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ORIGINAL ARTICLE

Autochthonous Lyme Borreliosis in Humans and Ticks in Korea

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Abstract
Objective: This study aimed at finding epidemiological and clinical features of autochthonous Lyme borreliosis in humans through epidemiological investigations and identifying its vectors and pathogens through analysis of ticks.
Method: Epidemiological investigations, including review of the retrospective medical records and patient interviews, were conducted in two cases that occurred in 2012. To identify the vectors and pathogens, ticks were collected between September 23 and October 6, 2012 from the area where the tick bite in the first patient occurred. The ticks were classified, and polymerase chain reaction (PCR) tests and cultures were performed.
Results: The first patient, a 46-year-old female, visited a forest in Gangwon province, which was 900 m above sea level, where the tick bite occurred. Two weeks after the tick bite, erythema migrans (12×6 cm^2 in size) appeared on the site of tick bite, along with fever, chill, fatigue, myalgia, and arthralgia on shoulders, knees, and hips. The second patient, a 44-year-old male, visited a mountain in Gangwon province, which was 1200 m above sea level, where a tick bite occurred. One month after the tick bite, erythema migrans appeared at the site of the tick bite, along with fatigue, myalgia, and arthralgia on the right shoulder and temporomandibular joint. Indirect fluorescent antibody testing and Western blotting were carried out in these two cases for diagnosis, and positive findings were obtained. As a result, Lyme borreliosis could be confirmed. To estimate the pathogens and vectors, the ticks were collected. A total of 122 ticks were collected and only two species, *Haemaphysalis japonica* and *Haemaphysalis flava*, were identified. PCR and culture were performed on ticks. However, *Borrelia burgdorferi* sensu lato was not isolated from any collected ticks.

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Conclusions: This study is significant to confirm Lyme borreliosis officially at first by the national surveillance system, although identification of the mites and pathogens failed.

1. Introduction

Lyme borreliosis is the most common tick-borne infection in the United States and Europe. It is caused by the spirochete bacteria *Borrelia burgdorferi* sensu lato, which is transmitted through the bite of *Ixodes* spp. More than 20,000 people in the USA are reported to suffer from Lyme borreliosis each year and the number is increasing [1]. In Europe, particularly in Germany, Austria, Slovenia, and Sweden, Lyme borreliosis was widely reported in forested areas [2]. In Asian countries such as China and Japan, Lyme borreliosis has also been reported [3,4]. In Korea, patients with autochthonous Lyme borreliosis were rarely reported; only six patients were reported in previous studies [5−9]. However, it was known that the ticks that could transmit *B. burgdorferi* sensu lato inhabited groves in Korea [10,11], and bacterial strains such as *B. burgdorferi, Borrelia afzelii*, and *Borrelia garinii* were isolated from them [12,13]. Moreover, in a study on seropositive patients conducted during 2005−2009, 37 cases were suspected to have been infected domestically [14]. These studies support the hypothesis that there would be more patients in Korea who were overlooked. Therefore, surveillance of Lyme disease is significant for public health. Lyme borreliosis was designated as an infectious disease in 2010 and surveillances were conducted by Korea Centers for Disease Control and prevention (KCDC) since then. Autochthonous Lyme borreliosis had not been reported in any patient since 2010, and the first patient was officially confirmed in 2012. Two patients with autochthonous Lyme borreliosis were reported to the surveillance system in KCDC in 2012. This study aimed at finding the epidemiological and clinical features of autochthonous Lyme borreliosis in humans through epidemiological investigations and identifying vectors and pathogens though an analysis of the ticks collected from the regions where tick bite occurred for the first patient.

2. Materials and Methods

The first patient was reported to the KCDC on July 31, 2012, and epidemiological investigations, including the review of retrospective medical records and the patient interview, were conducted immediately by an epidemiological intelligence service (EIS) officer. A blood specimen collected from the patient was sent to the Division of Zoonoses in Korea National Institute of Health (KNIH) for diagnosis. Some tissue from the tick was found on the patient's skin, which was sent to the Division of Medical Entomology in KNIH for identification. The second patient was reported on November 22, 2012, and epidemiological investigations, including the review of retrospective medical records and the patient interview, were undertaken by an EIS officer. To identify the vectors and pathogens, ticks were collected by the EIS officer from the region where the patient was bitten by ticks and sent to the Infectious Disease Intelligence Division in Gangwon Institute of Health and Environment between September 23 and October 6, 2012. The ticks were classified by the Division of Medical Entomology in KNIH; to identify the pathogens; polymerase chain reaction (PCR) tests and cultures were also performed on the ticks by the Division of Zoonoses in KNIH.

3. Results

The first patient was a 46-year-old female. She lived in Chunchon city in Gangwon province. She was a housewife and did not have any underlying diseases. She had never traveled abroad. On June 15, 2012, she visited the forest in Mt Hwaak in Gangwon province, which was 900 m above sea level, where she drifted off the trail and entered the deep forest to collect herbs. Approximately 36 hours after she visited the forest, she found a tick bite with the tick still attached to her left back and removed it herself. However, the tick was not removed completely and some tissue from the tick still remained on her skin. She visited the local clinic and it was removed. Two weeks after the tick bite, erythema migrans (12×6 cm^2) appeared at the site of the tick bite, along with fever, chills, fatigue, myalgia, and arthralgia on her shoulders, knees, and hips (Figure 1). Because the erythema progressed over the next 2 weeks, she visited a general hospital; she was reported as a suspected patient of Lyme disease to the KCDC.

The second patient was a 44-year-old male. He lived in Seoul. He worked in an office and did not have any underlying diseases. He had not traveled abroad recently. On May 26, he climbed Mt Odae in Gangwon province, which was 1200 m above sea level, to collect herbs. Three days after he visited there, he found a tick bite with the tick being attached to his right axillar and removed it himself. One month after the tick bite, erythema migrans appeared at the site of tick bite,

Table 1. Epidemiological features and clinical manifestations in Cases 1 and 2

	Case 1	Case 2
Date of tick bite	June 15, 2012	May 26, 2012
Location of tick bite	Mt Hwaak in Gangwon province (altitude: 900 m)	Mt Odae in Gangwon province (altitude: 1200 m)
Feeding time of the ticks	36 h	72 h
Incubation period	2 wk	1 mo
Clinical manifestations	Erythema migrans	Erythema migrans (disappeared)
	Fever, chills, fatigue	Fatigue
	Myalgia and arthralgia (Both shoulders, knees and hips)	Myalgia and arthralgia (Right shoulder and temporomandibular joint)
Clinical stage	Early localized infection	Chronic Lyme disease (Post Lyme Syndrome)

along with fatigue, myalgia, and arthralgia on the right shoulder and temporomandibular joint. The erythema expanded until it became 70 × 80 cm^2 in size and disappeared in 2 weeks. Although the erythema disappeared, myalgia and arthralgia remained. Therefore, he visited a general hospital in November 2012, and he was reported as a suspected patient of Lyme disease to the KCDC.

In the laboratory tests, every patient in this study was found to have normal white blood cell counts, hemoglobin concentrations, platelet counts, and liver function tests including aspartate aminotransferase (ALT) and alanine aminotransferase (ALT) concentrations. To diagnose Lyme disease, an indirect fluorescent antibody test was conducted by the Division of Zoonoses in KNIH. In the first case, the IgM titer was found to be below 1:16 and the IgG titer 1:256. Therefore, Western blotting was performed to confirm Lyme borreliosis, and positive findings were detected. In the second case, the IgM titer was found to be below 1:16 and IgG 1: 512. Western blotting showed positive result. Although Lyme borreliosis could be confirmed using this method, pathogens could not be identified because no pathogens were detected in the cultures (Table 1).

To identify the vectors, the ticks that had bitten the patents were needed. In the first case, some tissue from the tick could be collected from the hospital where the patient had had it removed from her skin (Figure 2). The tissue was part of the hypostome of the tick. However, the exact species of the tick could not be identified because the tissue was too small and decayed.

Because the pathogens and vectors were still not identified, a further study was conducted. To estimate the pathogens and the vectors, ticks from the region where the first patient was bitten were analyzed. A total of 122 ticks were collected (Table 2) and only two species, *H. japonica* and *H. flava*, were isolated. After classification of the ticks, PCR and cultures were performed on the ticks by the Division of Zoonoses in KNIH to isolate the pathogens. However, *B. burgdorferi* sensu lato was not isolated from any collected ticks.

4. Discussion

Clinical manifestations of Lyme borreliosis are usually classified into three stages: early localized infection, early disseminated infection, and late infection [15]. Early localized infection usually begins with the development of erythema migrans after an incubation period of 3–30 days [16]. Approximately 80% of patients exhibit that skin lesion [1,17]; 50% of the patients had systemic symptoms such as fever, chill, headache, and arthralgia [18]. Clinical manifestations of

Figure 1. Erythema migrans at the site of tick bite.

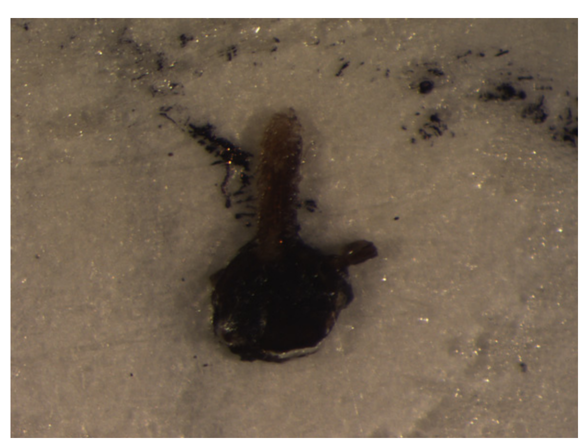

Figure 2. Hypostome of the tick found on a patient's skin.

Table 2. Results of the ticks collected from Mt Hwaak in Gangwon province

	Date	Adult (male)	Adult (female)	Nymph	Total
H. flava	9/25	0	0	6	6
	10/2	2	3	11	16
	10/9	0	2	4	6
H. japonica	9/25	2	2	19	23
	10/2	9	1	31	41
	10/9	10	1	19	30
Total		23	9	90	122

early disseminated infection appeared within days of weeks after the onset of erythema migrans. During this period, 30−50% of patients may develop secondary annular skin lesions. Skin lesion is often accompanied by nonspecific systemic symptoms including headache, fever, chills, myalgia, and arthralgia [18]. Of untreated patients, 15% may have neurologic abnormalities, including meningitis, encephalitic signs, cranial neuritis, peripheral neuropathy, cerebral ataxia, or myelitis, and 8% may have cardiac problems including atrioventricular block [17]. Months after the onset of infection, 60% of untreated patients can have clinical symptoms of late infection [17]. The clinical symptoms usually include arthritis or acrodermatitis chronica atrophicans, and, although rare, approximately 5% of patients may develop chronic neurologic d abnormalities [19,20]. Several patients may have post Lyme syndrome such as musculoskeletal pain, neurocognitive manifestations, and fatigue symptoms after recovery of Lyme borreliosis [15]. In the first case, erythema migrans developed 2 weeks after the tick bite and in the second case it developed 1 month after the tick bite. Incubation periods of these two cases were similar to the incubation periods reported in previous studies. The first case showed clinical characteristics of early localized infection, whereas the second case showed clinical manifestations of post Lyme syndrome.

To transmit Lyme borreliosis from ticks to human, the ticks should have enough pathogens and they have to feed for more than 36 hours [20,21]. In this study, the patients found the ticks more than 36 hours after the tick bite, and it was assumed that the ticks could have enough time to transmit Lyme borreliosis.

Laboratory test results show that white blood cell count, hemoglobin concentrations, platelet count in the patients with Lyme borreliosis are usually within normal range, and about 35% of patients in the USA and up to 20% in Europe can have slightly raised liver function test results, especially AST and ALT [20]. In this study, the patients had normal laboratory test results, which were similar to the results of previous studies.

For diagnosis of Lyme borreliosis, characteristic clinical findings are important. Typical erythema migrans is sufficient to make a clinical diagnosis in the absence of laboratory tests. However, if erythema migrans is not seen, at least one of the clinical manifestations of Lyme borreliosis and the laboratory evidence of infection are needed to diagnose Lyme borreliosis [15,20]. Culture of *B. burgdorferi* in the Barbour−Stoenner−Kelly medium is one of the laboratory tests that can be used for a definite diagnosis of Lyme borreliosis. However, the culture is not routinely performed because it is very insensitive and ineffective [20]. PCR testing was used in several previous studies for the diagnosis of Lyme borreliosis, and it was found to be superior to culture for isolating *B. burgdorferi* sensu lato from joint fluid. However, it had limitations in detecting the pathogens in cerebrospinal fluid, blood, and urine samples. Therefore, culture and PCR are not recommended for clinical use [16,20,22]. The mainstay of laboratory diagnosis is two-tier serological testing [15,20,23,24]. The first tier is generally the enzyme-linked immunosorbent assay (ELISA) and the second tier is Western blotting. If ELISA is positive or equivocal, Western blotting is conducted to confirm Lyme borreliosis. In Korea, laboratory tests for Lyme borreliosis are conducted only by the laboratory of the Division of Zoonoses in KNIH. Diagnoses of the patients in this study were also confirmed by that laboratory. Although *B. burgdorferi* sensu lato was detected in the ticks that inhabit Korea, it has not been isolated from humans yet. Therefore, cultures from the patients were conducted to identify the pathogens. However, there were no positive findings in the cultures and it was assumed that the failure might be due to a delayed visit to the hospital.

Because isolation of pathogens and detection of the vectors failed in this study, a further study was conducted. To estimate the pathogens and the vectors, ticks were collected. The ticks were collected only from the region where the first patient was bitten because the second patient was reported in winter, 6 months after the tick bite and the distributions of ticks might have changed within this period.

In previous studies on ticks in Korea, *B. burgdorferi* was isolated from *Ixodes persulcatus*, which was collected in Korea, and three species of the ticks were known to be the vectors of Lyme borreliosis in Korea: *I. persulcatus, Ixodes nipponensis*, and *Ixodes granulatus* [10−13]. Distributions of these ticks were different from each other. *I. nipponensis* was detected across Korea, whereas *I. granulatus* was found frequently in the southern regions, including Jullanam province. *I. persulcatus* was detected in the eastern Alpine range, including Gangwon province. In the studies on *B. burgdorferi* sensu lato isolated from the ticks in Korea, *B. afzelii* was isolated from the ticks collected from the eastern, central, and southern regions, and *B. garinii* was isolated from the ticks collected only from the southern regions. In this study, a total of 122 ticks were collected and only two species, *H. japonica* and *H. flava*, were detected. *B. burgdorferi* sensu lato was not isolated from any collected ticks. In conclusion, identification of pathogens and vectors failed.

According to the studies on seasonal distributions of ticks in Korea, ticks that transmitted Lyme borreliosis had not been found until April and were detected from May through October [11]. The ticks were found most frequently between July and August, and they disappeared rapidly after September. In this study, the tick bite occurred in June, whereas the collection of the ticks was conducted between September and October. It was assumed that the failure of the identification of the vectors might be due to different seasonal distributions of the ticks.

Although this study could not identify the mites and pathogens, it is still significant to confirm Lyme borreliosis officially at first since introduction of the national surveillance system. Moreover, the epidemiological investigations found that the places where the tick bites occurred were on a mountain situated at a high altitude in Gangwon province, in the eastern Alpine range. Because *I. persulcatus*, known to be one of the vectors of Lyme borreliosis, inhabit this province and the number of people visiting this province for leisure is increasing rapidly, more cases of tick bite may occur. Therefore, a continuous surveillance system for Lyme borreliosis is essential and, based on the data from the surveillance system, further studies can be conducted.

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