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Brief Original Article

Seroprevalence of *Borrelia burgdorferi* among the indigenous people (Orang Asli) of Peninsular Malaysia

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Abstract

Introduction: Lyme disease has been well-described in the North America and European countries. However, information is still very limited in the developing countries including Malaysia. The Orang Asli (OA), the indigenous people of Peninsular Malaysia reside mostly in the forest and forest fringe areas abundant with the vector for Lyme disease. Here, we described the seroprevalence of *Borellia burgdorferi* (*B. burgdorferi*) among the OA and demographic variables that could be associated with seroprevalence.

Methodology: A total of 16 OA villages distributed across 8 states in Peninsular Malaysia participated in this study. Sera obtained from 904 OA volunteers were screened for anti-*B. burgdorferi* IgG antibodies. ELISA results obtained and demographic information collected were analysed to identify possible variables associated with seroprevalence.

Results: A total of 73 (8.1%) OA tested positive for anti-*B. burgdorferi* IgG antibodies. Among all the variables examined, village of residence ($p = 0.045$) was the only significant predictor for seropositivity. High (> 10.0%) prevalence was associated with three OA villages. Those living in one particular village were 1.65 times more likely to be seropositive as compared to other OA villages. Age, gender, marital status, household size, level of education, monthly household income and occupation were not significant predictors for seropositivity.

Conclusion: Results of the present study support earlier findings that *B. burgdorferi* infection among Malaysians is currently under-recognized. Further studies will be needed at these locations to confirm the presence of Lyme disease among these populations.

Key words: Lyme disease; *Borrelia burgdorferi*; Malaysia; Orang Asli; ticks; infectious diseases.

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Introduction

The tick is an important human and animal disease vector, capable of harbouring a wide range of pathogenic microorganisms. Among the diseases transmitted by ticks, Lyme disease, a disease caused by the spirochetes from *Borrelia burgdorferi* (*B. burgdorferi*) *sensu lato* (*s.l.*) complex, is one of the most recognised tick-borne diseases in humans [1,2]. The disease typically manifests as a skin rash and infection often results in more severe complications involving the joints, heart, and nervous system. Proper diagnosis and treatment during the early phase is important in limiting the severity of the disease [3].

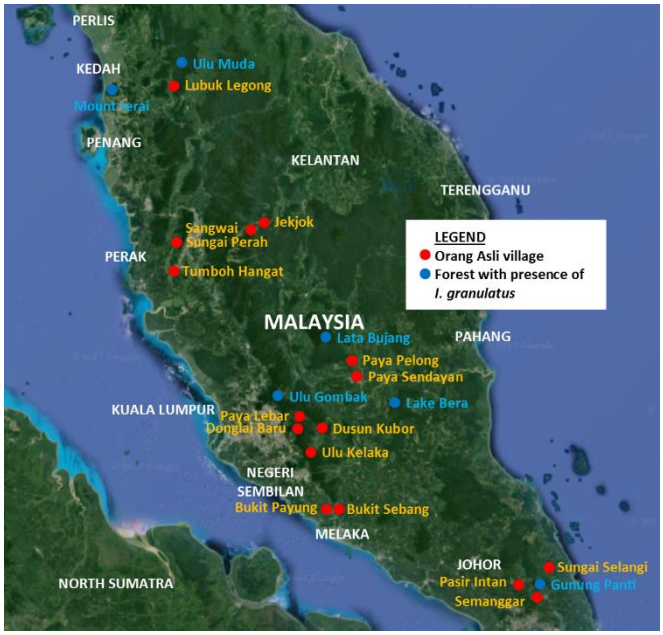
Lyme disease is endemic in more than 70 countries worldwide [1,4]. The United States alone records about 30,000 annual cases, but the actual number has been estimated to be as high as 300,000 cases [5]. High incidences of Lyme disease have also been reported in European countries with the highest annual number of cases recorded in Austria and Germany [2]. As surveillance efforts are often limited in most developing countries, the epidemiology of Lyme disease is not well known. In Malaysia, a previously conducted serological study found the presence of anti-*B. afzelii* IgG antibodies in 3.3% of patients and blood donors [6]. Since prevalence of Lyme disease is highly dependent on the abundance of its vector, the prevalence is

expected to be higher in communities living in forested or semi-forested areas where ticks are abundant.

Ticks propagation is highly influenced by factors such as abundance of hosts, environmental temperature and environmental humidity. The abundant wildlife and the tropical climate of Malaysian forests are optimal for ticks [7]. Of the various species of ticks found in the Malaysian forests, the tick species capable of acting as a vector for Lyme disease, *Ixodes granulatus*, has previously been reported in various location across Peninsular Malaysia (Figure 1) [8-13].

The indigenous people of the Peninsular Malaysia, locally known as the Orang Asli (OA), form a minority population which comprises of only 0.58% of the total population of Malaysia [14]. Most Orang Asli reside in OA settlements which are often located in the forest or forest-fringe areas. The OA depend significantly on surrounding forests where they forage for wild fruits, ornamental plants, and wood products, and hunt wild animals as a source of income and food. These activities predispose them to tick bites and increase their risk for tick-borne diseases. Report of tick-borne diseases among these populations, however, has been scarce. Their remote locations and lack of appreciation of tick-borne diseases could be among the reasons. In the present study, we investigated the seroprevalence of *B. burgdorferi* among this unique community and identify demographic variables that could influence the seroprevalence.

Figure 1. Participating OA villages and locations with known presence of *I. granulatus*.



Methodology

Study Area and Sample Collection

The 16 OA villages from 8 states in Peninsular Malaysia were approved by JAKOA as study sites. These villages were Lubuk Legong (LL), Sungai Perah (SP), Tumboh Hangat (TH), Jekjok (JJ), Sangwai (SW), Paya Pelong (PP), Paya Sendayan (PS), Donglai Baru (DB), Paya Lebar (PL), Dusun Kubur (DK), Ulu Kelaka (UK), Bukit Payung (BP), Bukit Sebang (BS), Pasir Intan (PI), Semanggar (SM) and Sungai Selangi (SS) (Figure 1). Sampling activities conducted from July 2012 to June 2013 at the study sites resulted in 945 blood samples from healthy OA volunteers aged 5-years of age and older. Approximately 5 to 10ml of blood was withdrawn from each participant through venipuncture using blood tubes with clot activator. The blood samples were centrifuged to separate serum from blood cells before the sera were aliquoted into storage tubes. All samples were stored at -80°C prior to analysis. Adult volunteers were also given a questionnaire asking for their basic personal data, including marital status, education level, monthly household income, household size and occupation.

Detection of anti-B. burgdorferi IgG antibodies

The presence of IgG antibodies reactive against *B. burgdorferi* in serum samples was determined using the NovaLisa *Borrelia burgdorferi* IgG – ELISA (recombinant) kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). All procedures were performed strictly following the protocol provided by the manufacturer.

Statistical analysis

ELISA results and questionnaire data were analysed using the Statistical Package for the Social Sciences (SPSS) v24 (IBM Corp., New York, USA). Binary logistic regression was used to test the association between *B. burgdorferi* seropositivity, the dependant variable, with age and education level, gender, monthly household income, household size, marital status, village of residence and occupation as independent variables. Samples with equivocal results were excluded. Orang Asli villages with fewer than ten participants were excluded due to low participation rates.

Results

From the 16 approved study sites, 945 OA consented to participate in the study. Of the 16 study sites, BS and SS were excluded from the study due to the low participation. Samples with an insufficient

volume for analysis were also excluded, along with samples with incomplete demographic data or equivocal ELISA results. In total, 904 sample results from 14 villages were considered for the study (Table 1). The mean age of participants was 23.2 years old (standard deviation = 16.0, range = 5 - 83) and 385 (42.6%) of them were male. Serological detection performed found positive reaction for anti-*B. burgdorferi* IgG antibodies in 73 (8.1%) of the serum samples. Among the 904 participants, 381 adult participants from 13 OA villages responded to the questionnaire and were included in the following statistical analysis (Table 1). OA village (PP) was excluded due to low rate of questionnaire completion (N = 4, 16.7%). Most of the OA respondents (86.6%) were married with household size of 5.5 (range = 1 to 16) people on average. A large percentage of the OA (42.3%) received formal education up to the primary school level (primary education) and were earning less than RM500 (≤ USD 125) a month (66.9%). Most of the respondents were housewives (44.7%) followed by plantation workers (17.4%), odd-job workers (8.8%), students (4.4%), retirees & unemployed (1.8%) and others (8.6%). Some (14.3%) of the occupation were grouped as unknown because the respondent chose not to state their occupation in the questionnaire. Using marital status, household size, level of education, monthly household income, village of residence and occupation as independent variables, all variables tested were found not to be significant predictors ($p \geq 0.05$) for *B. burgdorferi* seropositivity except village of residence (Table 2). Among the villages, volunteers from TH (16.9%), LL (14.5%), SM (12.8%) and DK (12.2%) were found to have a high percentage of seropositivity (Table 1). Volunteers from TH were 1.65 times more likely to be seropositive as compared to other OA villages (TH vs non-TH).

Discussion

Based on the findings from this study, 8.1% (0.0% - 16.9%) of the recruited OA were reactive to the recombinant *B. burgdorferi* antigens. These results are comparable to findings from similarly conducted studies in China (2.9% - 14.9%) [15], India (9.1% -

Table 1. Seroprevalence of *B. burgdorferi* among the Orang Asli of Malaysia.

	Total participants, N = 904					**Questionnaire respondents, N = 381**					
State	Village of residence	n	Male (%)	Age, mean (SD)	*B. burgdorferi* IgG positive, n (%)	Respondent, n (%)	Education level[a] (%)	Monthly household income[a], MYR (%)	Married, n (%)	Household size, mean (range)	*B. burgdorferi* IgG positive, n (%)
Kedah	LL	83	42 (50.6)	26.3 (14.1)	12 (14.5)	51 (61.4)	Primary school (45.1)	< 500 (94.1)	42 (82.4)	5.5 (1-13)	7 (13.7)
Perak	SP	92	36 (39.1)	28.4 (16.7)	6 (6.5)	44 (47.8)	Primary school (43.2)	500 - 1000 (54.5)	38 (86.4)	4.7 (2-10)	2 (4.5)
	TH	89	40 (44.9)	22.7 (15.2)	15 (16.9)	45 (50.6)	Primary school (53.3)	500 - 1000 (51.1)	34 (75.6)	5.8 (2-11)	7 (15.6)
Kelantan	JJ	69	26 (37.7)	21.2 (15.3)	3 (4.3)	31 (44.9)	Secondary school (61.3)	< 500 (64.5)	30 (96.8)	5.5 (2-12)	1 (3.2)
	SW	105	42 (40.0)	19.6 (15.2)	3 (2.9)	34 (32.4)	No formal education (52.9)	< 500 (85.3)	31 (91.2)	5.9 (3-13)	1 (2.9)
Pahang	PP	24	10 (41.7)	16.0 (14.8)	1 (4.2)	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]
	PS	97	46 (47.4)	17.0 (11.6)	7 (7.2)	26 (26.8)	Primary school (53.8)	< 500 (76.9)	21 (80.8)	5.8 (2-9)	1 (3.8)
Selangor	DB	46	21 (45.7)	22.1 (19.0)	1 (2.2)	15 (32.6)	No formal education (66.7)	< 500 (80.0)	12 (80.0)	5.9 (2-10)	0 (0.0)
	PL	47	18 (38.3)	26.1 (15.6)	3 (6.4)	26 (55.3)	Primary school (46.2)	< 1000 (92.4)[c]	22 (84.6)	6.1 (3-11)	3 (11.5)
Negeri Sembilan	DK	123	55 (44.7)	23.1 (17.3)	15 (12.2)	42 (34.1)	Primary school (54.8)	< 500 (66.7)	37 (88.1)	5.7 (1-16)	5 (11.9)
	UK	51	21 (41.2)	23.3 (15.5)	2 (3.9)	25 (49.0)	Primary school (44.0)	< 500 (68.0)	24 (96.0)	5.5 (1-11)	2 (8.0)
Melaka	BP	20	4 (20.0)	27.1 (15.7)	0 (0.0)	15 (75.0)	Primary school (53.3)	< 500 (80.0)	15 (100.0)	5.2 (3-8)	0 (0.0)
Johor	PI	19	7 (36.8)	29.2 (14.6)	0 (0.0)	11 (57.9)	No formal education (45.5)	< 500 (63.6)	9 (81.8)	4.2 (1-7)	0 (0.0)
	SM	39	17 (43.6)	31.5 (19.4)	5 (12.8)	16 (41.0)	No formal education (43.8)	< 500 (68.8)	15 (93.8)	5.3 (3-7)	1 (6.3)
Total		**904**	**385 (42.6)**	**23.2 (16.0)**	**73 (8.1)**	**381 (42.1)**	**Primary school (42.3)**	**< 500 (66.9)**	**330 (86.6)**	**5.5 (1-16)**	**30 (7.9)**

[a]Education level and Monthly household income are both categorical variable with more than 2 levels. Categories with highest percentage are displayed; [b]Not included in the analysis due to low number of respondents (N = 4); [c]The percentage of OA with annual household income of ''< 500'' and ''500–1000'' are the same (46.2%).

Table 2. Univariate analysis on predictors for *B. burgdorferi* seropositivity.

Variables	N	*p*
Age	904	0.804
Gender	904	0.227
Education level	381	0.531
Household income	381	0.740
Household size	381	0.419
Marital status	381	0.993
Village	904	**0.045**
Occupation	381	0.166

Significant p-value < 0.05 is bolded.

17.8%) [16] and Mongolia (1.9% - 14.0%) [17]. It was, however, lower in comparison to the seroprevalence studies conducted in selected-populations from Poland [18] and Sweden [19] where anti-*B. burgdorferi* IgG antibodies were found to range from 13.7% to 25.7%. The observed inter-study differences in the seroprevalence could be attributed to a number of factors, foremost is the risk of contact with the disease vector [20]. The risk of contact, in turn, is influenced by the abundance of disease vectors in the area and activity pattern of the human host. Geographical and climatic factors such as abundance of animal hosts [21], temperature and humidity [22] significantly influence the abundance of ticks in an area. At the same time, outdoor activities, such as farming or foraging in forested areas, can increase the risk of tick-bites and the potential for exposure to *B. burgdorferi*. Occupation, the independent variable describing daily activities of the OA was, however, found to be statistically insignificant as a predictor for *B. burgdorferi* seropositivity (Table 2) despite some among the occupations described frequent plantations and forests (i.e. plantation workers and odd-job workers). The outcome of the statistical analysis might be caused by lack of sufficient statistical power in this study. Less than half (n = 381, 42.1%) of the volunteers responded to the questionnaires and only a small percentage (n = 73, 8.1%) were tested positive for *B. burgdorferi* IgG antibodies. The three independent variables not affected by the low questionnaire completion rate, age, gender and village of residence, were analysed separately to allow the full use of data from 904 volunteers. In addition to these real epidemiological factors, some of the inter-study variability in seroprevalence may also be a function of the specificity of assays used across the different studies.

Lyme disease is predominantly caused by *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii*. However, more than 20 new genospecies in *B. burgdorferi s.l.* complex have been discovered over the past decades and seven of them (*B. bavariensis* [23,24], *B. bissettii*, *B. kurtenbachii* [25], *B. lusitaniae*, *B. mayonii* [26], *B. spielmanii* and *B. valaisiana*) have exhibited evidence of being able to cause human infections [27]. *B. burgdorferi sensu stricto*,

B. garinii, *B. afzelii*, *B. bavariensis* and *B. valaisiana* were among the *borrelia* species found in circulating in China [28], Japan [29], Korea [8] and Taiwan [30]. The discovery of the new genospecies revealed a higher genomic variation among genospecies in *B. burgdorferi s.l.* complex which could potentially affect the accuracy of serological tests. Most of the existing serological tests however, were designed and formulated without being validated using the new genospecies. It is unknown if the serological tests can detect new Lyme disease causing genospecies or cross-react with non-Lyme causing genospecies [31]. Existing detection or diagnostic assays should be validated against all reported genospecies in *B. burgdorferi s.l.* complex to determine sensitivity and specificity of the assays. In addition, the ELISA kit used may produced false-positive result due to cross-reactivity with *Treponema pallidum*, the causative agent for syphilis. Seroprevalence of syphilis among OA population remained unknown due to lack of serological data. As such, the extent of cross-reactivity caused by anti-*Treponema pallidum* IgG antibodies cannot be determined at this point.

Ultimately, clearly defining the true risk of *B. burgdorferi* exposure across regions and populations will require well-designed epidemiological studies with agreed upon standardized assays that take into account the environmental and behavioural variables outlined above.

Conclusion

The study described the seroprevalence of *B. burgdorferi* among OA of Peninsular Malaysia at 8.1%. Village of residence was the only significant predictor for seropositivity. OA from TH village were most likely to be seropositive for *B. burgdorferi*. The distribution of *B. burgdorferi s.l.* genospecies especially among the TH population requires further investigation.

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Ethics Statement

The study protocol was approved by the Medical Ethics Committee of the University of Malaya Medical Centre, Malaysia (MEC Ref. No: 824.11). Permission was also obtained from the Department of Orang Asli Development (JAKOA) (reference number: JHEOA.PP.30.052 Jld. 6 (19)), a department under the Malaysian Ministry of Rural and Regional Development entrusted to oversee the affairs of the OA. Subjects or legal guardians of the subject consented to participate in this study.

Author Disclosure Statement

The authors declare that they have no competing interests. BLP is a U.S. military service member. This work was prepared as part of his official duties. The opinions and assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Department of the Navy, Department of Defense, or the U.S. Government. Furthermore, Title 17 U.S.C. §105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

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