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ORIGINAL ARTICLE

Serological Detection of Lyme Borreliosis Agents in Patients From Korea, 2005–2009

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Abstract
Objectives: Laboratory tests are now being used to identify seropositive cases in patients suspected of having a Lyme borreliosis (LB) infection. From 2005 to 2009, we analyzed the serological and epidemiological characteristics of 53 LB positive cases in Korea using immunoblot assay.
Methods: During the five-year study period, a total of 1897 serum samples from suspected LB cases were referred to us for further laboratory diagnosis. The bacterial strains *Borrelia afzeli* pKo, *Borrelia garinii* 935T and *Borrelia burgdorferi* B31 were used for indirect immunofluorescent antibody assay. Immunoblot assay was performed using the recomBlot Borrelia.
Results: Based on the information from the clinicians, the main symptoms of LB infection were rash and fever (66.0%), neurological symptoms (30.2%), and arthritis (5.7%). Of the 53 cases, 16 (30.2%) were infected abroad and the remaining 37 cases (69.8%) were suspected to have been infected in Korea. Immunoblot assays detected high levels of the antigens p41 (FlaB) of *B. burgdorferi* and OspC of *B. garinii* in infected samples.
Conclusions: The causative bacteria of LB were not isolated from humans yet but from vector ticks and rodents in Korea, and a few cases were reported with serological diagnosis. Our results suggest that LB is present in all areas of Korea and indicate that *B. garinii* and *B. burgdorferi* may be the predominant bacteria in patients with LB. However, further studies are needed to isolate and identify the causative bacteria for LB in patients.

1. Introduction

Lyme borreliosis (LB) is an infectious disease transmitted by *Ixodes* spp. ticks and caused by the spirochete bacteria *Borrelia burgdorferi* sensu lato. These bacteria are classified into three human pathogens, *B. burgdorferi* sensu stricto, *Borrelia afzelii* and *Borrelia garinii*. It has been reported that the

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antigenicity and pathogenicity of LB varies depending on the region [1,2].

In 1993, *B. burgdorferi* and *B. garinii* were isolated from *Ixodes persulcatus* and *Apodemus agarius* captured in the Chungcheongbuk and Gangwon provinces in Korea [3]. *B. burgdorferi* and *B. afzelii* were also isolated from *I. persulcatus, I. nipponesis, I. granulatus* and *A. agarius* captured in Gangwon and Jeollanam provinces [3]. The causative bacteria have not yet been isolated from humans in Korea; however, clinical cases for which a diagnosis was made based on serological and molecular findings have recently been reported [4].

An initial symptom of LB is erythema chronicum migrans, a rash formed at the site of the tick bite. Other symptoms, such as fever, headache, myalgia and lethargy, are persistently present for several weeks. In the absence of prompt antibiotic treatment, these symptoms can lead to chronic myalgia, arthritis and impaired visual acuity [1,2].

LB is characterized by the variability of clinical symptoms and incidences. For a confirmatory diagnosis, both clinical diagnosis and laboratory tests are required. Laboratory tests for LB include indirect immunofluorescent antibody assay (IFA) and immunoblot assay. Immunoblot assay is also used to make a confirmatory diagnosis in areas where LB is not an endemic disease or where its incidence is relatively low [1,5]. In this study, we used immunoblot assay to analyze the serological and epidemiological characteristics of 53 positive LB cases reported in Korea between 2005 and 2009.

2. Materials and Methods

2.1. Sera

During the period from 2005 to 2009, when a LB case was suspected, serum samples were referred to us for further laboratory diagnosis. Detailed information on an important clinical marker for LB and on other characteristic manifestations was obtained from the attending clinicians.

2.2. Bacterial strains and culture media

The bacterial strains *B. afzeli* pKo, *B. garinii* 935T, and *B. burgdorferi* B31 were cultured in BSK (Barbour-Stoenner-Kelly) -II medium (Sigma, USA) for 7−14 days. The bacteria were harvested by centrifugation and washed three times in PBS (pH 7.2). The bacteria were suspended at a concentration of 2×10^8 cell/mL and used to prepare slides for IFA.

2.3. Indirect immunofluorescent antibody assay

The concentration of the cultured bacterial strains was adjusted to approximately 2×10^6 cell/10 μL. These bacterial strains were evenly distributed to an 18-well spot slide glass and then dried. Following a 10-minute fixation using acetone, the slides were preserved at −70°C until required.

The test serum was diluted by serial 2-fold dilutions from a titer of 1:16 to 1:1024. 20 μL of the diluted serum was placed in each well of an 18-well spot slide glass and the slides were incubated in a humidified chamber at 37°C for 30 minutes. The wells were then washed twice with PBS (pH 7.2) and once with distilled water. FITC-conjugated anti-human immunoglobulin (MP Biomedicals, Ohio, USA) was added and the slides were incubated in a humidified chamber at 37°C for 30 minutes. Using the same methods, the slides were washed and then dried. The slides were treated with a mounting solution to prepare them for microscopy. Positive samples were defined as those in which the IgM titer was ≥1:16 or the IgG titer was ≥1:256. Samples with positive readings were subjected to immunoblot assay.

2.4. Immunoblot assay

Immunoblot assay was performed using the recomBlot Borrelia test (Mikrogen, Neuried, Germany) according to the manufacturer's instructions. The test serum was diluted using the reactant solution to a ratio of 1:200 for IgG and 1:10 for IgM. The reaction was performed at room temperature for 10−16 hours. The strips were washed four times and then treated with a peroxidase conjugate. When the color developed, the strip was examined and the results were determined. The recomBlot Borrelia immunoblot test can detect IgG and IgM antibodies directed against *B. burgdorferi* using recombinant antigens of *B. burgdorferi, B. afzelii* and *B. garinii* like p100, VlsE, p39, OspA, OspC, p41 and

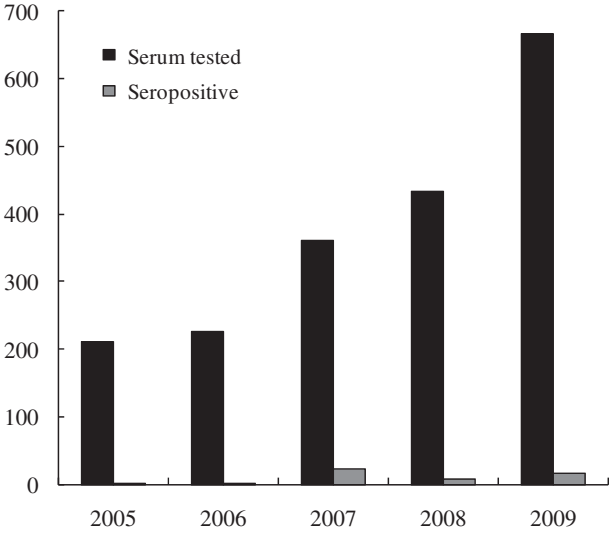

Figure 1. Annual distribution (from 2005 to 2009) of the number of serum samples from Lyme borreliosis suspected case and the number that tested seropositive for Lyme borreliosis.