

Mini review: surveillance of Lyme borreliosis in Southeast Asia and method of diagnosis

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Abstract. – Lyme borreliosis is caused by the Gram-negative spirochetes *Borrelia spp.*, particularly *Borrelia burgdorferi sensu lato* complex. The disease is transmitted through the bite of the infected black-legged *Ixodes* tick. Lyme borreliosis extensively occurs in the Northern Hemisphere, mainly in the United States. Lyme borreliosis cases are also detected in Asian countries including Korea, Nepal, China, Taiwan, and Japan. However, there is an inadequate understanding of Lyme borreliosis in the Southeast Asian region. Hence, this review aims to provide a brief update on the prevalence of Lyme borreliosis infection in Southeast Asia based on the latest literature on this issue. Lyme borreliosis has been discovered in human serum in Indonesia, Malaysia, and Singapore. The human serum samples were mainly examined with ELISA test using *Borrelia spp.* IgG and IgM antigens. *Borrelia spp.* also has been detected in ticks found on host animals such as *Sundamys muelleri* and *Python* in Malaysia, Thailand, and Laos. Polymerase chain reaction (PCR) is used to detect the presence of *Borrelia* DNAs in the samples. The published studies have demonstrated that *Borrelia spp.* exists in Southeast Asia and although the incidence is relatively low, it is believed that Lyme disease cases are under-reported.

Key Words:

Lyme disease, Lyme borreliosis, *Borrelia burgdorferi*, Southeast Asia, Diagnosis.

Introduction

In 1975, Lyme disease was first studied by a group of Yale researchers¹, when two mothers from Old Lyme, Connecticut, reported that many children and a few adults suffered from arthritis with other symptoms such as fever, headache, erythematous papule, and recurrent attacks of joint pain. The children were initially diagnosed with

juvenile rheumatoid arthritis (JRA). However, after a period of surveillance, the incident was described as a formerly unrecognized clinical entity and called “Lyme Arthritis”, as the researchers believed the disease was transmitted by arthropod vectors¹.

In later research², it was found that Lyme disease (also known as Lyme borreliosis) was provoked by the Gram-negative spirochetes *Borrelia spp.* Lyme borreliosis is mainly induced by the three genospecies of the *Borrelia burgdorferi sensu lato* complex: *Borrelia burgdorferi sensu stricto*, *Borrelia garinii* and *Borrelia afzelli*³. In the United States, Lyme disease is primarily caused by *Borrelia burgdorferi*⁴. It has been reported that *B. burgdorferi* exclusively occurs in North America, while *B. garinii* and *B. afzelli* are found in Europe and parts of Asia⁵. Infected black-legged *Ixodes* tick is the main vector for transmitting Lyme borreliosis. It has been reported that *Ixodes scapularis* (the deer tick) is extensively found in the Northeastern and Midwestern United States, while *Ixodes pacificus* widely propagates in the Pacific Northwest⁶. *Borrelia spp.* survives in the tick’s midgut. At least 48 to 72 hours is required for the infected tick to feed on its host, thus allowing the bacteria to wander to the salivary gland and be injected into the host before causing Lyme borreliosis⁷.

The clinical manifestations of Lyme borreliosis consist of three phases: the primary early localized disease, the secondary early disseminated disease, and the tertiary late disease. In early localized disease, erythema migrans (EM) begins with red macule (alteration of skin color) or papule at the site of the tick bite and is typically visible in patients within 3 to 30 days⁸. The erythematous lesion is bull’s eye-like, at least 5 cm in diameter and can continue to enlarge up to 70 cm. Patients may experience fever, malaise,

headache, regional lymphadenopathy, nuchal rigidity, myodynia or arthralgia at early stages. After 3 to 5 weeks of infection, multiple erythema migrans may occur, and some even experience cranial nerve palsies⁸. In rare incidences, patients may suffer from carditis, complete heart block, borrelial lymphocytoma and meningoradiculoneuritis⁸. The late manifestation of Lyme borreliosis occurs weeks to months, or even years after the primary infection. The common manifestation at this stage is arthritis (Lyme arthritis), whether monoarticular or oligoarticular, affecting large joints, especially in the knee. Some may even experience neuroretinitis, an optic nerve and peripapillary retina inflammation process^{9,10}.

Lyme borreliosis is the most prevalent tick-borne disease in the Northern Hemisphere, especially in the United States and cases are increasing. There is a significant increase in estimated clinician-diagnosed annual cases from previously ~329,000 to ~476,000 in the United States, according to the health insurance claims data from 2005 to 2010 and from 2010 to 2018, respectively^{11,12}. Lyme borreliosis also occurred in Europe¹³, Southern Canada^{14,15}, and some parts of Asia such as Korea¹⁶, China¹⁷, Nepal¹⁸, Taiwan¹⁹ and Japan²⁰. However, there is a lack of understanding of Lyme borreliosis in Southeast Asia.

This review provides insight into the surveillance of the prevalence of Lyme borreliosis infection in Southeast Asia and their method of diagnosis based on several published studies.

***Borrelia* spp. Collected from Human Serum**

Indonesia

In Indonesia, Rotan et al²¹ first reported that some forestry workers were diagnosed positive for Lyme borreliosis. The forestry workers from 4 different villages who suffered from unexplained joint pain for the past two years were tested for IgG anti-*Borrelia* ORG911G by applying enzyme-linked immunosorbent assay (ELISA) and further confirmed by running Rheumatoid factor (RF), C-reactive protein (CRP) and Anti-mutated citrullinated vimentin (MCV) test to rule out the joint pain that was occasioned by rheumatoid arthritis, osteoarthritis, gout, or septic arthritis. Three out of 41 patients (7.32%) tested positive for the IgG Lyme test; this was the earliest Lyme borreliosis case described in Indonesia.

Malaysia

Tay et al²² had done a study on Lyme borreliosis prevalence in Malaysia by using IgG and IgM ELISAs (Biotest, Dreieich, Germany) in the initial screening and followed by the Western Blot method for seropositive samples. In the initial screening, a total of 183 human sera including 30 blood donors, 121 patients that were initially clinically diagnosed to be infected with leptospirosis, melioidosis or rickettsia, 2 patients with erythema migrans and 30 patients who were exposed to tick typhus. Among these tested samples, 2 human sera had shown to be seropositive to both IgM and IgG, 28 seropositive to IgM, and 9 human sera seropositive to IgG. The seropositive sera were further tested with Western Blot (BIOTEST-BLOT, Biotest, Dreieich, Germany) whereby *Borrelia* IgM was detected in 6 patients, but neither one of them was positive for IgG. Tay et al²² also suggested the *B. afzelli* strain as the causative agent in Malaysia as the antibodies tested matched those of the *B. afzelli* antigens.

Recent research by Khor et al²³ studied seroprevalence among indigenous people from 16 villages in Malaysia. Among the 904 participants, 73 (8.1%) serum samples were found to be seropositive to ELISA anti-*B. burgdorferi* IgG antibodies (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). However, a single diagnostic test was carried out, suggesting the possibility of cross-reactivity with other serovars yielding false-positive results.

Singapore

In Singapore, 72 patients with annular erythema but negative for tick bite reaction were tested to estimate the prevalence of Lyme borreliosis. The patients underwent three serological tests: ELISA (3M IgG/IgM Fastline Test, 3M Diagnostic System, Inc, Santa Clara, CA, USA), Passive Hemagglutination (PHA) test (Lymix, Diagast Laboratories, Lille Cedex, France) and Indirect Immunofluorescence (IF) test (Lymag, Diagast Laboratories, Lille Cedex, France). The results showed that although five patients were seropositive to ELISA, all showed negative in further tests in passive hemagglutination and indirect immunofluorescence, thus suggesting that Lyme disease is not prevalent in Singapore²⁴.

In 2012, a patient was reported to be suffering from left eye neuroretinitis, with the patient's dog experiencing severe tick bite and anorexia²⁵. The patient was positive for *B. burgdorferi* IgG sero-

logical test and was suspected of Lyme neuroretinitis. After three weeks of treatment, a second serology test was repeated, and reconfirmed by western blot. Lyme titer was not elevated, and it was negative for Lyme IgG too. Taking into consideration the aetiology of the neuroretinitis, the patient had been treated with prednisolone, ceftriaxone and amoxicillin. Nevertheless, visual acuity was not improved but macular oedema and hard exudates improved, suggesting that the patient was infected with Lyme borreliosis²⁵. Table I shows the summary of *Borrelia spp.* collected from human serum.

***Borrelia spp.* in Vector Collected from the Animal Host**

Malaysia

Ixodes tick act as the primary vector of transmitting *Borrelia spp.* to humans and animals. Khoo et al²⁶ studied the exposure of tick-borne *Borrelia spp.* in Malaysia. They collected ticks from wild boar carcasses in the Orang Asli community and amplified the *16s rRNA* gene to examine the existence of *Borrelia spp.* in a tick. The ticks positive for *16s rRNA* were further confirmed by targeting the flagellin (*flaB*) genes. The result showed out of 37 total ticks collected, *flaB* gene was detected in 1 male *Haemaphysalis*

hystricis tick. The collected *Borrelia spp.* was found to be closely related to the *Borrelia spp.* collected in Japan and Portugal that clustered with relapsing fever^{27,28}.

In order to further study the prevalence of *Borrelia spp.* in Malaysia, Khoo et al²⁹ extended the research by collecting tick from recreational forests and semiurban residential areas located in Selangor, by targeting small mammals (order Scandentia and Insectivora) and rodents (order Rodentia). Nested PCR was used to target the *flaB* gene of *Borrelia spp.* The result showed out of 156 total ticks examined, 72 ticks (all were *Ixodes granulatus*) were positive for *Borrelia flaB* amplification, and most of the *Borrelia spp.* vector carrier was *Sundamys muelleri*. The *flaB* amplified sequence was then aligned with the sequences on the National Center for Biotechnology Information (NCBI) sequence database by using BLASTn search. The result showed the amplicon is highly similar to *B. yangtzensis*, *B. valaisiana*, *B. garinii* and *B. japonica*. It could be the first report of *B. yangtzensis*-like strain presence in Southeast Asia²⁹.

Another study³⁰ that purposed to detect and characterize *Borrelia* in vectors such as rodents and *Ixodes* ticks that could be found in primary forests (Gunung Gading National Park and Kubah National Park) and an oil palm plantation

Table I. Summary of *Borrelia spp.* collected from human serum.

Country	Location	Method	Total sample	Result	Reference
Indonesia	4 villages in Sibolangit	ELISA RF, CRP, Anti-MCV test	41 forestry workers (with unexplained joint pain)	3 forestry workers were seropositive for IgG	21
Malaysia	Nationwide (mainly in Selangor)	ELISA Western Blot	30 blood donors nationwide 121 patients 2 patients showed rythema migraines 30 patients exposed to tick typhus	1 blood donor showed IgM seropositive	22
	16 villages in Malaysia	ELISA	904 indigenous people	73 participants showed seropositive	23
Singapore	-	ELISA PHA IF	72 patients with annular erythema	No seropositive result reported	24
		ELISA Western Blot	A patient with left eye neuroretinitis	Showed seropositive in the beginning, but no increase in Lyme titer and negative in Western Blot after three weeks	25

in Sarawak, was carried out by Lau et al³⁰. This study is the first evidence of *Borrelia yangtzensis* and *Borrelia miyamotoi* being detected in rodents and ticks in Sarawak, although there has been no reported Lyme disease case from Sarawak. Besides, a new geographical record of the *Borrelia spp.* has been revealed from these findings.

Thailand

From 1999 to 2001, Hirunkanokpun et al³¹ collected ticks from 10 different locations in order to investigate tick-associated bacteria in Thailand. A total of 334 ticks of 14 different species were collected at research sites, with most of the samples collected from Khao Yai National Park. PCR was used to perceive the presence of bacterial DNA in ticks, including *Borrelia spp.*, *Francisella spp.*, *Rickettsia spp.* and *Wolbachia pipientis*. However, none of the ticks had shown specific positive for *Borrelia spp.* gene³¹.

Ticks had been globally reported to be infested snakes. Hence, Trinachartvanit et al³² investigated the existence of *Borrelia spp.* in ticks in different snake samples. A total of five different types of tick species were collected from five snakes of various species in Lophuri Province, and the presence of *Borrelia spp.* DNA within

the tick was then determined via PCR. The result showed that out of 12 ticks, the *Borrelia*-specific gene was amplified in three identified as *Amblyomma varanense* ticks obtained from *Python reticulatus*. The *Borrelia spp.* DNA sequences were then compared for *16s rRNA* and *flaB* in NCBI using the BLAST searching tool. Based on the constructed phylogenetic trees, the authors suggested that the *Borrelia spp.* collected has a distinct phylogenetic relationship between Lyme borreliosis and relapsing fever-related *Borrelia spp.*³².

Laos

In Laos, a total of 6,692 ticks were collected from 768 pools in Nakai District, Khammouan Province³³. Bacterial DNA within the tick was then screened with quantitative real-time PCR. A total of 12 ticks, including *Amblyomma testudinarium*, *Haemaphysalis spp.* and *Dermacentor auratus*, were found to be *Borrelia* positive. The collected *Borrelia spp.* showed high concordance to Shiretoko *Haemaphysalis Borrelia spp.*, which belongs to the relapsing fever group of *Borrelia* and is found in Japan.

Table II shows the summary of *Borrelia spp.* in vector collected from the animal host.

Table II. Summary of *Borrelia spp.* in vector collected from the animal host.

Country	Location	Method	Host	Result	Reference
Malaysia	Selangor (Orang Asli settlement)	PCR	Wild boar	12 tick samples were detected with <i>Borrelia</i> 16s rRNA 1 male <i>Haemaphysalis hystricis</i> (out of a total of 37 ticks collected) was detected with the <i>Borrelia</i> flaB gene, clustered with the Relapsing fever group <i>Borrelia</i>	26
	Selangor recreational forests and semiurban residential area	Nested PCR	Rodents, small mammals	72 out of 156 ticks collected were found to be <i>Borrelia</i> flaB positive (all positive samples were <i>Ixodes granulatus</i>) 10 out of 15 animal <i>Borrelia</i> carriers were <i>Sundamys muelleri</i> <i>Borrelia yangtzensis</i>	29
Thailand	10 different locations	PCR	-	None of the collected ticks was <i>Borrelia</i> positive	31
	Lophuri Province	PCR	Snake	3 out of 12 ticks collected were found to be <i>Borrelia</i> positive (all were collected from <i>Amblyomma varanense</i> on <i>Python reticulatus</i> skin)	32
Laos	Nakai District, Khammouan Province	Quantitative real-time PCR	Pools	12 out of 768 ticks collected were found to be <i>Borrelia</i> positive	33

Method of Diagnosis

There are a few methods that could be used to detect Lyme borreliosis in humans; culture persisted, being the gold standard for screening and confirming Lyme borreliosis. Nevertheless, *B. burgdorferi* has limited and restricted metabolic capacity, and complex growth media is needed for cultivation³⁴. Aside from that, the sensitivity of detection is low. Success rates using blood culture were reported ranged from 40-46%^{35,36}. The cultivation of bacteria needs long incubation time; culture samples are required to be kept for 8 to 12 weeks before being validated as negative³⁴. Despite the improved culture technique and the success rate surging to 94%, long-term of cultivation (up to 16 weeks) is needed to achieve a significant result³⁶. All the above factors could be the reason why this method was not popularly used for *Borrelia* detection.

Centers for Disease Control and Prevention (CDC) has recommended two-tiered tests for Lyme borreliosis diagnosis. The first step is using ELISA or Immunofluorescence Assay (IFA), followed by the second step, immunoblot test (Western Blot test), if a positive or equivocal result was shown in the first step³⁷. In Southeast Asia, the most common method practiced by researchers is ELISA for the first step of Lyme borreliosis diagnosis of human serum²¹⁻²⁴. The second step of immunoblot analysis is to reconfirm that the positive IgG and IgM antibodies are caused by Lyme borreliosis and not due to cross-reactivity³⁸. In Singapore, Goh et al²⁴ replaced the Western blot tests with PHA and IF due to the inaccessibility of western blot. Nevertheless, the limitation of two-tiered tests is the low sensitivity of detection during early infections due to the nature of delayed humoral immune response development³⁹.

A meta-analysis report³⁹ showed only a 46.3% positive rate at the primary early localized stage. IgM is only noticeable after two to four weeks of disease onset, while IgG is present after four to six weeks. Hence, immunoblot analysis done after 30 days shall be tested for IgG only. In a report, the appearance of IgG could persist in 25% of patients for 10 to 20 years, and in lower case IgM at 10% make it difficult to discriminate whether it is active or past infection⁴⁰.

PCR could be an alternative way for early detection of Lyme borreliosis. The sensitivity of using PCR for the acute phase was reported to be relatively higher than using two-tiered tests with high specificity⁴¹. The specimen for

PCR can be obtained through skin biopsy of patients with erythema migraine or acrodermatitis chronica atrophicans, or through body fluid samples like blood, cerebrospinal fluid in the brain and spinal cord or synovial fluid in joints⁴². Studies⁴³ showed the sensitivity of detection is highly reliant on the specimen used, low detection (10-30%) in blood and cerebrospinal fluid, and efficiency is highest with skin biopsies of the EM lesions (up to 88% of success rate). Although the overall sensitivity of using PCR in some cases might be lower, it could act as an early detection prior to the slow development of immunology response, as early and effective preclinical detection is crucial for successful patient management by establishing appropriate treatment and aiding in supervising the propagation of disease. Despite recognizing erythema migraine could help to differentiate Lyme borreliosis, not every patient will show similar symptoms, some giving a virus-like or flu-like symptom, or the presence of fatigue, muscle or joint pain, which could lead to confusion among clinicians⁴⁴. Hence, the cases of Lyme borreliosis in Southeast Asia could be under-reported. Late-stage Lyme borreliosis could lead to neurologic, musculoskeletal or cardiovascular complications if left untreated.

Ticks Correlated to Lyme Borreliosis in Southeast Asia

In Southeast Asia, there are 97 known continental tick species, covering around 10% of valid species⁴⁵. Among the continental ticks in Southeast Asia, 42 species of *Haemaphysalis* have occupied half of the ixodid fauna, while there are only 14 species of *Ixodes* in Southeast Asia although it is the most abundant worldwide⁴⁵. Tick is recognized as a vector of human bacterial diseases; compared to the soft tick *Asgasidae* and the new tick families called *Nuttalliellidae*, *Ixodids* have a relatively long period of feeding time (several days), and usually, their bites are painless. Since they feed in premature stages, such as larva (size ~0.8 mm) and nymph (size ~1.2x1.5 mm), it is challenging to notice them due to their small size⁴⁶. *Borrelia spp.* in Southeast Asia were collected from ticks that are prevalent in Asia and Southeast Asia regions, such as *Haemaphysalis spp.*, *Ixodes granulatus*, *Amblyomma varanense*, *Amblyomma testudinarium* and *Dermacentor auratus*⁴⁷⁻⁴⁹. This sets them apart from the common borreliosis vector in the United States and Europe region.

Rodents, birds, lizards and snakes have been reported to be the hosts of tick carrying *Borrelia spp.* Farm workers and indigenous people staying near the forest and semiurban regions could have a higher risk of exposure to tick infestation⁵⁰. Vectors such as infested birds having high mobility can increase the chance of disease transmission to a wider area. Moreover, human activity such as deforestation and urbanization could damage the habitat of the vector, causing the spread of tick-borne or zoonotic disease to a wider area, as the chances of human-animal interaction are increased.

To date, at least 18 genospecies in *Borrelia burgdorferi sensu lato* complex are distributed worldwide, with 9 species potentially human pathogenic, especially *B. burgdorferi s.*, *B. afzelli* and *B. garinii*, and 9 species that have not been reported to be detected in humans, including the *B. yangtzensis*-like strain found in Malaysia^{29,51}. There is a report²² suggesting that *B. afzelli* might be present in Malaysia as patient serum has shown positive for *B. afzelli* IgG and IgM antigen in the ELISA test (Biotest, Dreieich, Germany). Yet, *Borrelia* antigens are highly conserved among all Lyme borreliosis species. The *Borrelia* antibody responses will be similar when tested with ELISA⁵²⁻⁵⁴. Although ELISA is a completely standardized and automated platform coated with substrates such as well-characterized recombinant antigens⁵⁵, further confirmation tests shall be carried out in order to determine the species and strain of the particular Lyme borreliosis.

Conclusions

While focusing on the prevalence of Lyme borreliosis in the USA and EU countries, the occurrence of Lyme borreliosis in Asia, especially Southeast Asia, should not be neglected. Published studies demonstrated that *Borrelia spp.* exists in Southeast Asia, although the incidents are relatively low. However, the published studies only covered several countries in Southeast Asia, with an absence of research on Borreliosis in Vietnam, Philippines, Cambodia, Myanmar, Brunei, and Timor-Leste. Hence, it is believed that Lyme borreliosis cases in Southeast Asia are under-reported.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Authors' Contribution

KJT conceived of the idea for the article, performed the literature search and drafted the manuscript. HYT, HSP, and SYG collected the data and revised the manuscript. LSL and NSL provided critical feedback and helped to shape the manuscript. All authors reviewed and approved the final version of the manuscript.

Ethics Approval

Not applicable.

Informed Consent

Not applicable.

Availability of Data and Materials

The datasets are generated from online published articles and can be obtained from the corresponding authors upon appropriate request.

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