LEISHMANIASIS IN PENINSULAR MALAYSIA: THE ROLE OF IMMIGRANT WORKERS AND THE VECTOR

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Abstract. Visceral leishmaniasis is considered an emerging disease of public health concern in Malaysia, especially with the influx of immigrant workers from endemic countries. This concern was strengthened by the existence of the vectors, which were found in abundance in the country. The aims of this study were to investigate the seroprevalence of leishmaniasis among the workers and the potential for transmission of the disease to local people. Blood samples were collected randomly from registered immigrant workers and in parallel with the collection of the sand flies using CO_2 baited CDC light trap. Seroprevalence of leishmaniasis was determine by ELISA method; meanwhile, PCR- based method was used to detect the partial leishmanial ribosomal SSU rRNA gene in the sand flies. It was found that using a test kit, 55.3% were seropositive, with the highest was among the Nepalese (68.6%), followed by Indians (62.2%), Bangladeshi (54.9%), Myanmar (44.4%), Vietnamese (25.8%), and Indonesian (25.6%). A total of 1,218 sand flies were caught and microscopically identified, and it was found that 981 were *Phlebotomus* spp and 237 were *Sergentomyia* spp. None of the sandflies were positive for *Leishmania* spp by both microscopic examination and PCR. Our study showed that the seroprevalence of leishmaniasis among the immigrant workers was relatively high, although it was negative for the vectors.

Keywords: leishmaniasis, immigrant workers, sandflies, CDC-light trap, seroprevalence, Malaysia

INTRODUCTION

Leishmaniasis is a zoonosis and an arthropod-borne disease transmitted between vertebrate host by Phlebotomine and Lutzomyia sand flies (Kamhawi, 2006; Bates *et al*, 2015). Leishmaniasis, the term used for the disease cause by the protozoan parasite *Leishmania*, which can be categorized by two types of diseases: a cutaneous (skin) reaction and a visceral (abdominal organ) reaction. The parasites responsible for cutaneous leishmanisis are *Leishmania major*, *L. tropica*, *L. aethiopica*, and *L. chagasi*; and for visceral leishmaniasis, the common parasites are *L. donovani* and *L. infantum*. Infection can be acquired when sand flies transmit the flagellated parasites into the skin of a host. The incubation period from infection to

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symptoms is generally from one month to several years.

The leishmaniasis has occured in tropical and subtropical countries, with cases of visceral (more than 300,000) and cutaneous (more than 1 million) leishmaniasis reported worldwide, and fatality cases have increased by 10-20% (WHO, 2010; Ready, 2014). Although visceral leishmaniasis topped with 90% of the cases occurred in India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil, cutaneous leishmaniasis was more widely distributed in 3 other regions which are the Americas, Mediterranean basin and Western Asia (from Middle East to Central Asia) (Alvar *et al*, 2012).

Southeast Asian countries (Cambodia, East Timor, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam) have been known to be non-endemic countries for leishmaniasis. However, with improvement of air travelling and human migration all over the world, the disease could be transmitted from one country to another. Today, leishmaniasis is considered to be an emerging infection across Southeast Asian countries (Viroj, 2010). Cases of leishmaniais occurring among the Southeast Asian countries where mixed cases of indigenous or imported, either cases of immigrants from endemic areas or travellers from non-endemic countries who travelled to the endemic countries (Hamidah *et al*, 1995; Thisyakorn *et al*, 1999; Lew *et al*, 2007; Kashfi *et al*, 2011; Wiwanitkit, 2011; Pothirat *et al*, 2014; Siriyasatien *et al*, 2016).

In the Malaysian situation, the perceptible growth of the economics of the country since the seventies has created a regular and increasing demand for foreign workers in all sectors of the economy. Therefore, the employment of foreign workers in Malaysia has now become a regular phenomenon. There are more than 7 million foreign workers in Malaysia, including legal and illegal workers from Indonesia, Thailand, Bangladesh, Philippines, India, Nepal, Myanmar and other countries. Most of the foreign workers are concentrated in agriculture, construction, and manufacturing sectors. The recent influx of immigrant workers has raised many issues and implications including the transmission of communicable diseases.

Being a tropical country, Malaysia also rich with flora and fauna where some of the infectious disease vector can flourish favorably, such as the mosquitoes, acari, flies, and sand flies. However, the sand fly fauna of Malaysia is poorly known. It has been reported that 22 species and subspecies are found in Malaysia. Of these, 16 belong to the genus *Sergentomyia*, 4 to *Phlebotomus*, and 2 to *Idiophlebotomus*. *Phlebotomus argentipes, P. bêtise* and *P. kiangsuensis* have been observed feeding on man (Rudnick, 1971). Phlebotomine sand flies from the genera *Sergentomyia* and *Phlebotomus* were abundant in Malaysia, especially in limestone and cave areas (Khadri *et al*, 2008; Shahar *et al*, 2011). Therefore, the objective of this study was to estimate the prevalence of leishmaniasis among the immigrant workers and to establish the risk of transmission to the local population.

MATERIALS AND METHODS

Study sites

The study was carried out among the asymptomatic immigrant workers working in the construction, manufacturing, farming, and agricultural sectors in selected areas in Peninsular Malaysia. Ten districts from seven states in Peninsular Malaysia

Fig 1–Map of Malaysia showing study sites.

were selected based on the presence of immigrant workers and the existence of sandflies. The states (districts) were Pahang (Lipis), Kelantan (Gua Musang), Terengganu (Kuala Terengganu, Marang and Kemaman), Perak (Kinta), Selangor (Klang and Kuala Kubu Baru), Johor (Johor Bahru) and Perlis (Kangar) (Fig 1).

Blood collections

About 50 µl-500 µl blood were taken from finger pricking using sterile lancet in accordance with hospital laboratory procedure among consenting volunteers. Blood samples were collected into 0.5-3 ml microtainer tubes. The serums was collected by centrifugation of the blood at 3-4,000 rpm for 3-5 minutess. Serum was transfered into a collection tube, labeled with a registration number and date, and kept in portable liquid nitrogen tank for temporary storage, prior to transfer to the Institute for Medical Research for analysis. From the same prick, blood film for leishmania or other parasites was also made.

Sandflies collection, identification and dissection

Sand flies were caught in the same district as the blood collections, and the period of collection also approximately as same as blood collections. The sand fly traps were placed overnight over extended hours using uniform trapping methods in a limestone areas, construction sites, near the coastal areas, or recreational areas. The sand flies were trapped using the CDC-light trap with dry ice as a source of carbon dioxide (CO_2). The traps were

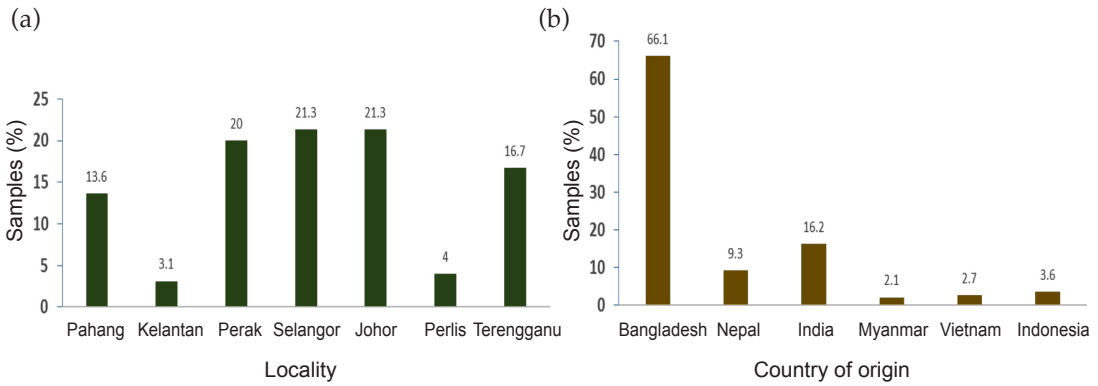


Fig 2–Percentage of blood / serum samples collected for leishmaniasis seroprevalece among the states where the migrants reside (a) and their country of origin (b).

set-up in the evening (started at 06.00 PM until 07.00 PM) and left overnight. Collection of the trapped sand flies were done as early as 06.00 AM in the morning. The sand flies were brought back to the temporary lab in the field for identification and dissected for promastigote stages.

Serological test

Enzyme link immunoassay for the qualitative determination of antibodies against *Leishmania* in human serum was carried out using the RIDASCREEN® Leishmania Ab (K7121) Test kit (R-Biopharm AG, Darmstadt, Germany). The procedure employed to carry out the test was strictly followed as provided in the manual by the manufacturer. The absorbance was determined using the photometric machine, or ELISA reader, (OPSYS MR™ Microplate Reader; Dynex Technologies, Chantilly, VA), at 450nm.

Determination of DNA of *Leishmania* spp from sand flies

Collected sand flies were grouped 2 to 10 sand flies / group according to location of collection. Extraction of *Leishmania* spp DNA from sand flies were carried out using DNeasy ®Blood / Tissues DNA Extraction Kit (Qiagen™, Hilden, Germany). Procedure for DNA extraction were strictly followed as provided by the manufacture. Polymerase chain reaction (PCR) was carried out using a published primers R221 and R332 and are *Leishmania*-specific and amplify a region of the 18 SSU rRNA gene, generating a product of 603 bp (van Eys Guillaume *et al*, 1992).

Statistical analysis

Data were stored in Excel 2003 (Microsoft, Armonk, NY) and tabulated in SPSS 11.0 Categorical variables were analysed with the χ^2 with significance set at $p<0.05$.

Ethical considerations

The Medical Research Ethic Committee granted ethical approval for this research.

RESULTS

Sample collection

A total of 2,153 blood / serum samples were collected from migrant workers located in seven states of peninsular Malaysia, with highest contributions from Selangor and Johor States (21.3% each) (Fig 2a). Of 2,153 samples collected, 1,422 were from Bangladeshi workers and the fewest samples, 45 (2.1%), were from Myanmar (Fig 2b).

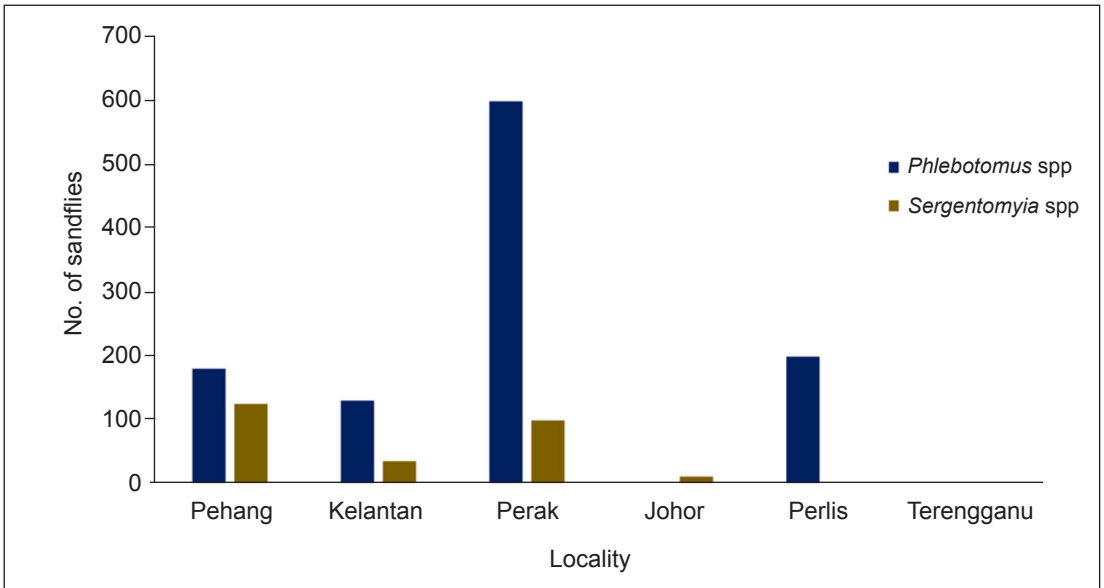
Table 1
Seroprevalence of leishmanaisis among migrant workers according to country of origin and locality of samples collected.

	ELISA serology		Total (%)
	Positive (%)	Negative (%)	
(a) **Ethnic group**			
Bangladeshi	780 (54.9) (65.5)*	642 (45.1) (66.7)*	1,422 (100.0) (66.1)*
Nepalese	138 (68.6) (11.6)*	63 (31.4) (6.5)*	201 (100.0) (9.3)*
Indian	217 (62.2) (18.2)*	132 (37.8) (13.7)*	349 (100.0) (16.2)*
Myanmar	20 (44.4) (1.7)*	25 (55.6) (2.6)*	45 (100.0) (2.1)*
Vietnamese	15 (25.8) (1.3)*	43 (74.2) (4.5)*	58 (100.0) (2.7)*
Indonesian	20 (25.6) (1.7)	58 (74.4) (6.0)*	78 (100.0) (3.6)*
Total	1,190 (55.3) (100.0)*	963 (44.7) (100.0)*	2,153 (100.0) (100.0)*
(b) **Locality**			
Pahang	149 (50.9) (12.5)*	144 (49.1) (15.0)*	293 (100.0) (13.6)
Kelantan	28 (42.4) (2.4)*	38 (57.6) (3.9)*	66 (100.0) (3.1)
Perak	251 (58.5) (21.1)*	178 (41.5) (18.5)*	429 (100.0) (20.0)
Selangor	296 (64.5) (24.9)*	163 (35.5) (17.0)*	459 (100.0) (21.3)
Johor	223 (48.6) (18.7)*	236 (51.4) (24.5)*	459 (100.0) (21.3)
Perlis	26 (29.9) (2.2)*	61 (70.1) (6.3)*	87 (100.0) (4.0)
Terengganu	217 (60.3) (18.2)*	143 (39.7) (14.8)*	360 (100.0) (16.7)
Total	1,190 (55.3) (100.0)*	963 (44.7) (100.0)*	2,153 (100.0) (100.0)*

*Indicate % calculated by column.

Detection of leishmaniasis by microscopy and serology

Samples were microscopically identified and detection of antibodies was performed using the commercial test kit. All samples were microscopically negative, 1,190/2,153 (55.3%) samples were seropositive and 63/2,153 (44.7%) were



Locality	*Phlebotomus* spp			*Sergentomyia* spp		
	Male	Female	Total	Male	Femal	Total
Pehang	63	118	181	40	86	126
Kelantan	56	74	130	15	20	35
Perak	>300	>300	>600	>50	>50	>100
Johor	0	0	0	0	11	11
Perlis	>100	>100	>200	0	0	0
Terengganu	0	0	0	0	0	0
Total	>500	>500	>1,000	>100	>100	>200

Fig 3–Bar-chart indicats sandflies trapped. Table shows estimated number of sandflies catched.

negative. The highest positivities were among the Nepalese workers (68.6%), followed by the Indians (62.2%), Bangladeshi (54.9%), Myanmar (44.4%), Vietnamese (25.8%), and Indonesians (25.6%) (Table 1a). Chi-square test shows there was an association between seropositivity and ethnicity, except the Vietnamese.

Leishmaniasis infection in sandflies by microscopy and PCR

Sandflies were trapped using CDC-light trap, which used dry ice as a sources of carbon dioxide (CO_2). The traps were set-up in the evening (started at 06.00-07.00 PM) and left overnight in the same in selected areas as blood / serum collection. Collection of the trapped sand flies were done as early as 06.00 AM in the morning. The sand flies were brought back to the temporary lab in the field for identification and dissection for promastigote stages. From the total collections of the sandflies, 981 of *Phlebotomus* spp and 237 of *Sergentomyia* spp were identified. Highest catches were from Perak State (Fig 3).

None of the sandflies were positive, either by microscopy or PCR.

DISCUSSION

Prior research has documented the emergence of leishmaniasis in Southeast Asian countries, although this region is considered to be non-endemic for the disease (WHO, 2016). Although it was not endemic, it should not be neglected, and this should be given a serious attention as many reports of leishmaniasis have emerged every year (Hamidah *et al*, 1995; Abraham *et al*, 1997; Thisyakorn *et al*, 1999; Lew *et al*, 2007; Suankratay *et al*, 2010; Kashfi *et al*, 2011). This might be due to the increased rate of travelling to endemic countries and migration of immigrants from endemic countries as well as tourist and education tourist (from African, Middle-East, China, and so forth). However, most of the cases reported were imported cases among the immigrant workers from Bangladesh and Nepal, or there were some cases among the local people but have the history of travelling to the endemic country (Viroj, 2010).

Leishmaniasis can also be considered as an emerging disease among the travellers (Pavli and Maltezou, 2010). Fortunately not many indigenous cases were reported and some cases shown to be co-exist with other diseases such as malaria and HIV. Abraham *et al* (1997) and Kashfi *et al* (2011), for example, reported that visceral leishmaniasis were detected in Nepalese workers in Malaysia and Bangladeshi workers in Singapore and indigenous cases of visceral and cutaneous leishmaniasis were also reported in Thailand (Thisyakorn *et al*, 1999; Suankratay *et al*, 2010).

In Malaysia, immigrant workers are from Bangladesh, Nepal, India, Indonesia and small numbers from African countries. As a reference, cases of visceral leishmaniasis have been reported since 1820s in the Indian subcontinent (Gibson, 1983). Although visceral leishmaniasis was nearly eliminated in this region in the 1960s; since then, the parasite reshaping the genome to survive and exist until today (Imamura *et al*, 2016). Nepal, Bangladesh, Iran, Brazil and many other countries have managed to establish studies to determine the epidemiology, prevalence, and risk factors of the disease (Addy and Nandy, 1992; Evans *et al*, 1992; Jeronimo *et al*, 1994; Garg *et al*, 2001; Ahluwalia *et al*, 2003; Amusategui *et al*, 2004; Bern *et al*, 2005; Berman, 2006; Schenkel *et al*, 2006; Maia-Elkhoury *et al*, 2008; Rijal *et al*, 2010; Das *et al*, 2014; Picado *et al*, 2014).

It is worrying that most of the immigrant workers in Malaysia are from this region, and this concern is based on the experiences of our neighboring country, Thailand, where the vector has already been established, and they also found new species of leishmania which are *Leishmania siamensis* and *L. martiniquensis* contributed to the cases in Thailand (Suankratay *et al*, 2010; Bualert *et al*, 2012).

In this study we established a baseline data on the seroprevalence of leishmaniasis among the immigrant workers in Malaysia, and we extend the research on its potential vectors contributing to the transmission of the disease. In our studies, we found that, seroprevalence of leishmaniasis tested among the immigrant workers in selected areas in peninsular Malaysia were highest from the Nepalese ethnicity followed by the Bangladeshi and the Indian and the lowest were from the Indonesian. Some of the positive cases have a very high titer, however during blood sampling, the workers were healthy with no signs and symptoms of visceral

or cutaneous leishmaniasis. The results might indicate that the high serum titer among the immigrant workers was acquired in the past. This is worrying because Nepal, Bangladesh, and India are regarded as countries with higher cases of leishmaniasis (Alvar *et al*, 2012).

Natural infection of sand flies with *leishmania* spp has been reported worldwide. In Nepal, where visceral leishmaniasis is endemic, *Phelebotomus argentipes* is responsible for the transmission of the disease (Pandey *et al*, 2008; Bhattarai *et al*, 2009). While *P. ariasi*, *P. perniciosus,* and *Sergentomyia minuta* have been reported in eastern France (Rioux *et al*, 2013), *P. longicuspis* and *P. sergenti* in northern Morrocco (Es-Sette *et al*, 2014), *Lutzomyia ayacuchensis* in Ecuador (Kato *et al*, 2005), *P. longiductus*, *P. wui* and *P. chinensis* in China (Zhang and Leng, 1997; Guan *et al*, 2000) and *P. argentipes*, *P. major,* and *Sergentomyia (Neophlebotomus) gemmea* in Thailand (Suankratay *et al*, 2010, 2014).

In this study, due to the concern of the potential transmission among the local population, the vector identification was studied in parallel with the blood survey. Sandflies of *Phlebotomine* and *Sergentomyia* genera were identified. However, PCR and microscopic examination showed none of the sandflies were positive with leishmania. Presently, there is no evidence of local transmission even though the suitable vectors are present in Malaysia.

Our finding only focused on a small scale of survey among the immigrant and the vectors compared to the real numbers of immigrants and vectors. We believe, not only will leishmaniasis be introduced to Malaysia, but other emerging infectious disease as well. A thorough understanding of the transmission mechanism of any infectious agent is crucial to implementing an effective intervention strategy. Detection and identification of *Leishmania* spp in naturally infected sand flies is important for prediction of the risk and expansion of the disease.

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