Original Article
Seroprevalence of Lyme disease and associated risk factors in rural population of Beijing

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Abstract: A seroepidemiological survey of 801 local residents from 28 villages was conducted to assess the seroprevalence of Lyme disease and to identify the risk factors of becoming seropositive for Lyme disease in the northern suburb of Beijing. Forty-one serum samples were positive for IgG against B burgdorferi and the seroprevalence was 5.1% (41/801), indicating that Lyme disease is endemic in the rural population. In the multivariable analysis, sowing and harvesting in summer (OR, 2.377, 95% CI, 1.233-4.583), weed in the yard (OR, 1.914, 95% CI, 1.003-3.655) were positively associated with Lyme disease, while wearing protective clothes (OR, 0.173, 95% CI, 0.041-0.732) was negatively associated with Lyme disease. People living in the area are easily infected just near the house or in the cropland. They were barely diagnosed and cured. Without clear tick knowledge, the people are at high risk of exposure to tick bite and Lyme disease.

Keywords: Lyme disease, risk factors, immunoglobulin g, seroepidemiologic studies

Introduction

In China, The first human Lyme disease was identified in the forest regions of Heilongjiang province in 1986 [1]. In 1990s, seroepidemiological findings indicated that some residents in forest areas had a high antibody titer for the Borrelia (B.) burgdorferi sensu lato (s.l.) with positive rate 5.1% in China [2], which was revealed by Immunofluorescence assay (IFA) using homemade slides. Since then, the B. burgdorferi s.l. were isolated and genotyped from tick vectors [3-5], and rodents [4, 6]. Also, Natural foci of Lyme disease were confirmed in different places among China [3, 6-9].

In the northern suburb of Beijing, Ixodes persulcatus and Haemaphysalis longicornis were distributed, and B. afzelii and B. garinii were isolated and genotyped from ticks and host rodents [6, 10]. It is proved that Ixodes persulcatus can transmit Lyme disease [11] and play the leading role in transmission in north China [12, 13].

Insufficient attention was paid to human Lyme disease in China, as there is no obligatory notification system established in China. In recent 10 years, epidemiological data on human Lyme disease were rare, and the risk factors for human Lyme disease in the country remained unknown. Here, we reported the results of a seroepidemiological survey in Beijing to assess the seroprevalence of Lyme disease in rural population and to identify the associated risk factors for people becoming seropositive for Lyme disease.

Materials and methods

Ethics statement

The Institutional Review Board (IRB) of Beijing Center for Diseases Prevention and Control reviewed and approved all protocols to conduct this study. Written informed consent was obtained prior to the interview and venous blood samples collections.
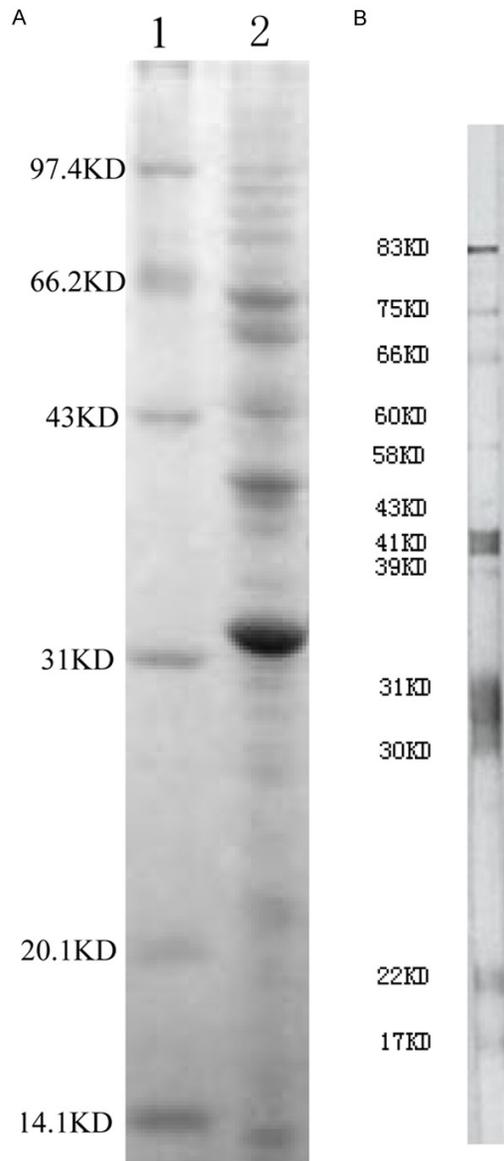
Figure 1. SDS-polyacryla mide gel and Immublots of the strain with positive rabbit serum of the strain PD91. A. Coomassie brilliant blue-stained SDS-poly-acryla mide gel of the antigen. B. Immublots of PD91 strain with positive rabbit serum.

Subjects

The study consisted of 801 local residents lived in 28 villages near to Miyun Reservoir in the northern part of Beijing, China. The villages in northern suburb of Beijing were selected by their geographic locations and the residents were selected randomly applying probability proportional to size method based on the official population census data.

Data collection

In March and April, 2013, the subjects were personally interviewed by well-trained interviewers according to a structured questionnaire specifically designed for the study. The venous blood samples were collected for each subject at the same time. Three main categories of variables were considered in the questionnaire: socioeconomic status (age, sex, education level, and residential environment), the outdoor activities, and the potential risky or protective behaviors during outdoor activities. Mountainous and plains regions were defined mainly according to the official document. Outdoor activities included picking, fishing, and exercise in the wild field.

Serological tests

The presence of IgG antibodies against specific antigens of *B. burgdorferi* s.l. was detected by combined enzyme-linked immunosorbent assay (ELISA) and western blot (WB). Serum IgG antibodies to *B. burgdorferi* s.l. were firstly determined by a qualitative ELISA test. The test was performed with commercial ELISA kits (IDEIA™ *B. burgdorferi* IgG; Oxoid, UK) according to the manufacturer's instructions. All sera with equivocal or positive results were tested for IgG by a Western blot, which is performed by previously described [9]. Samples were considered positive only if they were confirmed by WB. The positive results of Western Blot were determined according to the CDC-recommended criteria for WB in China [9] as followed: when one or more band of P83/100, P58, P39, P30, OspC, P17, P66 and OspA were shown, the sample was considered as positive for IgG antibodies to *B. burgdorferi* s.l (**Figure 1**).

Statistical analysis

Continuous variables were compared by the student's *t* test or ANOVA, when appropriate. Categorical variables were given as percentage (n/N) and compared by Pearson chi-square test. Multivariate logistic regression was employed to evaluate the association of seroprevalence with the influence factors of Lyme disease. All tests were two-sided and $P < 0.05$ was considered statistically significant. All data were analyzed with the SPSS software package (Version 13.0, SPSS Inc., Chicago, Illinois, USA).

Table 1. Demographic characteristics and stratified seroprevalence of IgG antibodies against *B. burgdorferi* detected by ELISA in rural population, 2013, Beijing, China

Characteristic	No.	Prevalence (%)	P value	Crude OR (95% CI)
Sex			0.482	
M	19	5.78		1.254 (0.667-2.355)
F	22	4.66		
Age, y			0.036	
-50	18	3.77		
51-	23	7.10		1.949 (1.034-3.672)
Occupation			0.284	
Farmer	34	5.59		1.574 (0.686-3.610)
Non-farmer	7	3.63		
Education, y			0.145	
≤ 6	16	4.24		
6-9	21	7.05		1.711 (0.876-3.339)
> 9	4	3.17		0.74 (0.243-2.255)

Results

Study group

The study consisted of 801 local residents lived in 28 villages near to Miyun Reservoir in the northern part of Beijing, China. The mean age was 43.5 years (range 6-91 years, SD, 19.1 years) and 57.2% were female. Most of the subjects were farmers (75.91%) and with lower level of education (≤ 9 years, 84.3%). None of 801 subjects were diagnosed with Lyme disease. According to the narrative of the subjects, erythema migrans were only reported in two of them, and swollen lymph nodes were reported in eleven of them, Arthralgias caused by unknown reasons were reported in 144 subjects (17.98%). In addition, antibiotic misuse was founded in 299 subjects (37.33%).

Seropositivity

Forty-one serum samples out of 801 were tested positive for IgG against *B. burgdorferi* s.l., and the seroprevalence tested was 5.1% (41/801). **Table 1** showed the seroprevalence rate stratified for sex, age group, occupation and education levels, a significant higher rate was observed in the over 50 years old group.

Influence factors

The environment and behaviors factors within the past year were analyzed and the results were showed in **Table 2**. In the univariate logistic regression analysis for seropositive cases, sowing and harvesting in summer, and living in a house where there is weeding in the yard were potential risk factors for becoming seropositive for Lyme disease. Tick bite prevention by wearing protective clothing with collars and cuffs and long pants was a potential protection factor for seropositivity. Not as expected, the seroprevalence of subjects lived in mountainous regions were significantly lower than those in plain area. No significant difference in seroprevalence was observed stratified for presence of pets (dog and cat) in a household and stratified for outdoor activities.

Results of the multivariable analysis for seropositive cases indicated that sowing and harvesting in summer (OR, 2.377, 95% CI, 1.233-4.583), living in a house where there is weeding in the yard (OR, 1.914, 95% CI, 1.003-3.655) were significantly associated with an increased chance of seropositivity and wearing protective clothing with collars and cuffs and long pants (OR, 0.173, 95% CI, 0.041-0.732) was protective against seropositivity (**Table 3**).

Discussion

This study showed that the seroprevalence of Lyme disease was 5.1% in the rural population in Beijing, One previous study revealed that the positive rate was slightly lower than 5.1% in 1990s [2]. Although results of the two studies were not comparable, they all indicate that Lyme disease is in endemic to the rural region of Beijing and highlight the need to pay more attention to surveillance and additional investigation.

Under the high seroprevalence, patients with irreversible physical damage at advanced stage of Lyme disease were only occasionally found in the last decade, less of them were clinically diagnosed. Under-diagnosis of early Lyme disease and physical damage at advanced stage are huge problem in this area.

To the best of our knowledge, there is little research that has described risk factors associated with the development of Lyme disease in

Table 2. Associations between risk factors within a year and seroprevalence of IgG antibodies against *B. burgdorferi* detected by ELISA in rural population, 2013, Beijing, China

Characteristic	No.	Prevalence (%)	P value	Crude OR (95% CI)
Residence located at			0.016	
Mountainous regions	32	4.47		
Plain area	9	10.59		2.531 (1.164-5.503)
Sowing and harvesting in summer			0.008	
No	16	3.39		
Yes	25	7.60		2.344 (1.231-4.463)
Outdoor activities			0.448	
No	21	4.61		
Yes	20	5.80		1.275 (0.680-2.391)
Pet in household			0.188	
No pet	15	4.02		
Any pet	26	6.07		1.544 (0.805-2.961)
Weeding in yard			0.029	
No	18	3.73		
Yes	23	7.21		2.003 (1.063-3.775)
Wearing protective Clothing			0.023	
No	39	5.95		
Yes	2	1.37		0.038 (0.052-0.919)

Table 3. Associations between risk factors within a year and seroprevalence of IgG antibodies against *B. burgdorferi* detected by ELISA in multivariable analysis

Characteristic	B	S.E.	Wald	df	P	OR (95% CI)
weeding in yard	0.649	0.33	3.873	1	0.049	1.914 (1.003-3.655)
Wearing protective clothing	-1.755	0.736	5.682	1	0.017	0.173 (0.041-0.732)
Sowing and harvesting in summer	0.866	0.335	6.689	1	0.010	2.377 (1.233-4.583)

China. Most information regarding risk comes from studies performed in Europe [14] or North America [15, 16] where the lifestyle and habits are completely different.

The most previous studies about ticks and *B. burgdorferi* s.l. were carried out in mountainous areas in China. It was believed that the density of ticks was higher and the pathogen was easily isolated. However, we found that living in mountainous areas and outdoor activities in these regions were not increasing the chance for people getting infected as expected. We think that the chance for people getting infected by Lyme disease is decided mostly by the probability of exposure to tick bite and the pathogen. Highest density of ticks was found in summer in northern Beijing [6]. And activities in cropland in summer was the risk factor, the residents in mountainous areas needed fewer activities in croplands in summer because of the different kind of crops.

Unlike the results in Europe [17], pets in household were not the risk factors of becoming seropositive for Lyme disease. Pets were less likely to be living in close proximity to their owners in these rural regions, as pets aren't vaccinated or de-wormed.

Wearing protective clothing with collars and cuffs and long pants, which was commonly used as a protection from mosquitoes, was protective against seropositivity. The risk of a tick bite can be reduced by preventive measures [15, 18], but in this survey, knowledge of tick bite was found to be insufficient. Wearing protective clothing is the only way used to protect the residents from the tick bites in Beijing.

In summary, Lyme disease is in endemic in the rural population of Beijing, people living in the area are readily infected, just near the house or in the cropland. Without clear tick knowledge, the people are at high risk of exposure to tick

bite and Lyme disease. Further studies are needed to fully understand the risk or protective factors for infection with LB in China. The poor knowledge about ticks cannot help to prevent tick bites and LB. Greater efforts should be made in popularizing the basic prophylactic knowledge about ticks.

Disclosure of conflict of interest

The manuscript represents an original work, and is not under consideration for publication elsewhere. All the authors meet criteria for authorship, and have read and approved the submission of manuscript. No conflict interest exists in connection with the submitted article.

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