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Entamoeba infections and associated risk factors among migrant workers in Peninsular Malaysia

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Abstract. The influx of low skilled migrant workers to Malaysia from low socio-economic countries where gastrointestinal parasitic infections are prevalent has raised concerns about transmission to the local population. Three methods for detection (serology, microscopy and molecular techniques) were utilized to identify *Entamoeba* infections amongst the targeted cohort and determine risk factors associated with infection. Serological screening of 484 migrant workers from five working sectors in Peninsular Malaysia using IgG4 ELISA based on the rPPDK antigen showed an overall seroprevalence of 7.4% (n = 36; CL95 = 5.3–10.1%) with only one factor statistically associated with seropositivity of anti-amoebic antibodies, i.e. years of residence in Malaysia ($\chi^2_1 = 4.007$, $p = 0.045$). Microscopic examination of 388 faecal samples for protozoan cysts and trophozoites showed a slightly higher prevalence (11.6%; n=45; CL95: 8.4–14.8%). Meanwhile, amplification of the 16S rDNA gene detected two species i.e. *Entamoeba dispar* (23/388; 5.9%; CL95: 3.6–8.3%) and *E. histolytica* (11/388; 2.8%; CL95: 1.2–4.5%) and mixed infections with both parasites in only three samples (3/388; 0.8%; CL95: 0.2–2.2%). *Entamoeba dispar* infection was significantly associated with those employed in food and domestic services ($\chi^2_4 = 12.879$, $p = 0.012$). However, none of the factors affected the prevalence of *E. histolytica* infection. Despite the low prevalence of *E. histolytica* in faecal samples of the study cohort, the presence of this pathogenic parasite still poses potential public health risks and calls for tighter control strategies based on better availability of chemotherapeutic treatment and accessibility to appropriate health education.

INTRODUCTION

Human intestinal protozoan infections by *Entamoeba* spp. have been reported worldwide (Norhayati *et al.*, 2003; Ngui *et al.*, 2011) and in extreme cases, can affect up to 50% of the population particularly in areas of Central and South America, Africa, and Asia (Tengku & Norhayati, 2011). Approximately 50 million cases of invasive disease caused by *E. histolytica* have been estimated to occur worldwide each year (World Health Organization, 1997). Six species (*Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *E. coli*, *E. hartmanni* and *E. polecki*) (Tengku & Norhayati, 2011; Ngui *et al.*, 2012) are known to reside in the human gastrointestinal tract, although only *E. histolytica* is considered to be the etiological agent of human amoebiasis. The other species are commensals and cause no harm to humans (Ngui *et al.*, 2012). Transmission of all six species is primarily through person-to person contact, although contaminated water also plays an important role. Subjects infected with *E. histolytica* develop colitis and/or additional intestinal pathologies, and collectively 40,000–110,000 deaths/annum

are attributable to this species (Hopkins, 1992).

The standard method for diagnosis is through microscopic fecal examination, however the cysts and trophozoites of *Entamoeba* species are morphologically very similar to each other and indistinguishable in the case of the pathogenic *E. histolytica* and the non-pathogenic *E. dispar* and *E. moshkovskii* (Ngui *et al.*, 2012). In order to avoid unnecessary treatment and to have a better understanding of the epidemiology of these parasites in the human population it is important to be able to reliably distinguish infections with *E. histolytica* from those caused by the other non-pathogenic species (Ngui *et al.*, 2012). Methods for the specific detection of *E. histolytica* in stool samples include the enzyme-linked immunosorbent assay (ELISA) targeting species specific copro-antigens, and for these several commercial ELISA kits are available enabling differentiation between *E. histolytica* and *E. dispar* in stool samples (Garcia *et al.*, 2000). Tests based on the detection of parasite specific antibody in serological samples are also informative about exposure to different species of *Entamoeba*, but these generally reflect previous exposure to infection and not necessarily current infection. Perhaps the most sensitive method for detection of current infection is the polymerase chain reaction (PCR) which enables amplification of DNA in stool samples using primers for informative segments of genes whose sequences differ between species, thereby enabling subsequent identification via product size on gels (if different between species) and/or sequencing of the amplicons (Khairnar & Parija, 2007).

Malaysia emerged as a multi-sector economy in the 1970s attracting multinational workers from neighbouring countries (i.e., Indonesia, Bangladesh, Nepal, Myanmar, India and Vietnam) (Bardan, 2014; Ministry of Human Resources Malaysia, 2015). Its growth has been largely facilitated through the influx of low-skilled migrant workers, however there is concern that infectious diseases endemic in neighbouring countries may also be inadvertently imported. Despite compulsory medical screening of foreign workers prior to entering the Malaysian workforce, screening for parasitic infections particularly amoebiasis is lacking. In view of the considerable large numbers of workers employed particularly in the food and domestic services, transmission of amoebiasis may also be facilitated which is likely to have a significant impact upon the local community.

Although many studies have been conducted already in Malaysia through conventional microscopy, ELISA and PCR and primarily among the rural communities (Tengku & Norhayati, 2011), this study is the first to combine microscopy with serology and molecular methods among migrant workers in Malaysia. This three-pronged approach has enabled us to provide more precise information on the prevalence of this parasite among low-skilled migrant population in the country and to differentiate between the *Entamoeba* species, as well as to identify the risk factors associated with disease transmission.

MATERIALS AND METHODS

Recruitment of study participants and data collection

Low and semi-skilled migrant workers employed in Malaysia are only allowed to serve in five working sectors namely; manufacturing, food services, construction, domestic services, agriculture and plantation. Participants were recruited voluntarily from various agencies and companies from September 2014 to August 2015. A minimum sample size (n=355) was calculated using a formula by Leedy and Ormrod (2001) based on earlier estimates of infection prevalence (36%) values in Malaysia (Suresh *et al.*, 2002). A total of 610 migrant workers from different categories were recruited.

Questionnaires were distributed to participants in order to gather relevant information related to the study. An individual clinical interview was performed in order to collect socio-demographic information, history of taking anthelminthic drugs and

symptoms of intestinal infection at the time or prior to sampling. It is possible that some workers may have experienced intestinal symptoms but chose not to disclose their condition due to the prospect of losing their job. The interview process was mediated through an interpreter in situations of difficulty in understanding either spoken Malay or English.

Ethical clearance and samples collection

All participants were fully informed of the nature of the study in order to enable maximum co-operation and completion of consent forms. Ethical clearance was obtained from the ethics committee, University Malaya Medical Centre (UMMC), Malaysia prior to commencement of the study (Reference number: MECID NO: 20143-40). All adults provided written, informed consent to participate in the study. All individual tested positive was notified of their condition through their respected employers.

Approximately 5 ml of venous blood were drawn into a plain tube (without anticoagulant) by trained medical assistants and nurses using disposable syringes and needles. The blood samples were transported to the Parasitology Laboratory, Institute of Biological Science, Faculty of Science, University of Malaya. Blood samples were spun at 1,500 x *g* for 10 minutes and the serum samples were kept in -20°C until use.

Each individual was provided with a plastic container marked with a specific identification number and the name of the participant. The participants were instructed to scoop a thumb size faecal sample into the container, ensuring that the sample was not contaminated with urine. All samples were preserved in 2.5% potassium dichromate solution and brought back to the laboratory at the Institute of Biological Science, Faculty of Science, University of Malaya.

Serological analysis

The assay was performed using a standard in-house protocol at Universiti Sains Malaysia. Briefly, wells of Nunc MaxiSorp microtitre plate (Nalge Nunc International, Rochester, NY) were coated with 100 µL of 10 µg/mL of recombinant *E. histolytica* pyruvate phosphate dikinase (rPPDK) as antigen in 0.06 M carbonate buffer, pH 9.6 and incubated overnight at 4°C. The next day, the wells were washed with phosphate buffered saline, pH 7.2 containing 0.05% Tween-20 (PBS-T), then blocked with 3% (w/v) bovine serum albumin (Sigma Aldrich Co, St. Louis, MO, USA) in PBS for 1 h at 37°C. Washings between the incubation steps were performed using PBS-T on a plate shaker (700 rpm). Serum samples were diluted at 1:100 in PBS, added in duplicate at 100 µL per well. They were incubated for 2 h at 37°C with shaking (300 rpm). A secondary antibody (mouse monoclonal anti-human IgG4 HRP; Millipore, Billerica, MA, USA) was diluted 1:4,000 in PBS and added for 30 min at 37°C, followed by incubation with ABTS substrate solution (Roche Diagnostics, Mannheim, Germany) at 100 µL per well for 30 min at 37°C. The optical density (OD) values of the wells were determined using a Thermo Multiskan Spectrum Reader (Multiskan Spectrum, Thermo Scientific, Rockford, IL, USA) at 405 nm and 490 nm. The cut-off value for the ELISA was OD 0.122, derived from the mean plus two standard deviations of OD values from testing the sera of 30 healthy individuals.

Microscopic examination of faecal samples

All the collected faecal samples were screened using the formalin-ether concentration technique. Approximately 1 to 2 g of sample were mixed with 7 ml of formalin and 3 ml ethyl acetate and centrifuged 2,500 × g for 5 minutes. After centrifugation, 4 layers were visible composed of ethyl acetate, debris, formalin and pellets containing parasites. A drop of pellet was taken and stained with Lugol's iodine on a clean glass slide. The slides were examined under a light microscope at 40X magnification for *Entamoeba* spp. Microscopical examination did not involve permanent staining, and instead molecular diagnosis was used for confirmation.

Molecular detection of faecal samples by nested PCR

Genomic DNA was extracted from all faecal samples collected using NucleoSpin® Soil kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's instructions. Approximately, 250-500 mg fresh sample material were transferred to a NucleoSpin® Bead Tube containing ceramic beads with the presence of cell lysis buffer provided by the manufacturer. To lyse the sample, the NucleoSpin® Bead Tubes was vortexed at full speed at room temperature for 5 minutes. The extracted DNA was stored at –20°C until used for PCR amplification.

Nested PCR targeting the 16S-like ribosomal RNA gene was used to genetically characterize *E. histolytica*, *E. dispar* and *E. moshkovskii* according to Que and Reed (1991). Primers E1 (5'- TAA GAT GCA GAG CGA AA -3') and E2 (5'- GTA CAA AGG GCA GGG ACG TA -3') were used for detection of the *Entamoeba* genus (Khairnar & Parija, 2007; Parija *et al.*, 2010; Ngui *et al.*, 2012). Nested PCR was performed in a Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology, Inc.) in 20 µl volume reaction. The reaction contained i-Taq™ DNA polymerase (5U/µl) (2.5U), deoxynucleoside triphosphate (dNTPs) (2.5 mM each), 1X reaction buffer (10x) and gel loading buffer (1x). DNA template (2 µl), primers (100 pM each) and distilled water were added to the premix. PCR cycling consisted of 96°C for 2 minutes, followed by 35 cycles of 92°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute 30 seconds and a final extension at 72°C for 7 minutes. Subsequently, 2 µl of the primary PCR products were subjected to secondary PCR for *Entamoeba* species-specific characterization using primer sets EH1 (5'-AAG CAT TGT TTC TAG ATC TGA G -3') and EH2 (5'- AAG AGG TCT AAC CGA AAT TAG - 3') to detect *E. histolytica* (439 bp); ED1 (5'-TCT AAT TTC GAT TAG AAC TCT -3') and ED2 (5'-TCC CTA CCTATT AGA CAT AGC - 3') to detect *E. dispar* (174 bp); Mos1 (5'-GAA ACC AAG AGT TTC ACA AC -3') and Mos2 (5'- CAA TAT AAG GCT TGG ATG AT - 3') to detect *E. moshkovskii* (553 bp) (Khairnar & Parija, 2007; Parija *et al.*, 2010; Ngui *et al.*, 2012). The cycling conditions for the second round of amplification were 96°C for 2 minutes, followed by 35 cycles at 92°C for 1 minute, 47°C for 1 minute and 72°C for 1 minute, and a final extension at 72°C for 7 minutes. PCR products were subjected to electrophoresis through 2% (w/v) agarose and visualized in a UV trans-illuminator after staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea). Positive and negative control reactions were included with each batch of samples analyzed and the final results were reported on the basis of species-specific product size (Khairnar & Parija, 2007; Parija *et al.*, 2010; Ngui *et al.*, 2012).

Statistical analysis

Prevalence data are shown with 95% confidence limits (CL_{95}) were analyzed using maximum likelihood techniques based on log linear analysis of contingency tables using the software package SPSS (Version 22). Infection was considered as a binary factor (presence/absence of parasites). Full factorial model was fitted with the intrinsic factors: sex (2 levels: males and females), age (5 age classes: those <25 years old, 25-34 years old, 35-44 years old, 45-54 years old and those >54 years) and nationality (5/6 countries: Indonesia, Bangladesh, Myanmar, India, Nepal and Vietnam). Other extrinsic factors analyzed include employment sector (5 sectors: construction, manufacture, plantation, food service and domestic), years of residence in Malaysia (2 categories: less than 1 year and more than 1 year), accommodation type (2 types: squatter and non-squatter) and level of education (4 levels: primary school, secondary school, university and no formal schooling).

RESULTS

Socio-demographic characteristics

A total of 610 participants were recruited of which, 484 (79.3%) provided blood and 388 (63.6%) faecal samples. Only 306 volunteers provided both blood and faecal samples (Figure 1).

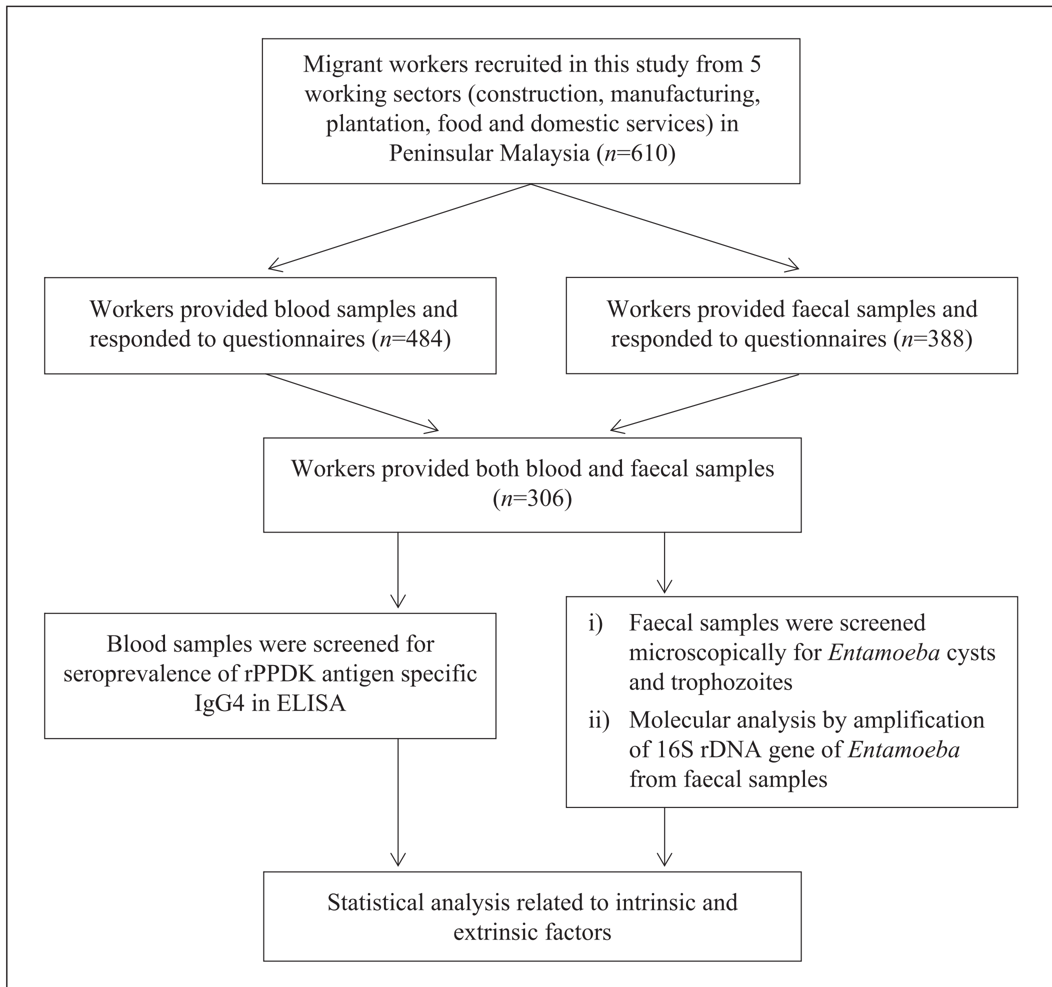

Figure 1. Sample collection and screening process among migrant workers from five working sectors in Peninsular Malaysia.

All blood donors originated from rural areas, with more men (*n*=375; 77.5%) and with the majority less than 45 years old (*n*=436; 90.1%). Slightly more than half were Indonesians (*n*=246; 50.8%) followed by Nepalese (*n*=103; 21.3%).

Slightly more than half who provided stool were Indonesian (*n*=167; 43.0%), men (*n*=304; 78.4%) and less than 35 years old (*n*=259; 66.8%). The socio-demographic details are as described in Table 1 and Table 2.

Seroprevalence of amoebiasis based on rPPDK-IgG4 ELISA

The overall seroprevalence of rPPDK antigen specific IgG4 in ELISA among 484 migrant workers was 7.4% (*n*=36; CL_{95}= 5.3–10.1%). Seropositivity of *Entamoeba* infection was analyzed statistically in relation to sociodemographic factors. In the minimum sufficient model identified by the backwards-stepwise selection procedure, the intrinsic factors including sex, age and nationality were not statistically associated with infection. Meanwhile, of the four extrinsic factors considered (employment sectors, years of residence in Malaysia, type of accommodation and level of education), only one factor i.e. years of residence in Malaysia ($\chi^2_1 = 4.007$, $p = 0.045$) (Table 1) was significantly associated with seropositivity of anti-amoebic antibodies.

Table 1. The prevalence for amoebiasis among migrant workers based on the IgG4-rPPDK ELISA relative to sex, age, nationality, employment sector, years of residence in Malaysia, type of accommodation and education attainment (n=484)

Factor(s)		rPPDK-ELISA	
		% [95% CL]	*P*-value
Intrinsic Factors			
Sex	Men (n=375)	7.7 [5.2-10.9]	0.641
	Women (n=109)	6.4 [2.6-12.8]	
Age class (years)	<25 (n=142)	4.9 [2.0-9.9]	0.143
	25-34 (n=183)	8.2 [4.7-13.2]	
	35-44 (n=111)	5.4 [2.0-11.4]	
	45-54 (n=35)	17.1 [6.6-33.6]	
	>55 (n=13)	15.4 [1.9-45.4]	
Nationality	Indonesia (n=246)	8.1 [5.0-12.3]	0.244
	Bangladesh (n=69)	11.6 [5.1-21.6]	
	Myanmar (n=14)	14.3 [1.8-42.8]	
	India (n=51)	5.9 [1.2-16.2]	
	Nepal (n=103)	2.9 [0.6-8.3]	
	Vietnam (n=1)	0.0 [0.0-0.0]	
Extrinsic Factors			
Employment Sector	Construction (n= 67)	10.4 [4.3-20.3]	0.244
	Manufacturing (n= 93)	3.2 [0.7-9.1]	
	Plantation (n=102)	5.9 [2.2-12.4]	
	Food service (n=116)	10.3 [5.5-17.4]	
	Domestic (n=106)	7.5 [3.3-14.3]	
Years of Residence*	< than 1 year (n=180)	4.4 [1.9-8.6]	0.045
	> than 1 year (n=304)	9.2 [6.2-13.0]	
Accommodation	Squatter (n=48)	10.4 [3.5-22.7]	0.430
	Non-squatter (n=436)	7.1 [4.9-9.9]	
Education attainment	Primary (n=227)	6.2 [3.4-10.1]	0.684
	Secondary (n=202)	7.9 [4.6-12.5]	
	University (n=10)	10.0 [0.3-44.5]	
	No formal schooling (n=45)	11.1 [3.7-24.1]	

* significant at 0.05.

Microscopy and molecular detection of *Entamoeba* spp.

Microscopical examination recorded 11.6% (n=45) positive samples with significantly more women infected compared to men ($\chi^2_1 = 5.2, p = 0.022$). Significant associations were also noted in relation to employment sectors ($\chi^2_4 = 23.2, p < 0.001$) and educational attainment ($\chi^2_3 = 18.8, p < 0.001$).

The 16S rDNA gene of *Entamoeba* was successfully amplified in 31/388 (8.0%) samples, resulting in the identification of *E. dispar* (23/388; 5.9%), *E. histolytica* (11/388; 2.8%) and mixed infections of *E. dispar* and *E. histolytica* (3/388; 0.8%). Only one factor affected the prevalence of *E. dispar* infection, i.e. employment sector, with infection being primarily associated with those working in food and domestic services ($\chi^2_4 = 12.879, p = 0.012$) (Table 2). Although not significant, the percentages of *E. histolytica* was observed higher among workers in domestic and manufacturing sectors and those age >55.

Summary of *Entamoeba* infections

From the 306 participants who provided both blood and fecal samples, only 4 serum samples were positive for IgG4 to the rPPDK antigen from 25 PCR positive faecal samples. In addition, 19 positive sera for IgG4 to the rPPDK antigen were recorded among the 272 microscopy negative samples (Table 3). Overall, *Entamoeba* positive participants

Table 2. The prevalence of *E. dispar* and *E. histolytica* infections, based on PCR for detection of species-specific rDNA, among migrant workers relative to sex, age, nationality, employment sector, years of residence in Malaysia and education attainment (*n*=388)

Factor(s)		*E. dispar*		*E. histolytica*	
		% [95% CL]	*P*-value	% [95% CL]	*P*-value
Intrinsic Factors					
Sex	Men (n=304)	4.9 [2.8-8.0]	0.135	2.3 [0.9-4.7]	0.258
	Women (n=84)	9.5 [4.2-17.9]		4.8 [1.3-11.7]	
Age class (years)	<25 (n=114)	5.3 [2.0-11.1]	0.499	3.5 [1.0-8.7]	0.624
	25-34 (n=145)	8.3 [4.3-14.0]		2.8 [0.8-6.9]	
	35-44 (n=90)	4.4 [1.2-11.0]		1.1 [0.0-6.0]	
	45-54 (n=29)	3.4 [0.1-17.8]		3.4 [0.1-17.8]	
	>55 (n=10)	0.0 [0.0-0.0]		10.0 [0.3-44.5]	
Nationality	Indonesia (n=167)	6.6 [3.3-11.5]	0.224	3.6 [1.3-7.7]	0.307
	Bangladesh (n=70)	1.4 [0.0-7.7]		0.0 [0.0-0.0]	
	Myanmar (n=23)	8.7 [1.1-28.0]		4.3 [0.1-21.9]	
	India (n=47)	10.6 [3.5-23.1]		2.1 [0.1-11.3]	
	Nepal (n=81)	4.9 [1.4-12.2]		3.7 [0.8-10.4]	
Extrinsic Factors					
Employment Sector*	Construction (n= 47)	0.0 [0.0-0.0]	0.012	0.0 [0.0-0.0]	0.225
	Manufacturing (n= 61)	4.9 [1.0-13.7]		4.9 [1.0-13.7]	
	Plantation (n=71)	1.4 [0.0-7.6]		1.4 [0.0-7.6]	
	Food service (n=104)	9.6 [4.7-17.0]		1.9 [0.2-6.8]	
	Domestic (n=105)	8.6 [4.0-15.6]		4.8 [1.6-10.8]	
Years of Residence	< than 1 year (n=134)	5.2 [2.1-10.5]	0.667	3.7 [1.2-8.5]	0.448
	> than 1 year (n=254)	6.3 [3.6-10.0]		2.4 [0.9-5.1]	
Education attainment	Primary (n=166)	4.8 [2.1-9.3]	0.136	2.4 [0.7-6.1]	0.745
	Secondary (n=160)	8.8 [4.9-14.2]		3.8 [1.4-8.0]	
	University (n=8)	0.0 [0.0-0.0]		0.0 [0.0-0.0]	
	No formal schooling (n=54)	1.9 [0.0-9.9]		1.9 [0.0-9.9]	

* significant at 0.05.

Table 3. *Entamoeba* infections among migrant workers (n=306) based on microscopy, serology and PCR screening

			N	rPPDK-ELISA +ve
Microscopy +ve	PCR +ve	Eh	9	2
		Ed	16	2
	PCR -ve		9	0
Microscopy -ve			272	19
Total			306	23

Eh – Positive for either *E. histolytica* or *E. dispar* + *E. histolytica*.
Ed – Positive for *E. dispar* only.

did not present with any clinical disease symptoms.

DISCUSSION

The strong economic presence since the early 1970's encouraged mass migration of workers to Malaysia particularly from rural parts of the neighboring countries where intestinal protozoan infections remain endemic. Malaysia has undergone major urbanization (96.0%), with good sanitation facilities accessible to three-quarters of its population and the majority has clean drinking water (98.2%) (Central Intelligence Agency, 2016; Sahimin *et al.*, 2016). A small fraction (3.8%) of the population still lives below the poverty line. *E. histolytica*, a pathogenic intestinal protozoan is prevalent in the Indian subcontinent, Africa, the Far East, and areas of South and Central America (Ali *et al.*, 2008). In Southeast Asia, amoebiasis is commonly found among the aborigines, the immigrants and communities with low socioeconomic status and poor sanitation in both rural and urban areas (Mahmud *et al.*, 2013).

Little change has been observed over the last decade as very similar prevalence values were reported earlier (Noor Azian *et al.*, 2006; Hakim *et al.*, 2007; Hartini & Mohamed Kamel, 2009; Ngui *et al.*, 2011; 2012; Shahrul Anuar *et al.*, 2012), however, the earlier published findings were based on microscopical screening that did not allow for species differentiation. Morphologically, *E. histolytica* cysts and trophozoites are similar and difficult to differentiate from the non-pathogenic *E. dispar* and *E. moshkovskii*. The latest techniques adopted for the detection of *E. histolytica* in this study were the antigen-detection enzyme-linked immunosorbent assays (ELISA) (Gonin & Louise, 2003; Redondo *et al.*, 2006; Zeehaida *et al.*, 2008) and *Entamoeba* DNA amplification by PCR (Katzwinkel-Wladarsch *et al.*, 1994; Troll *et al.*, 1997; Fotedar *et al.*, 2007; Ngui *et al.*, 2012). This resulted in the identification of *E. dispar* as the most predominant species, followed by *E. histolytica* and mixed infections of *E. dispar* and *E. histolytica*.

The distribution of *E. dispar* and *E. histolytica* in the present study was in agreement with worldwide findings, with the non-pathogenic *E. dispar* being more dominant than the pathogenic *E. histolytica* (Braga *et al.*, 2001; Verweij *et al.*, 2003; Gonin & Louise, 2003; Visser *et al.*, 2006; Khairnar & Parija, 2007; Fotedar *et al.*, 2007; Nazemalhosseini Mojarad *et al.*, 2010). *E. dispar* has been observed often among certain risk groups such as homosexual males, HIV patients, institutionalized populations, immigrants and travelers. A study in South African public hospitals and primary schools showed that *E. histolytica* was detected in 18.8% and 2.1% of samples, respectively, whereas 25.3% and 8.5% had *E. dispar*, respectively (Samie *et al.*, 2006). In Brazil, a study in an urban slum area in Fortaleza reported higher prevalence of *E. dispar* (90.0%) compared to *E. histolytica* (10.0%) (Braga *et al.*, 2001). In Sweden, a clinical setting study showed that 79.7% were positive for *E. dispar* and only 4.8% were positive with *E. histolytica* (Lebbad & Svard, 2005). In India, similar findings have been reported with 49.5% patients being infected with *E. dispar*, meanwhile only 7.4% were infected with *E. histolytica* (Khairnar & Parija, 2007). High prevalence of *E. dispar* (91.4%; n=3,825) compared to *E. histolytica* (3.5%) and *E. moshkovskii* (3.5%) has also been reported from Iran (Nazemalhosseini Mojarad *et al.*, 2010).

There is very little information is available on the prevalence of the multiple *Entamoeba* species in Malaysia. This is because all community and hospital studies were based entirely on microscopic examination of fresh stool samples for parasite identification. Two local reports highlighted higher prevalence of *E. histolytica* compared to *E. dispar*; the first was a study of five rural villages in Peninsular Malaysia (75.0% *E. histolytica*, 30.8% *E. dispar*) (Noor Azian *et al.*, 2006) while the second study was among the aboriginal community in Pahang (13.2% *E. histolytica*, 5.6% *E. dispar*) (Ngui *et al.*,

2012). Ngui *et al.* (2012) also reported the *E. moshkovskii* (5.8%) for the first time with infections observed among children. *E. moshkovskii* infections were also common among Bangladeshi children aged between 2 to 5 years (Ali *et al.*, 2003). Although not significant, the present study observed higher infection among older workers (>55 years old) unlike previous studies where most cases were reported mainly among children (Ali *et al.*, 2003; Ngui *et al.*, 2012; Shahrul Anuar *et al.*, 2012). It is unclear why older adults were infected in this study however, this could be due to their personal hygiene behavior infections particularly during eating, defecation (Singh *et al.*, 2013). Infections are particularly widespread in landscape that are inadequate in sanitation and poor water treatment (Norhayati *et al.*, 2003) or related to lower host immune responses in the aged.

In the present study, *E. dispar* infections showed significant association with those working in food service and domestic sector. Similarly, *E. histolytica* also was also observed higher among workers in the same sectors despite not significantly associated. The health risk for transmission to the public is higher among workers from these sectors compared to the rest as its presence is predominantly dependent on human carriers or cyst passers. Food and drink may become contaminated with the cysts of *Entamoeba* from infected food handlers and domestic maids through unwashed hands after defecation (Mahmud *et al.*, 2013; Woh *et al.*, 2016).

Despite the majority of the study subjects being provided with accommodation, tap water and sewage toilets, a small proportion still harbored the infection. In addition, more than half (62.8%) had resided in Malaysia for more than a year prior to being tested. Therefore, it is possible that in the case of those who had arrived within the last year (37.2%), the infection was acquired in their home country and compounded with behavioral factors such as bad hygiene practices that continued to persist after their entry into Malaysia. However, continued transmission in Malaysia among the longer-term resident workers could also be due to bad personal hygiene, and living in crowded conditions as most workers were accommodated in hostels provided by their employers. Therefore, it is pertinent that all workers be educated on good hygiene practices and knowledge of disease transmission.

The IgG4 assay based on rPPDK has been shown to be a good diagnostic marker for extraintestinal amoebiasis (Wong *et al.*, 2011; Saidin *et al.*, 2014). The assay has been used to develop a lateral flow rapid with high diagnostic sensitivity and specificity for detection of amoebic liver abscess (ALA) (Saidin *et al.*, 2014). In this study the ELISA format of the assay was used in view of the large number of serum samples. Overall, the prevalence of seropositive results among the migrant workers was moderate (7.4%), with years of residence in the country associated with the seropositive individuals. Antibody detection is usually performed to detect extraintestinal amoebiasis, and typically the organism is not present in faeces. Thus, it is not surprising that 19 of 272 microscopically-negative samples were positive by rPPDK-IgG4 ELISA, and all 19 were also PCR-negative. Nevertheless, patients with intestinal amoebiasis may also be antibody positive (Haque *et al.*, 2000). For example, a study in Taiwan showed that 66.7% (24/36) patients who were seropositive for amoebiasis had intestinal infection (Chang *et al.*, 2008). In this study, 4 of 25 (16%) of *E. histolytica* PCR positive people were seropositive by the rPPDK-IgG4 ELISA. However, it should be noted that there were no corresponding faecal samples for direct detection of *E. histolytica* among 178 of those that had provided serum samples (out of a total of 484 serum samples that were tested by the ELISA). Thus, the percentage of PCR positive people may have been higher if stool samples from all participants had been available. Furthermore, the stool PCR assay may have missed some people with intestinal infection due to various factors such as intermittent cyst shedding, presence of inhibitors or extremely low amount of target DNA. These factors may explain the

higher prevalence of positive serology results compared to the molecular detection in stool samples, although the most likely explanation lies in that the presence of antibody normally reflects earlier exposure to infection, and most of the antibody positives in this case may have already recovered from infection by the time faecal samples were made available. Thus, IgG-based antibody positivity in some individuals is likely to be a sign of previous infections due to the long-lived IgG antibodies but may be due also to cross-reaction. In terms of cross-reactivity, IgG4-based assays are more specific than IgG or IgG1-based assays (Saidin *et al.*, 2014). Studies have shown also that IgG4-based Western blot and lateral flow dipstick using rPPDK were 100% specific (Wong *et al.*, 2011; Saidin *et al.*, 2014). Thus, it is unlikely that the seropositivity in the present study was due to cross-reactions. The 19 antibody positive subjects may have cryptic extra-intestinal infection, or may have recently recovered from the infection. As a rule of thumb, antibody results should always be interpreted together with supporting data from assessment of clinical symptoms and imaging results. The seropositive but PCR negative individuals in this study should be monitored and treated when symptoms of ALA appear.

The outcome of this study has provided important information on the health status of the migrant workers. Although the current study did not show a high prevalence of pathogenic *E. histolytica*, this parasite nevertheless has the potential to become a public health problem if left unheeded. Therefore, appropriate control strategies should be put in place such as ensuring proper treatment of infected individuals, and the introduction of health education programs by local authorities to instill in the workers understanding of transmission routes, symptoms and preventive measures.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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