, its sensitivity lies between the reported European and U.S. standard values (IgG 73.2%, IgM 50.6%). From 2000, WB has been used in Xinjiang, and has proved suitable for serological diagnosis, which explains why ELISA was not used in the study. The asymptomatic IgG conversion rate was 34.07% (46/135), which is much higher than the prevalence rate of clinical LD (3/135, 2.22%). A potential explanation is that the symptoms reported by the research participants were inadequate, which would have caused researchers to fail to record nonspecific flu-like symptoms. It is also possible that

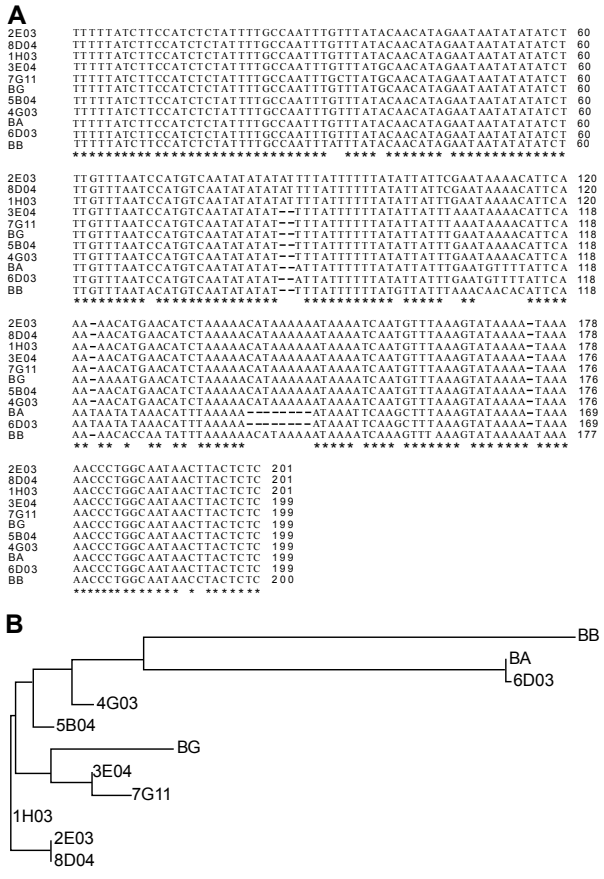

Figure 2: Sequence analysis of the 5S-23S rRNA intergenic spacer of Borrelia DNA amplified from the patient urine samples.
A. Sequence alignment of the 8 spacer sequences derived from this study (1H03, 2E03, 3E04, 4G03, 5B04, 6D03, 7G11, and 8D04) along those of *B. garinii* PBi strain (BG), *B. afzelii* PKo strain (BA), and *B. burgdorferi* B31 strain (BB) using the Clustal W2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/).
B. Phylogenetic analysis showed that 7 sequences cluster with that of *B. garinii*, 1 with *B. afzelii*, and whereas none with *B. burgdorferi*.

No. with positive serology in 2006		
IgG	IgM/IgG	Total(%)
29	19	46(34)

Table 3: Asymptomatic seroconversion prevalence of IgG anti-Borrelia antibodies (Western blot).

Urine PCR	Serum positive result		TOTAL
	+	-	
+	11	11	22
-	67	68	135

Table 4: Serological analysis and PCR detection.

patients were unable to recollect any infectious symptoms or signs that they experienced or that some of the erythema had developed in unnoticeable areas (some untreated erythema may even disappear within a few weeks). Each of these possibilities could have contributed to *B. burgdorferi* antibody-positive individuals as having been classified with asymptomatic infections. LD may be similar to other infectious diseases, such as rubella or toxoplasmosis, in that it mainly remains asymptomatic as a latent infection. This can be due to differences between hosts or geographical differences of the *B. burgdorferi* strains. However, this possibility requires further investigation. In comparison to other countries, research in the U.S. has demonstrated that the ratio of the existence of dominant infections to latent infections is 1:1, which is significantly different from our findings. However, research in Switzerland has shown that the majority of infections are latent, and this does support our results. There have been reports to suggest that the main protein of the Chinese *B. burgdorferi* strain carries high polymorphism and a unique structure, which is significantly different from that of the North American bacterial strain but similar to the European bacterial strain [7-11]. Thus, this may explain the geographic difference between the reported pathogenicities of *B. burgdorferi*. The reason for this discrepancy remains unknown.

This study, from the perspective of molecular biology confirmed the presence of *B. burgdorferi* in urine specimens of the population and obtained information about the pathogen genotypes. In table 4, we found 11 participants with asymptomatic antibody-positive and PCR-positive nature, which confirmed that their urine contained *B. burgdorferi* DNA. This indicated that *B. burgdorferi* DNA could be discharged from the body during asymptomatic infection. There were 11 PCR-positive individuals among the serum antibody-negative cases. This suggests PCR method can detect the pathogens in urine specimens during the early, middle, and late stages. When serological reaction has disappeared, PCR still remains positive. PCR can still be positive even when *B. burgdorferi* is dead. Both serological antibody measurement and PCR detection from urine specimens have deficits as well as benefits. However, simultaneous approach measurements using both approaches could increase the accuracy of diagnosis. This study discussed the 5S-23S rRNA gene spacer region RFLP analysis methods in clinical urine specimens. Further examinations should address the collection of patients' specimens, data processing, and the links between serology and PCR results.

Conclusions

Our study confirmed that for the local human population living within the natural foci of LD in the Xinjiang region, the spontaneous progression of *B. burgdorferi* infection was most often benign, and clinical LD rarely occurred, although asymptomatic infection was commonly observed. The bacterial strains causing the *B. burgdorferi*

infections consisted of two genospecies, *B. garinii* and *B. afzelii* and *B. garinii* was the predominant genospecies.

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References

1. Jiang Y, Wan K, Geng Z, Hou X (2005) Standard criteria of western blot for the diagnosis of Lyme disease caused by *Borrelia garinii* in China. Chin J Microbiol Immunol 25: 594-598.
2. Pícha D, Moravcová L, Zdárský E, Maresová V, Hulínský V (2005) PCR in lyme neuroborreliosis: a prospective study. Acta Neurol Scand 112: 287-292.
3. Shi C, Zhang Z, Wan K (2000) Analyzing clinical samples of Lyme disease with 5S-23s rRNA gene spacer RELP analysis. Chin J Zoonosis 16: 21-23.
4. Tan Y, Sun H, Liu Y, Yu L (2005) Investigation of applied diagnostic technique for Lyme disease. Chin J Clin Neurosci 13: 269-272.
5. Wilske B, Fingerle V, Schulte-Spechtel U (2007) Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49: 13-21.
6. Yu X, Ye R, Zhang Y, Liu X (1993) Geographical distribution and medical importance of *Ixode Persulcatus* Xinjiang. Endemic Disease Bulletin 4: 105-108.
7. Bao F, Liu A (2007) Current progress in research of *Borrelia burgdorferi* and Lyme disease. Trop Med 7: 1125-1127.
8. Ekerfelt C, Forsberg P, Svenvik M, Roberg M, Bergström S, et al. (1999) Asymptomatic Borrelia-seropositive individuals display the same incidence of Borrelia-specific interferon-gamma (IFN-gamma)-secreting cells in blood as patients with clinical Borrelia infection. Clin Exp Immunol 115: 498-502.
9. Fahrer H, van der Linden SM, Sauvain MJ, Gern L, Zhioua E, et al. (1991) The prevalence and incidence of clinical and asymptomatic Lyme borreliosis in a population at risk. J Infect Dis 163: 305-310.
10. Steere AC, Sikand VK, Schoen RT, Nowakowski J (2003) Asymptomatic infection with *Borrelia burgdorferi*. Clin Infect Dis 37: 528-532.
11. Wormser GP, Nadelman RB, Nowakowski J, Schwartz I (2001) Asymptomatic *Borrelia burgdorferi* infection. Med Hypotheses 57: 435-438.