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Original Article

Prevalence of *Borrelia burgdorferi* sensu lato in rodents from Jiangxi, southeastern China region

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Abstract: In order to investigate the prevalence of *B.burgdorferi* sensu lato in rodents from Jiangxi province of southeastern China. Isolation of *B.burgdoferi* strains and PCR-based studies were carried out in 204 mice collected from six counties of Jiangxi province in May of 2011 and 2012. The results showed the prevalence of Lyme spirochetal infection among seven species of wild and peridomestic rodents in Jiangxi. 3 strains isolated from 204 mice were all belonged to *Borrelia yangze* sp.nov. The study firstly showed the role of rodents in maintaining the pathogen of Lyme disease in the environment from Jiangxi province and there existed at least one genotype of Lyme spirochetes in Jiangxi.

Keywords: *B.burgdorferi* sensu lato, rodents, *Borrelia yangze* sp.nov, southeastern China

Introduction

Lyme disease is the most prevalent vector-borne disease in temperate regions of the northern hemisphere. Its agent, *Borrelia burgdorferi* sensu lato comprise a group of complex bacteria which are transmitted among vertebrate hosts by hard ticks. To date, 16 species has been named within the group of LB spirochetes [1-3]. Among which 5 species are associated with disease in humans: *Borrelia burgdorferi* (sensu stricto), *Borrelia garinii*, *Borrelia afzelii*, *Borrelia lusitaniae*, and *Borrelia spielmanii* [4].

In China, an epidemiological investigation of Lyme disease have been conducted since 1986, more than 20 provinces were confirmed the existence of natural foci of Lyme disease [5]. There are at least four species reported by several studies: *Borrelia burgdorferi* (sensu stricto), *Borrelia garinii*, *Borrelia afzelii*, and *Borrelia yangtze* sp.nov. [6, 7]. While most of the investigations concentrate on the northeast, northwest and southwest China. Study on Lyme disease of southeastern china was limited, especially in rodents.

Jiangxi province is located in southeast China, there are dense forests and rich vegetation in the province. The humid climate is suitable for the growth of ticks. So we think ticks and tick-borne diseases may be an important public health problem in this area. But until now, there is no report about the epidemiology and pathogen of Lyme disease in this area. In order to investigate the prevalence of *B.burgdorferi* sensu lato in rodents from Jiangxi province of southeastern China. We have an investigation in rodents in six counties of Jiangxi province.

Materials and methods

Sample collection and Borrelia isolation

A total of six survey sites were chosen in Jiangxi province (**Figure 1**), they are: Fuliang county, Longnan county, Shangrao county, Shangyou county, Shanggao county and Luoan county. In 2011 and 2012, mices were collected by trap method. Kidney and bladder of mice were inoculated into 5 ml BSKII medium (Sigma, St. Lousis, MO), incubated at 33°C, examined once a week by dark-field microscope.

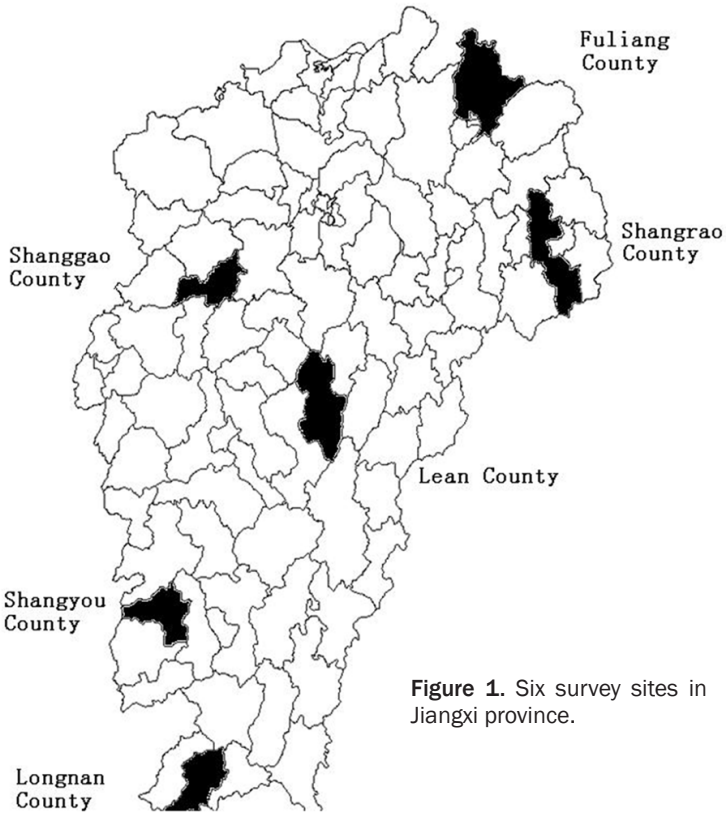

Figure 1. Six survey sites in Jiangxi province.

Table 1. Results of PCR test of mice samples in six counties of Jiangxi province

Site	Mice numbers	Positive numbers (rates)
Fuliang county	51	20 (39.22%)
Shanggao county	51	22 (43.14%)
Shangrao county	39	20 (51.28%)
Shangyou county	21	10 (47.62%)
Lean county	28	9 (32.14%)
Longnan county	14	2 (14.29%)
Total	204	83 (40.69%)

Test of mice samples

DNA extraction from samples of mice: Kidney and spleen of mice were collected for DNA extraction. Commercial kits (Qiagen, QIAamp DNA Mini Kit (250)) were used for DNA extraction from samples of mice. Protocol was provided by the kit.

Polymerase chain reaction: A total of 204 mice samples were tested by *rrf-rrl* intergenic spacer nested PCR [9]. The primers of nested PCR were as follows: of the first step, the forward primer 5'-CGACCTT-CTTCGCCTTAAAGC-3' and the reverse primer 5'-TAAGCTG-ACTAATACTAATTACCC-3'; of the second step, the forward primer 5'-TCCTAGGCATTCACCATA-3' and the reverse primer 5'-GAGTTCG-CGGGAGA-3'. The PCR was performed in 50 μl mixture containing 8 μl of sample DNA, 1 μM of each primer and 25 μl of 2× Taq buffer (CWBIO, Bejing, China). 1 μl of the first PCR products was used as template DNA for the second PCR reaction. The PCR condition of the first step was as follows: 95°C for 5 min; 35 cycles at 95°C for 45 s, 53°C for 45 s, and 72°C for 45 s; and a final extension at 72°C for 5 min. The condition of the second step was the same as the first step except the annealing temperature was 55°C. The PCR products were visualized by gel electrophoresis with 1% TBE agarose gel stained with Goldenview™ (Aidlab, Beijing, China).

Borrelia identification

DNA extraction: All isolates were cultured in BSKII medium at 33°C for 5-7 days, after which spirochetes were harvested by centrifugation at 12,000×*g* for 30 min. The pellet was washed twice in 0.01 M phosphate-buffered saline (PBS, pH 7.4) and finally resuspended in 1 ml of sterile PBS. The DNA was extracted by boiling in water at 100°C for 10 minute and stored at -20°C until use.

MLSA: Seven loci, rrs, hbb, groEL, recA, fla, ospA, and the rrf-rrl intergenic spacer, were used for MLSA and amplified under conditions described previously [6, 8]. All loci were amplified by a single PCR. The reaction was performed in a final volume of 50 μl, comprising 2× Taq PCR Master Mix (TIANGEN BIOTECH, Beijing), 50 μM of each primer of a primer pair, and 1 μl of temperate DNA. PCR was performed as follows: 1 min at 94°C; 35 cycles of 1 min at 94°C, 45 s at 52°C, and 45 s at 72°C; and a final extension step of 5 min at 72°C. The products were sequenced by the BGI Company.

Sequence analysis and nucleotide sequence accession number: The CLUSTAL_X [9] algorithm was used for sequence alignments, and

Table 2. Prevalence of DNA of *B.burgdorferi sensu lato* in different species of mice in Jiangxi province

sites	Apodemus agrarius		Rattus rattoides		Rattus flavipectus		Rattus norvegicus		Sorex aran eus Linnaeus		Niviventer confucianus		Mus musculus Linnaeus	
	N	n	N	n	N	n	N	n	N	n	N	n	N	n
Fuliang county	39	17			3	1			2	0	7	2		
Shanggao county	37	13	2	2			4	3	3	1			5	3
Shangrao county	22	10	5	3	7	4			5	3				
Shangyou county	4	3	17	7										
Lean county					4	0	17	7					7	2
Longnan county			11	1			3	1						
Total	102	43	35	13	14	5	24	11	10	4	7	2	12	5

N: Number of mice collected; n: Number of PCR positive sample.

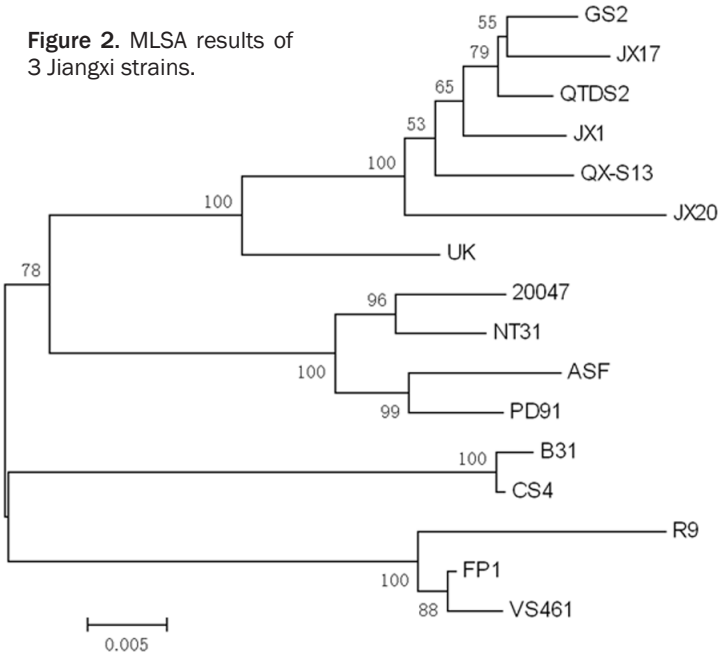

Figure 2. MLSA results of 3 Jiangxi strains.

MEGA 4 software was used for phylogenetic analyses of both individual and concatenated sequences. Distan-ces were calculated using the neighbor-joining method. All the reference sequences were acquired from the GenBank.

Results

PCR test of mice samples

Among 204 mice, 83 were tested positive for specific DNA of *B.burgdorferi sensu lato*, the average positive rate is 40.69%. The positive rates were different in six counties (**Table 1**).

Specific DNA were detected in seven species of rodent hosts (**Table 2**). The highest positive rate (45.83%, 11/24) was detected in *Rattus norvegicus*. Higher species were also detected in the species of *Apodemus agrarius* (42.16%, 43/102), *Mus musculus Linnaeus* (41.67%, 5/12), and *Sorex araneus Linnaeus* (40%, 4/10). In addition, the positive rates for *Rattus rattoides*, *Rattus flavipectus* and *Rattus niviventer* were 37.14% (13/35), 35.71% (5/14) and 28.57% (2/7), respectively.

Borrelia isolation and identification

3 strains (JX1, JX17, JX20) were isolated from 204 mice. JX1 is isolated from *Rattus rattoides* in Longnan county, JX17 and JX20 are isolated from *Apodemus agrarius* in Fuliang county. 3 isolates were identified by MLSA methods. The result shows that 3 strains from Jiangxi province were adjacent to Guizhou strains (GS2, QTDS2, QX-S13) which belong to *Borrelia yangze* sp.nov (**Figure 2**).

Discussion

In this survey, 204 mice from six county of Jiangxi province were investigated. According to the results, *Apodemus agrarius* was the dominant species in Fuliang, Shanggao and Shangrao counties, which located in the North area of Jiangxi province. However, *Rattus rattoides* was the dominant species in Shangyou and Longnan counties, which located in the south area of Jiangxi province. Our results show the prevalence of Lyme spirochetal infection among seven species of wild and peridomestic rodents in Jiangxi.

Our report describes the first isolation of Lyme spirochetes from rodents in Jiangxi province. 2 strains were isolated from *Apodemus agrarius* and 1 strain was from *Rattus rattoide*s. The MLSA result showed that 3 strains were all belonged to *Borrelia yangze* sp.nov, which was a group of *B. valaisiana*-related strains. *Borrelia yangze* sp.nov has been reported in Guizhou, Zhejiang and Sichuan provinces of southwestern China [6, 7, 10, 11]. So it is possible that *Borrelia yangze* sp. distributed widely in the south areas of China. In addition, strains of the same species have been isolated previously from *I. granulatus* ticks, *I. nipponensis* ticks and a variety of rodents in China [6, 7, 12]. These findings suggested that the existence of zoonotic transmission of *Borrelia yangze* sp. is likely. Thus, an epidemiologic survey on vectors of this area should be required.

It remains determined whether *B.valaisiana* and *B. valaisiana*-related strains can cause a disease in humans. But previous reports shows specific DNA for *B. valaisiana* has been detected by PCR from skin biopsy specimens of two erythema migrans patients and from CSF of a patient with slow progressive spastic paraparesis [13, 14]. Indirect evidence suggests that *B.valaisiana* is involved in some chronic clinical manifestations [15, 16]. A case infected with B. valaisiana-related genospecies was reported for the first time in northeast China recently [17].

Further investigation is needed about whether other genospecies of *Borrelia burgdorferi* exist in Jiangxi province and whether Lyme patients exist in Jiangxi province.

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Disclosure of conflict of interest

None.

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