PARASITIC INFECTIONS AMONGST MIGRANT WORKERS IN MALAYSIA

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FACULTY OF SCIENCE
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ABSTRACT

Sociodemographic background of 610 migrant workers employed in Malaysia was collected via questionnaire to determine their parasitic health status. Six nationalities were recruited with most workers from Indonesia (49.5%), followed by Bangladesh (19.2%), Nepal (16.4%), India (10.5%), Myanmar (4.3%) and Vietnam (0.2%) and employed in five working sectors namely; domestic service (24.3%), construction (22.8%), food service (21.0%), plantation (16.7%) and manufacturing (15.2%). A total of 388 individuals provided faecal samples for parasitic screening via microscopy. Four nematode species (*Ascaris lumbricoides, Trichuris trichiura Enterobius vermicularis,* and hookworms), one cestode (*Hymenolepis nana*) and three protozoan species (*Entamoeba histolytica/dispar, Giardia* spp. and *Cryptosporidium* spp.) were recovered. High prevalence of infections with *Ascaris lumbricoides* (43.3%) was recorded followed by hookworms (13.1%) and *E. histolytica/dispar* (11.6%) with infections significantly influenced by nationality, years of residence in Malaysia, employment sector and education level. *Toxoplasma gondii* infections were screened serologically from 484 workers with more than half of the workers were seropositive (57.4%) with 52.9% seropositive for anti-*Toxoplasma* IgG only, 0.8% seropositive for anti-*Toxoplasma* IgM only and 3.7% seropositive with both IgG and IgM antibodies. Samples positive for both IgG and IgM antibodies were further tested for IgG avidity showed high avidity suggesting latent infection in 18 workers. Four significant factors recorded namely; age, nationality, employment sector and length of residence in Malaysia. Three diagnostic methods were tested and compared to detect *Strongyloides stercoralis* infections in 306 migrant workers with 37.6% were seropositive. Subsequent confirmation using a nested PCR showed successful amplification from three males

(2.6%) with target amplicon of approximately 680bp. For the three methods, nested PCR was the most sensitivity method in the detection for strongyloidiasis and should be applied in future studies. PCR method was also applied to determine the species level for four parasite's genus recovered in the population. Internal transcribed spacer 2 and 28S ribosomal RNA region of *N. americanus* and *Ancylostoma* spp. was successfully amplified and resulted in *A. duodenale* reported for the first time in Malaysia. Nested PCR targeting 16S-like ribosomal RNA gene successfully recovered *E. dispar* as the most dominant infection among workers. Despite the low presence of *E. histolytica* in the population, it still carries a public health risk. Amplification of the triosephosphate isomerase (TPI) gene from *G. duodenalis* isolates successfully obtained the presence of assemblage B and sub-assemblage AII suggesting the mode of transmission was human-to-human. Based on the SSU rRNA gene, the *C. parvum* amplicons were successfully detected in 9 human isolates.

ABSTRAK

Latar belakang sosiodemografi 610 pekerja asing yang bekerja di Malaysia telah dikumpul melalui soal selidik untuk menentukan tahap kesihatan parasitik mereka. Enam warganegara telah direkrut dengan majoriti pekerja dari Indonesia (49.5%), diikuti oleh Bangladesh (19.2%), Nepal (16.4%), India (10.5%), Myanmar (4.3%) dan Vietnam (0.2%) dan bekerja dalam lima sektor iaitu; perkhidmatan domestik (24.3%), pembinaan (22.8%), perkhidmatan makanan (21.0%), perladangan (16.7%) dan pembuatan (15.2%). Seramai 388 individu memulangkan sampel najis untuk pemeriksaan parasit melalui mikroskop. Empat spesies nematoda (*Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius vermicularis* dan cacing tambang), satu cestoda (*Hymenolepis nana*) dan tiga spesies protozoa (*Entamoeba histolytica / dispar*, *Giardia* spp. dan *Cryptosporidium* spp.) ditemui. Jangkitan parasit tertinggi dicatatkan oleh *Ascaris lumbricoides* (43.3%), diikuti oleh cacing tambang (13.1%) dan *E. histolytica / dispar* (11.6%) dan faktor jangkitan dipengaruhi oleh kewarganegaraan, jangka masa menetap di Malaysia, sektor pekerjaan dan tahap pendidikan. Jangkitan *Toxoplasma gondii* telah disaring secara serologi dari 484 pekerja dan lebih daripada separuh pekerja adalah seropositif (57.4%) dengan 52.9% seropositif untuk anti-*Toxoplasma* IgG sahaja, 0.8% seropositif untuk anti-*Toxoplasma* IgM sahaja dan 3.7% seropositif dengan kedua-dua antibodi IgG dan IgM. Sampel positif untuk kedua-dua antibodi IgG dan IgM kemudiannya diuji dengan ujian IgG aviditi dan keputusan menunjukan aviditi tinggi yang mencadangkan jangkitan terpendam dari 18 pekerja tersebut. Empat faktor penting didapati iaitu; umur, kewarganegaraan, sektor pekerjaan dan jangka masa menetap di Malaysia. Tiga kaedah diagnostik telah diuji dan dibandingkan untuk mengesan jangkitan *Strongyloides stercoralis* di kalangan 306 pekerja asing dengan 37.6% adalah

seropositif. Pengesahan berikutnya menggunakan tindak balas polimer berantai (PCR) menunjukkan tiga lelaki (2.6%) dijangkiti parasit ini dengan sasaran amplicon kira-kira 680bp. Daripada tiga kaedah tersebut, tindak balas polimer berantai (PCR) adalah kaedah paling sensitiviti dalam pengesanan jangkitan strongyloidiasis dan disyorkan diguna pakai dalam kajian di masa hadapan. Tindak balas polimer berantai (PCR) juga digunakan bagi menentukan spesies daripada empat genus parasit yang dijumpai di dalam populasi. Internal transcribed spacer 2 dan 28S ribosomal RNA daripada *N. americanus* dan *Ancylostoma* spp. telah berjaya dikesan dan jangkitan *A. duodenale* dilaporkan buat kali pertama di Malaysia. Tindak balas polimer berantai menyasarkan gen 16S-like ribosomal RNA berjaya mendapati *E. dispar* sebagai jangkitan yang paling dominan di kalangan pekerja. Walaupun penemuan *E. histolytica* yang rendah di dalam populasi, ia masih boleh membawa risiko kesihatan awam. Penguatan gen triosephosphate isomerase (TPI) *G. duodenalis* berjaya menemui kehadiran himpunan B dan sub-himpunan AII yang mencadangkan jangkitan dari manusia ke manusia. Berdasarkan gen SSU rRNA, amplicon *C. parvum* telah berjaya dikesan daripada 9 pekerja asing.

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LIST OF SYMBOLS AND ABBREVIATIONS

n	:	sample size
%	:	percentage
µg/µl	:	microgram per microliter
µl	:	microliter
°C	:	degree Celsius
bp	:	base pair
CI	:	confidence interval
CL	:	confidence limit
cm	:	centimeter
dH_2O	:	distilled water
DNA	:	deoxyribonucleic acid
dNTP	:	deoxyribonucleoside triphosphate
EDTA	:	ethylenediaminetetraacetic acid
g	:	gram
GE	:	gel extraction
IgG	:	immunoglobulin G
IgM	:	immunoglobulin M
ITS	:	Internal Transcribed Spacer
IPIs	:	Intestinal parasitic infections
min	:	minute
mL	:	milliliter
mM	:	miliMolar
mm	:	millimeter
ng	:	nanogram

nm	:	nanometer
PCR	:	Polymerase Chain Reaction
rDNA	:	ribosomal DNA
RFLP	:	Restriction Fragment Length Polymorphism
Taq	:	*Thermus aquaticus*
TAE	:	Tris Acetic acid EDTA
U	:	Unit
UV	:	Ultraviolet
V	:	Volt
w/v	:	weight/volume
x	:	Times
X^2	:	chi-square

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Malaysia

Malaysia comprises of Peninsular Malaysia, Sabah and Sarawak. It is situated in Southeastern Asia, with the peninsula bordering Thailand in the north and Singapore in the south, and one-third of the island of Borneo, which borders Indonesia and Brunei. The location is very strategic along the Strait of Malacca and southern South China Sea. The total population is approximately 30.5 million with Malays (50.1%) as the dominant ethnic group, followed by Chinese (22.6%), indigenous (11.8%), Indian (6.7%) and others (0.7%) (Central Intelligence Agency, 2016).

The standard of living in this country is better compared the neighbouring countries in the region. A total of 74.7% of the population has undergone urbanization with a change rate of 2.66% annually (2010 – 2015) (Central Intelligence Agency, 2016). The population is made up in major urban cities such as the capital, Kuala Lumpur and Johor Bahru is approximately 6.8 million and 912,000, respectively with a majority having access to good sanitation (96%) and clean drinking water (98.2%).

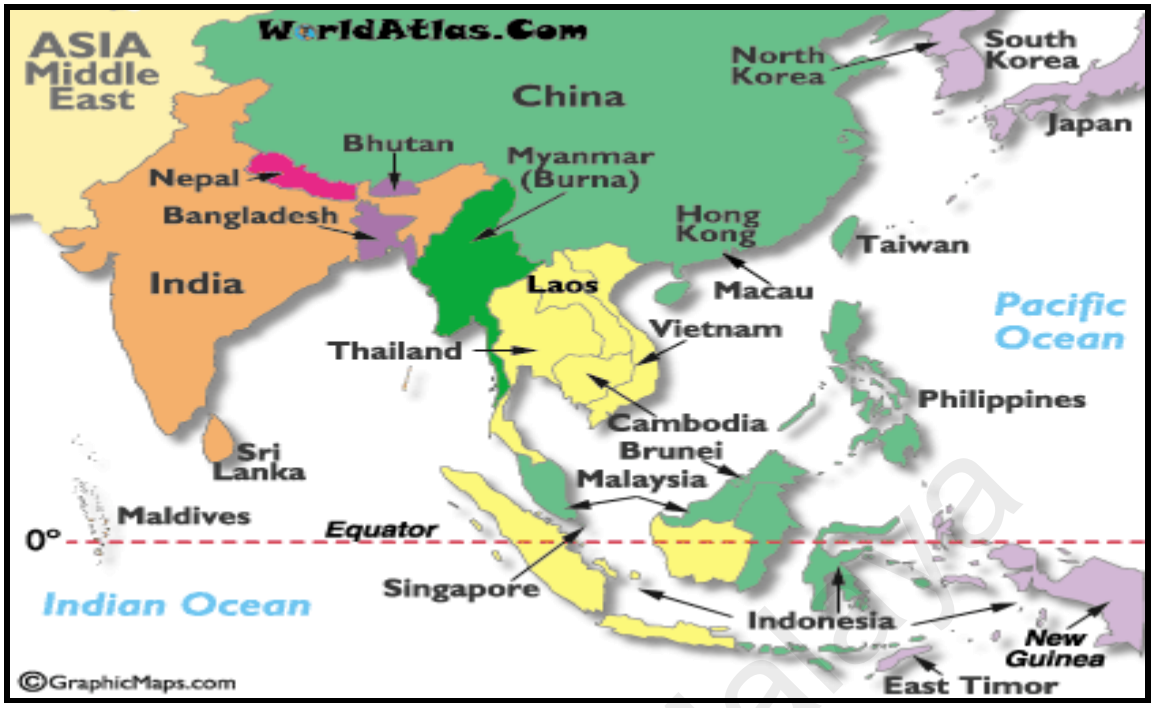

Figure 1.1: Malaysia and the neighbouring countries (Southeast Asia and South Asia countries; Thailand, Indonesia, The Philippines, Vietnam, Laos, Myanmar, India, Nepal, and Bangladesh) (Source: WorldAtlas.com, 2016).

1.1.1 Economy status of the ASEAN region

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) due to their adverse economic conditions such as poverty, high unemployment rates and lack of employment opportunities in their home countries. Indonesia, the largest economy in Southeast Asia region underwent slowdown in growth since 2012 due to the end of the commodities export boom (Central Intelligence Agency, 2016). Since then, Indonesia struggles with poverty, unemployment, inadequate infrastructure, corruption, a complex regulatory environment, and unequal resource distribution among its regions (Central Intelligence Agency, 2016).

Bangladesh is an underdeveloped country however, since 1996, the economy grew roughly 6% per year despite political instability, poor infrastructure, corruption, insufficient power supplies, slow implementation of economic reforms, global financial crisis and recession to becoming a country showing improvements in development (Central Intelligence Agency, 2016).

Nepal is among the poorest and least developed country in the world, with about one-quarter of its population living below the poverty line. Agriculture is the mainstay of the economy, providing a livelihood for almost 70% of the population and accounting for about one-third of the gross domestic product (GDP). Industrial activity mainly involves the processing of agricultural products, including pulses, jute, sugarcane, tobacco and grain (Central Intelligence Agency, 2016).

Socio and economic status between Malaysia and the neighbouring countries is described in Table 1.1. The data records Malaysia as one of the highest standard of living and stable economy compared with other neighbouring countries.

Table 1.1: Socio-demographic and economic status between Malaysia and neighbouring countries (Indonesia, India, Bangladesh, Nepal, Myanmar and Vietnam).

	Malaysia	Indonesia	India	Bangladesh	Nepal	Myanmar	Vietnam
Population (July 2015 est.)	30,513,848	255,993,674	1,251,695,584	168,957,745	31,551,305	56,320,206	94,348,835
Population growth rate (2015 est.)	1.44%	0.92%	1.22%	1.6%	1.79%	1.01%	0.97%
Net migration rate (2015 est.)	-0.33 migrant(s)/1,000 population	-1.16 migrant(s)/1,000 population	-0.04 migrant(s)/1,000 population	0.46 migrant(s)/1,000 population	3.86 migrant(s)/1,000 population	-0.28 migrant(s)/1,000 population	-0.3 migrant(s)/1,000 population
Urbanization	74.7%	53.7%	32.7%	34.3%	18.6%	34.1%	33.6%
Sanitation facility access	96%	60.8%	39.6%	60.6%	45.8%	77.4%	78%
Drinking water resources	98.2%	87.4%	94.1%	86.9%	91.6%	80.6%	97.6%
GDP – real growth rate (2015 est.)	4.7%	4.7%	7.3%	6.5%	3.4%	8.5%	6.5%
GDP by sector (2015 est.)	Agric.: 8.9% industry: 35% services:56.1%	Agric.: 13.6% industry: 42.8% services: 43.6%	Agric.: 16.1% industry: 29.5% services: 54.4%	Agric.: 16% industry: 30.4% services: 53.6%	Agric.: 31.7% industry: 15.1% services: 53.2%	Agric.: 36.1% industry: 22.3% services: 41.6%	Agric.: 17.4% industry: 38.8% services: 43.7%

	Malaysia	Indonesia	India	Bangladesh	Nepal	Myanmar	Vietnam
Industrial production growth rate (2015 est.)	5.5%	4.5%	2.8%	9.4%	2.6%	12.2%	7.5%
Labor force (2015 est.)	14.3 million	122.4 million	502.1 million	81.95 million	15.2 million	36.18 million	54.93 million
Labor force by sector	Agric.: 11% industry: 36% services: 53% (2012 est.)	Agric.: 38.9% industry: 13.2% services: 47.9% (2012 est.)	Agric.: 49% industry: 20% services: 31% (2012 est.)	Agric.: 47% industry: 13% services: 40% (2010 est.)	Agric.: 69% industry: 12% services: 19% (2014 est.)	Agric.: 70% industry: 7% services: 23% (2001 est.)	Agric.: 48% industry: 21% services: 31% (2012 est.)
Unemployment rate (2015 est.)	2.7%	5.5%	7.1%	4.9%	46% (2008 est.)	5%	3%
Population below poverty line	3.8% (2009 est.)	11.3% (2014 est.)	29.8% (2010 est.)	31.5% (2010 est.)	25.2% (2011 est.)	32.7% (2007 est.)	11.3% (2012 est.)
Household income: **Lowest 10%** **Highest 10%**	 1.8% 34.7% (2009 est.)	 3.4% 28.2% (2010 est.)	 3.6% 31.1% (2005 est.)	 4% 27% (2010 est.)	 3.2% 29.5% (2011 est.)	 2.8% 32.4% (1998 est.)	 3.2% 30.2% (2008 est.)
Inflation rate (2015 est.)	2.1%	6.7%	5.6%	5.7%	7.2%	9.2%	0.9%

	Malaysia	Indonesia	India	Bangladesh	Nepal	Myanmar	Vietnam
Industries	Rubber & oil palm processing & manufacturing, petroleum & natural gas, light manufacturing, pharmaceutical, medical technology, electronics & semiconductor, timber processing; logging and agriculture processing.	Petroleum & natural gas, textiles, automotive, electrical appliances, apparel, footwear, mining, cement, medical instruments & appliances, handicrafts, chemical fertilizers, plywood, rubber, processed food, jewelry and tourism	Textiles, chemicals, food processing, steel, transportation equipment, cement, mining, petroleum, machinery, software and pharmaceuticals.	Jute, cotton, garments, paper, leather, fertilizer, iron & steel, cement, petroleum products, tobacco, pharmaceuticals, ceramics, tea, salt, sugar, edible oils, soap & detergent, fabricated metal products, electricity and natural gas.	Tourism, carpets, textiles, small rice, jute, sugar, oil seed mills, cigarettes, cement and brick production.	Agricultural processing; wood & wood products, copper, tin, tungsten, iron, cement, construction materials, pharmaceuticals, fertilizer, oil & natural gas, garments, jade and gems.	Food processing, garments, shoes, machine-building, mining, coal, steel, cement, chemical fertilizer, glass, tires, oil and mobile phones.

Source: The World Factbook – Central Intelligence Agency (2016).

1.2 Migrant workers in Malaysia

1.2.1 Migrant workers

According to the International Convention on the Rights of Migrants Workers and Members of Their Families 1990, "migrant worker" refers to a person engaged, or previously engaged in a remunerated activity in a State of which he or she is not a national. The latest statistic shows an estimated 232 million migrant workers around the world. According to International Labour Organization (ILO, 2015), factors such as globalization, demographic shifts, conflicts, income inequalities and climate change are the push factor for workers and their families to cross borders in the search of employment and security. Migrant workers contribute to growth and development in the country of destination, while the country origin greatly benefit from their remittances and skills acquired during employment.

1.2.2 Migrant workers in Malaysia

The robust growth of Malaysia's economy led to the high demand of workforce from small to large-scale enterprises. This in turn, created an influx for workers to fulfill the work demand. It is a practice in this country that potential employers are obligated to advertise work opportunities to all potential registered job seekers at the Labour Department through the registration in Jobs Malaysia with priorities given to the locals.

A foreign worker is defined as non-citizen or Permanent Residence (PR) but is permitted for employment and temporary stay on Visit Pass (Temporary Employment) or Pas Lawatan (Kerja Sementara) (PLKS). Department of Labour Peninsular Malaysia

is the body given the mandate to process employment of foreign workers for them to enjoy protection and benefits prescribed by the labour law. However, Malaysia Employers Federation (MEF) (Bardan, 2014) denotes four categories of migrant workers; legal workers, expatriates, illegal workers and refugees.

Expatriates are those who are issued with and Employment Pass and largely professionals and highly skilled workers. There were about 80,000 expatriates in 1980s however, the numbers have dropped almost half to 44,938 in 2013 due to competitiveness and innovative capabilities of the local workforce. Presently, the majority of the expatriates are employed under the service (n=15,746; 35%), petroleum (n=8,654; 19.3%) and information technology (8,410; 18.7%) industries (Bardan, 2014).

The illegal or undocumented workers enter Malaysia illegally or subsequently failed to renew the work permit and work in the breach of the immigration laws. Therefore, employment is without protection and vulnerable to abuse and exploitation. The final category is the refugees. Asylum seeking refugees are issued with an identification card (ID) by the United Nations High Commissions for Refugees (UNHCR) for resettlement to a third country. Reports by MEF in March 2014, records a total of 143, 435 refugees and asylum-seekers registered with UNHCR in Malaysia (Bardan, 2014). Refugees are normally not allowed to work and are not issued with employment pass. However, they may seek employment normally in the informal sector until the process of resettlement is finalized.

1.2.3 Statistic of migrant workers

Malaysia experienced an economic bloom in the early 1970s, that led to high demand for low skilled and semi-skilled workforce from neighboring countries including from the South East Asian nations (e.g., Indonesia, Cambodia, Vietnam, the Philippines and Myanmar) and South Asian countries (e.g., Nepal, India and Bangladesh) particularly in five sectors including manufacturing, services, agriculture and plantation, construction and domestic. Statistics from 2000 to 2014 showed half of the workers were majority from Indonesia (Table 1.2).

Table 1.3 shows the employment breakdown of migrant workers in the different working sectors. Employment of migrant workers was primarily in the manufacturing sector followed by agriculture and plantation, construction, services and domestic works.

Table 1.2: Number of migrant workers employed in Malaysia according to country of origin (2000-2014).

Year	Country of Origin									
	Indonesia	**Bangladesh**	**Thailand**	**Philippines**	**Pakistan**	**Myanmar**	**Nepal**	**India**	**Others**	**Total**
2000	603,453	158,149	2,335	14,651	3,101	3,444	666	18,934	2,363	**807,096**
2001	634,744	114,308	2,508	11,944	2,392	6,539	48,437	26,312	2,645	**849,829**
2002	788,221	82,642	20,599	21,234	2,000	27,870	82,074	39,248	3,641	**1,067,529**
2003	988,165	94,541	10,158	17,400	2,141	48,113	109,067	63,166	4,229	**1,336,980**
2004	1,024,363	54,929	5,463	16,663	1,156	61,111	149,886	78,688	77,831	**1,470,090**
2005	1,211,584	55,364	5,751	21,735	13,297	88,573	192,332	134,947	91,655	**1,815,238**
2006	1,174,013	62,669	13,811	24,088	11,551	109,219	213,551	138,313	121,994	**1,869,209**
2007	1,148,050	217,238	18,456	23,283	16,511	104,305	189,389	142,031	185.542	**2,044,805**
2008	1,085,658	316,401	21,065	26,713	21,278	144,612	201,997	130,265	114,607	**2,062,596**
2009	991,940	319,020	19,402	24,384	21,891	139,731	182,668	122,382	96,728	**1,918,146**
2010	792,809	319,475	17,209	35,338	28922	160,504	251,416	95,112	117,086	**1,817,871**
2011	785,236	116,663	5,838	44,359	26,229	146,126	258,497	87,399	102,714	**1,573,061**
2012	746,063	132,350	7,251	44,919	31,249	129,506	304,717	93,761	81,773	**1,571,589**
2013	1,021,655	322,750	17,044	69,126	50,662	161,447	385,466	124,017	98,155	**2,250,322**
2014	817,300	296,930	12,467	63,711	51,563	143,334	490,297	105,188	92,624	**2,073,414**

*** Others: Cambodia, China, Vietnam, Laos, Sri Lanka.**

Source: Temporary Work Visit Pass (PLKS), Immigration Department: Ministry of Home Affairs (Ministry of Human Resources, 2015).

Table 1.3: Employment distribution of migrant workers according to working sectors (2000-2014).

Year	Sectors					
	Domestic	**Services**	**Manufacturing**	**Construction**	**Agriculture/ Plantation**	**Total**
2000	177,546	53,683	307,167	68,226	200,474	**807,096**
2001	194,710	56,363	312,528	63,342	222,886	**849,829**
2002	232,282	64,281	323,299	149,342	298,325	**1,067,529**
2003	263,465	85,170	385,478	252,516	350,351	**1,336,980**
2004	285,441	93,050	475,942	231,184	384,473	**1,470,090**
2005	320,171	159,662	581,379	281,780	472,246	**1,815,238**
2006	310,662	166,829	646,412	267,809	477,497	**1,869,209**
2007	314,295	200,428	733,372	293,509	503,201	**2,044,805**
2008	293,359	212,630	728,867	306,873	520,867	**2,062,596**
2009	251,355	203,639	663,667	299,575	499,910	**1,918,146**
2010	247,069	165,258	672,823	235,010	497,711	**1,817,871**
2011	184,092	132,919	580,820	223,688	451,542	**1,573,061**
2012	142,936	138,823	605,926	226,554	457,350	**1,571,589**
2013	169,936	269,321	751,772	434,200	625,093	**2,250,322**
2014	155,591	270,048	747,866	411,819	488,090	**2,073,414**

Source: Temporary Work Visit Pass (PLKS), Immigration Department: Ministry of Home Affairs (Ministry of Human Resources, 2015).

The regulatory agencies responsible for the admission of the migrant workers are as described in Table 1.4.

Table 1.4: Regulatory agencies of migrant workers according to working sectors in Malaysia.

Regulatory Agencies of Migrant Workers				
Manufacturing	**Services Sector**	**Construction**	**Agriculture & Plantation**	**Domestic Work**
• Ministry of Internatonal Trade and Industry (MITI)	• Ministry of Domestic Trade, Co-operatives and Consumerism (KPDNKK) • Ministry of Transportation (MoT) • Ministry of Women, Family and Community Development (KPWKM) • Ministry of Tourism and Culture Malaysia (MoTAC) • Royal Malaysia Police (PDRM)	• Ministry of Works Malaysia (KKR) • Construction Industry Development Board (CIDB)	• Ministry of Plantation, Industries and Commodities (MPIC) • Ministry of Agriculture & Agro-Based Industry Malaysia (MOA)	• Ministry of Human Resources (MoHR) • Ministry of Home Affairs (MoHA)

Source: Ministry of Human Resources (2015).

1.3 Health status of migrant workers

1.3.1 Heath screening of workers upon entry

Each worker is obligatory to undergo medical screening upon entry to Malaysia and the subsequent year up to the third year of service (under the same employer). Unitab Medic Sdn. Bhd. through FOMEMA is an agency involved in the implementation, management and supervision of a nationwide mandatory health screening programme for all legal migrant workers in Malaysia. FOMEMA ensures that the health status of each migrant worker is free from communicable diseases and promotes the well being of the society by safeguarding the health of the general community living.

The medical screening process is designed and managed by a group of medical professionals in public health, occupational health, radiology, laboratory services and other related specialties (Figure 1.2). All registration and payment is centralized with standardized fee. Employers are given the option a list of doctors in their registry to run the medical screening for their workers. Standardized medical examination is carried out stipulated by Ministry of Health, which is monitored and supervised through IT surveillance and inspectorate activities. Medical reports, X-ray and laboratory results are submitted independently and electronically to FOMEMA and to Immigration Department Headquarters to facilitate issuance of work pass or deportation. The results are obtained via online and those who failed the screening are certified 'UNFIT' and sent back to their country, with or without appeal to FOMEMA. Only 'FIT' workers are allowed to continue with their employment in Malaysia.

The medical examination is normally carried out within 30 days from the date of registration. Details of medical examination covered under the system stipulated by the Ministry of Health includes medical history, physical examination, system examination laboratory test and x-ray examination (Table 1.5).

Benefits of this system include the assurance of a healthier workforce, prevention of the spread of identified communicable diseases to other workers or the public at large, increased productivity through reduction in absenteeism due to illness, in addition to lower incidence of imported diseases, morbidity and mortality. As a result, an overall reduction in healthcare cost to employers, taxpayers and the Government and better use of local public health facilities for the citizens.

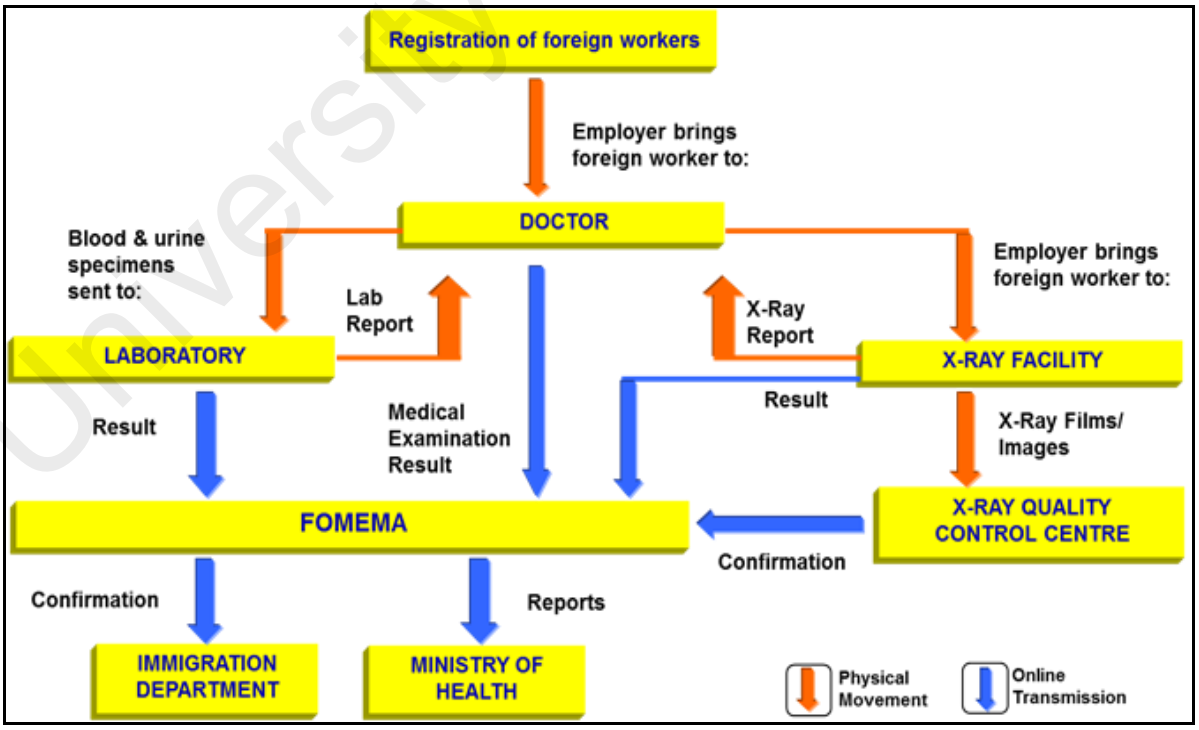

Figure 1.2: Medical screening process of foreign workers in Malaysia. Source: FOMEMA (2015).

Table 1.5: Categories of medical examination as stipulated by Ministry of Health.

Category	Examination
Medical History	• HIV/AIDS • TuberculoAsis • Leprosy • Viral Hepatitis • Peptic Ulcer • Epilepsy • Cancer • Kidney Disease • Malaria • Hypertension • Heart Diseases • Bronchial Asthma • Diabetes Mellitus • Phsyciatric Illnesses • Sexually Transmitted Diseases (STD) • Others
Physical Examination	• Height and Weight • Pulse Rate and Blood Pressure • Last menstrual Period (female) • Chronic Skin Rash • Anaesthetic Skin Patch • Deformities of Limbs • Anaemia • Jaundice • Lymph Nodes Enlargement • Vision Test • Hearing ability • Others
System Examination	• Cardiovascular System • Respiratory System • Gastrointestinal System • Nervous System • Mental Status • Genitourinary System
Laboratory Tests	Blood Test: • For Blood Grouping (A,B,AB or O and Rh). • For HIV, Hepatitis B, VDRL and Malaria.
	Urine Tests: • For colour, specific gravity, sugar, albumin and microscopic examination. • For opiates, cannabis and pregnancy (for female).
Chest X-ray	Physical examination of the foreign worker must be carried out first before chest X-ray examination.

Source: FOMEMA (2015).

Medical results are transmitted independently, thus averting physical handling or report tampering by employers or agents to ensure the integrity of the health-screening system, as well as to facilitate the employers' application or permit renewal in a timely manner and also plays a role as an access for the Government authorities to a centralized database. The system also provides timely information and vital statistics relating to communicable diseases and to facilitate immediate counter-action and preventive measures.

1.3.2 Common health problems

The Ministry of Health Annual Report 2012 recorded that a total of 48,734 (3.58%) were unfit for work from 1,361,228 screened with 36,731 (75.3%) cases categorized as communicable diseases and 12,003 (24.6%) as non-communicable diseases (Figure 1.3). The number of unfit cases was higher in 2012 compared to the previous year (24,416, 2.16%). Table 1.6 below shows the distribution of workers from the top 12 countries underwent health screening.

Of all communicable diseases, tuberculosis (abnormal chest X-ray findings) was the most common with 18,315 (37.6%) cases, followed by hepatitis B with 14,044 (28.8%) cases; syphilis with 3,520 (7.22%) cases; HIV with 821 (1.68%) cases and malaria with 31 (0.06%) cases (Figure 1.4). While there were also reports of 1,954 (4%) cases positive for pregnancy followed by 1,027 (2.1%) for urine opiates and 898 (1.84%) for urine cannabis, 208 (0.4%) cases with psychiatric diseases, 50 (0.1%) cases of epilepsy and 30 (0.06%) cases with cancer (Figure 1.5). Despite compulsory medical screening of workers for communicable diseases prior to entering the Malaysian workforce, screening for parasitic infections is grossly inadequate or lacking. Screening for non-

communicable diseases plays an important role as it is likely to impact significantly upon the local community through close contact, lost in productivity and the heighten cost of healthcare Therefore, there is an acute need for more accurate and up-to-date information on the parasitic infections in this particular group of workers and an understanding of the factors associated with transmission of these infections.

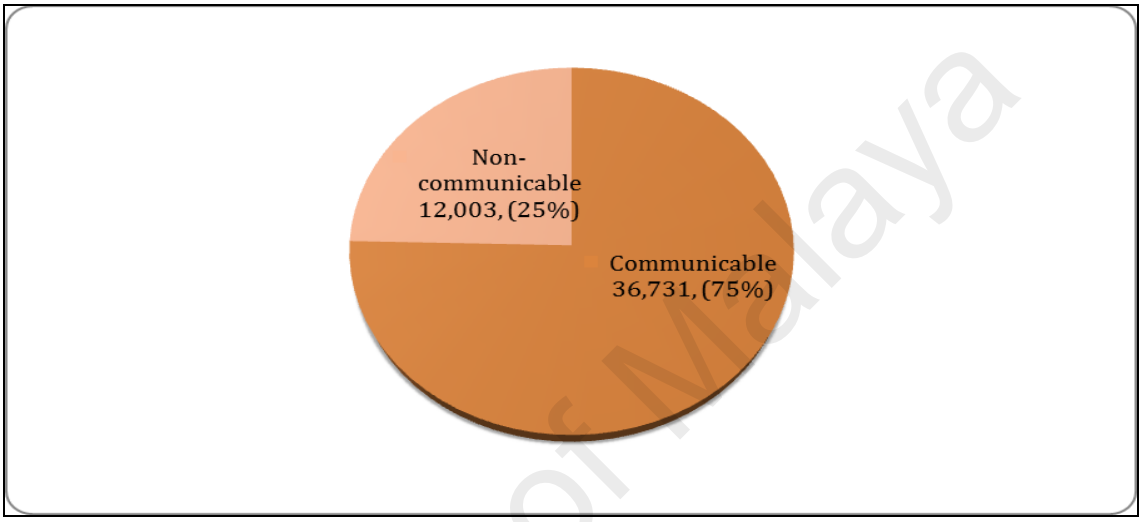

Figure 1.3: Prevalence of communicable and non-communicable diseases among foreign workers in 2012. Source: Disease Control Division, Ministry of Health (2012).

Table 1.6: Number of workers screened according to country of origin in 2012.

No.	Countries	Number screened	Percentage (%)
1	Indonesia	514,719	37.8
2	Nepal	291,856	21.4
3	Bangladesh	219,710	16.1
4	Myanmar	132,315	9.72
5	India	68,032	4.99
6	Vietnam	47,397	3.48
7	Philippines	28,505	209
8	Pakistan	25,722	1.88
9	Cambodia	21,059	1.54
10	Sri Lanka	5,767	0.42
11	Thailand	3,228	0.23
12	China	2,720	0.19

Source: Disease Control Division, Ministry of Health (2012).

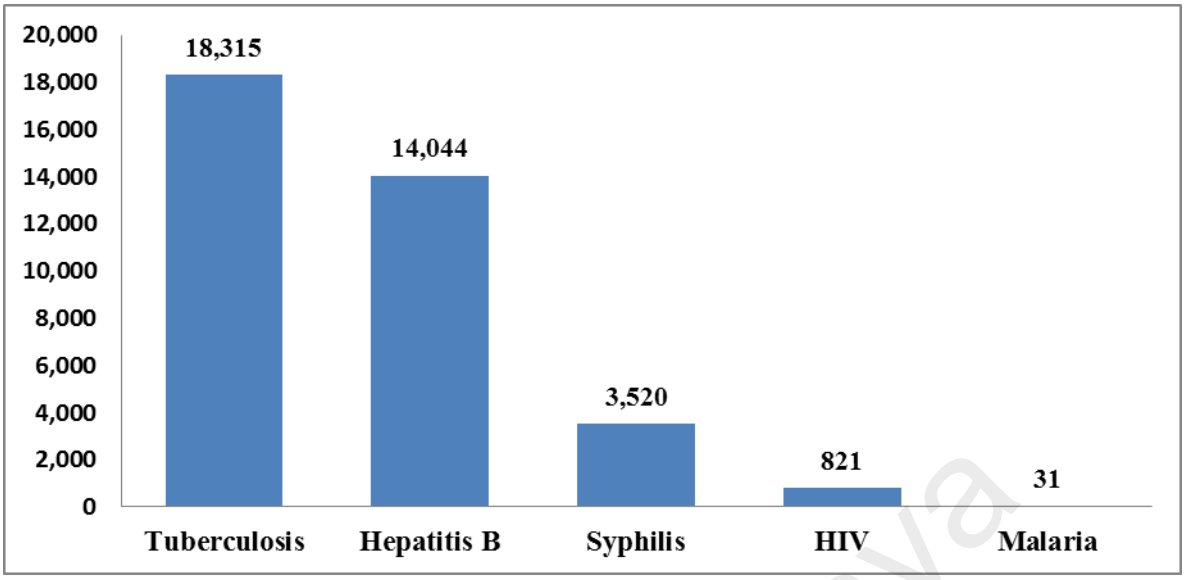

Figure 1.4: Five most common communicable diseases among foreign workers in 2012. Source: Disease Control Division, Ministry of Health (2012).

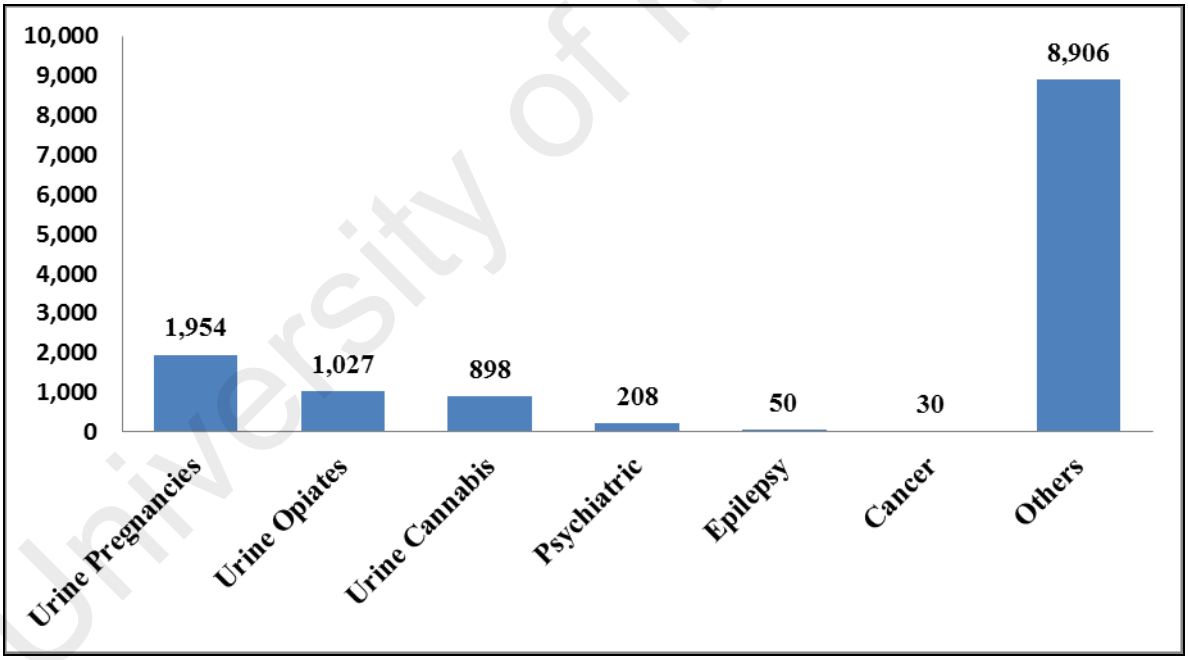

Figure 1.5: Other diseases reported among foreign workers in 2012. Source: Disease Control Division, Ministry of Health (2012).

1.4 Common parasitic infections in migrant workers

Parasitic infections are common in human with most infections are asymptomatic however others may have serious health implications. Intestinal helminthes infections are potentially pathogenic meanwhile many protozoan infections are nonpathogenic. High prevalence of infections is reported particularly among marginalized communities such as immigrants and refugees with intestinal parasites (protozoa and helminthes). Country of origin is known as the strongest predictor of intestinal parasites (Godue & Gyorkos, 1990; Stauffer *et al*., 2002; Cook & Zumla, 2003; Koroma *et al*., 2010). Global geographic distributions of intestinal parasitic infections are listed in Table 1.7.

Table 1.7: Geographic distribution of common human intestinal parasitic infections.

Location	**Helminth Parasites**	
Global	*Ascaris* *Trichuris* Hookworm *Strongyloides*	*Enterobius* *Fasciola* *Hymenolepis* All protozoa
Africa	*Schistosoma mansoni* *Schistosoma haematobium*	*Schistosoma intercalatum* *Taenia saginata*
Asia	*Fasciolopsis buski* **South Asia**: *T. solium*	**Southeast Asia:** *Opisthorchis viverrini* *Clonorchis sinensis* *Schistosoma japonicum* *Schistosoma mekongi*
Latin America	*Taenia solium* *Schistosoma mansoni*	*Opisthorchis guayaquilensis*
Middle East	*Echinococcus*	
Eastern Europe	*Diphyllobothrium latum* *Opisthorchis felineus*	

Source: Godue & Gyorkos, 1990; Stauffer *et al*., 2002; Cook & Zumla, 2003; Koroma *et al.* 2010.

1.4.1 Helminthes

The neglected intestinal parasitic infections (IPIs) such as soil-transmitted helminth (STH), is recognized as one of the most significant causes of illnesses and diseases especially among disadvantaged communities. World Health Organization (WHO) categorizes STH as one of the 17 neglected tropical diseases in the world population. More than 1.5 billion people, or 24% of the world's population are infected with single or multiple infections of common helminth such as roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*). Other helminth species include *Enterobius vermicularis* and *Hymenolepis nana*.

Figure 1.6 highlights distribution of global soil-transmitted helminth (STH) with limited studies conducted in Malaysia and neighbouring South East Asia and South Asia countries.

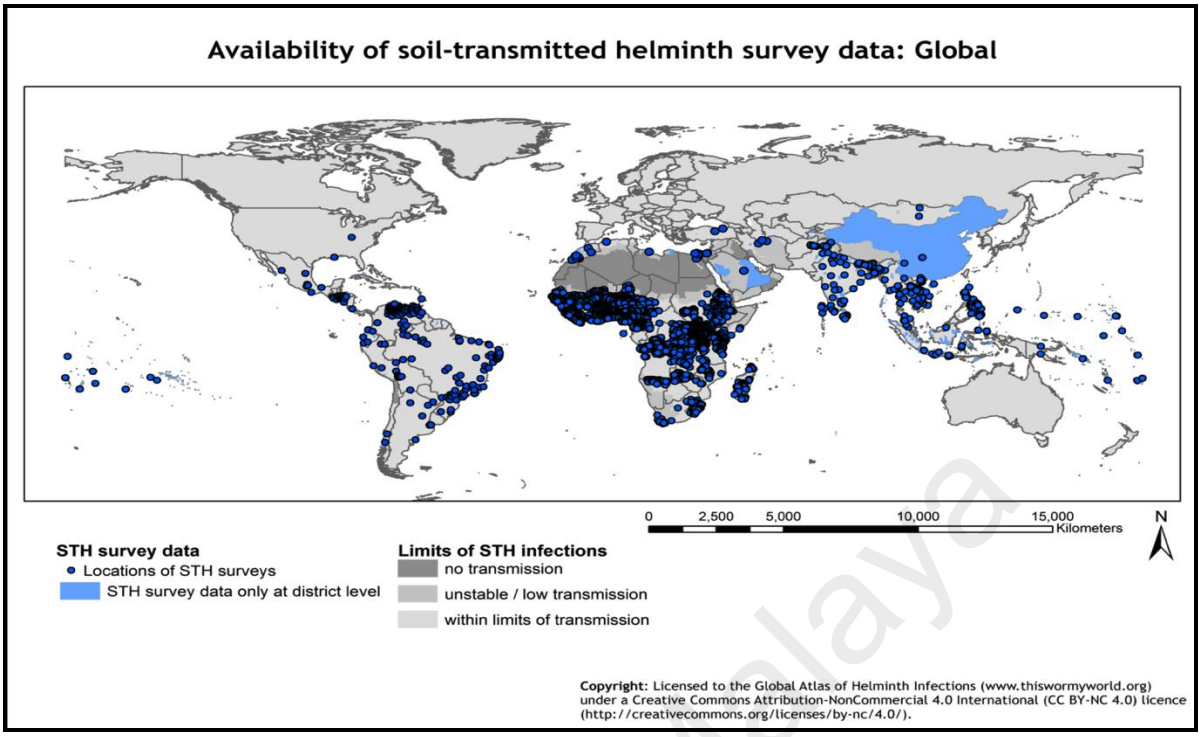

Figure 1.6: Worldwide distribution of soil-transmitted helminth. Source: Global Atlas of Helminth Infections (2016).

1.4.1.1 *Ascaris lumbricoides*

Ascaris lumbricoides (Table 1.8) is the most prevalent human parasitic infections and the largest nematode parasitizing human intestine infecting >1 billion persons globally (Bethony *et al*., 2006). The adult length for female ranges from 20 to 35cm, while the adult male from 15 to 30cm.

The adult worm establishes in the lumen of human intestine after ingestion of the infective eggs where a female can produce as many as 200,000 eggs per day. Eggs become infective after 18 days to several weeks dependent on the optimum moist, warm and soil. After ingestion, the eggs hatch into larvae and transported via the portal to the lungs and mature (10 to 14 days). The larvae then penetrate the alveolar walls to the throat before being swallowed. Upon reaching the small intestine, the worm matures

into an adult between 1 to 2 years (DPDx, 2013). Life cycle of *A. lumbricoides* is described in Figure 1.7.

Table 1.8: Scientific classification of *Ascaris lumbricoides*.

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Ascaridida
Family	Ascarididae
Genus	*Ascaris*
Species	*Ascaris lumbricoides*

Source: DPDx, 2013

Most human infections are asymptomatic but if symptoms do occur, it includes abdominal discomfort. Symptoms of heavy infections include acute lung inflammation, abdominal distension and pain, and intestinal obstruction (Bethony *et al*., 2006), while coughing is due to migration of worms in the body. Diagnosis requires careful microscopy examination of a fecal sample for eggs. World Health Organization (WHO, 2015) recommended albendazole (400 mg) and mebendazole (500 mg) for effective treatment that is not only inexpensive but also easy to administer by non-medical personnel. Both treatments have been extensive tested and used for treatment with few and minor side effects.

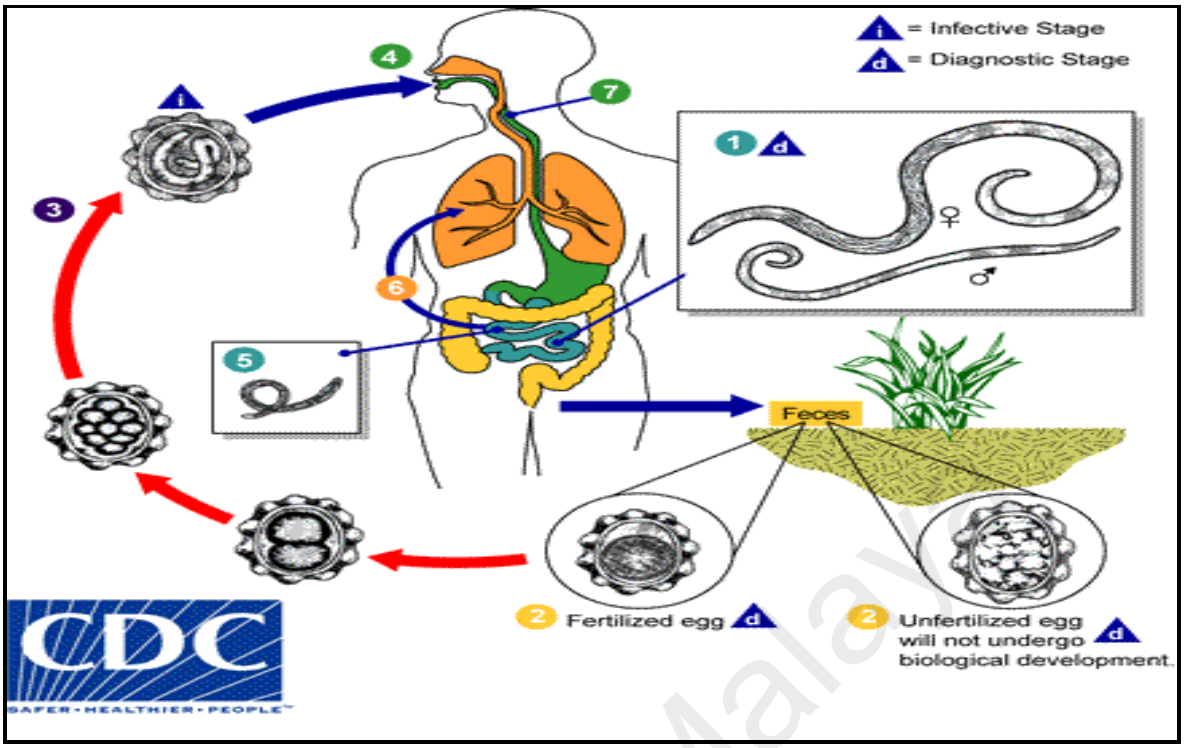

Figure 1.7: Life cycle of *Ascaris lumbricoides* (Source: DPDx, 2013)

1.4.1.2 Hookworm

Another important species of soil-transmitted helminthes is the hookworm (Table 1.9). Two species (*Ancylostoma duodenale* and *Necator americanus*) infect an estimated of 600 million people globally (Hotez, 2009). *N. americanus* infection is more common worldwide, while *A. duodenale* is more geographically restricted.

Unlike *Ascaris*, hookworm eggs are not infective. Eggs are passed in the stool of an infected person and the larvae hatch under favorable conditions (moisture, warmth, and shade) between 1 to 2 days. The released first stage (rhabditiform) larvae grow in the feces and/or the soil, and after 5 to 10 days develop into infective third stage (filariform) larvae which can survive 3 to 4 weeks in favorable environmental conditions. Upon contact with the human skin the larvae is transported via blood vessels to the heart and then lungs and penetrate the pulmonary alveoli before ascending to the bronchial tree

and pharynx, then swallowed. Once in the small intestine, the larvae mature into adults. Using the mouthpart, the adult worms pierce the intestinal wall, resulting in blood loss to the host. The presence of 40 to 160 adult hookworms can result in blood loss sufficient to cause anemia and malnutrition. Most adult worms are normally eliminated in 1 to 2 years, but they can live for several years (Figure 1.8).

Table 1.9: Scientific classification of the hookworm

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Strongylida
Family	Ancylostomatidae
Genus	*Ancylostoma / Necator*
Species	*Ancylostoma duodenale / Necator americanus*

Source: DPDx, 2013

Infections are normally asymptomatic although gastrointestinal symptoms can occur for those infected for the first time. Gastrointestinal symptoms include mild abdominal pain, nausea, vomiting, and anorexia. Infections may also be associated with skin reaction such as dermatitis. Iron-deficiency anemia due to blood loss is often associated with massive hookworm infection. The diagnosis is established through identification of eggs in feces under light microscopy. Quantitative methods of egg count (e.g., Kato-Katz) can be used to provide information on the intensity of infection. Regiments with mebendazole and albendazole are currently the treatment of choice for adult hookworms.

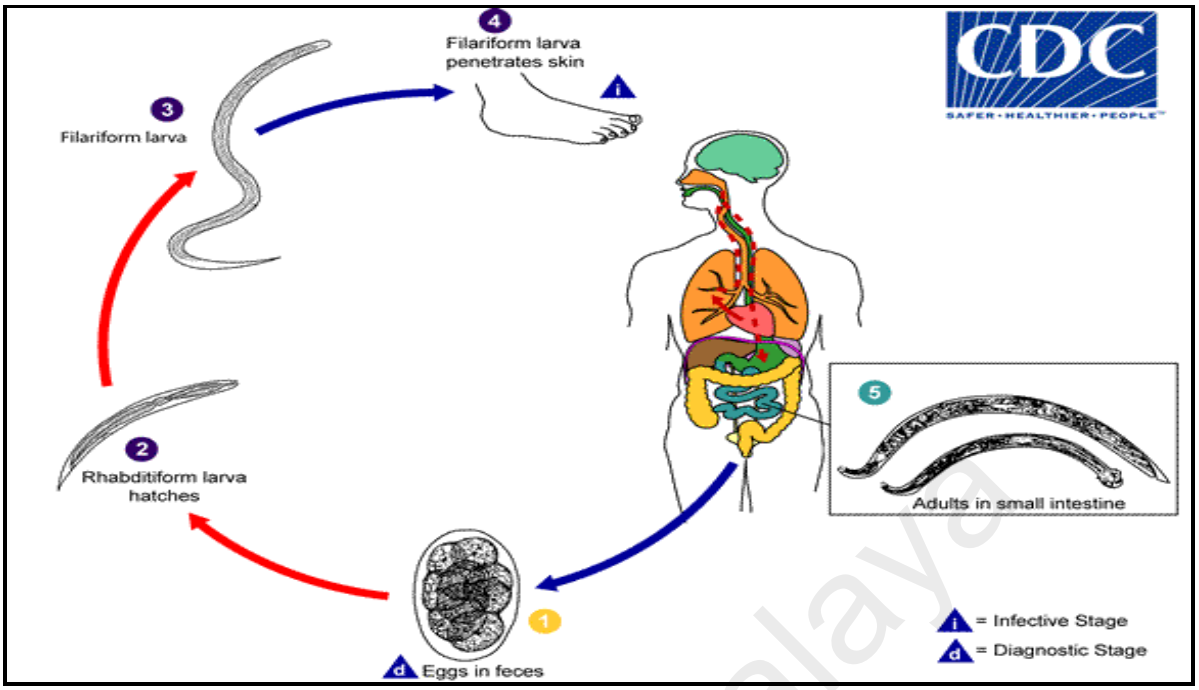

Figure 1.8: Life cycle of the hookworms (Source: DPDx, 2013).

1.4.1.3 *Trichuris trichiura*

The whipworm, *Trichuris trichiura* (Table 1.10) refers to the shape of the worm that looks like whips with wider "handles" at the posterior end. Globally, an estimated of 600 million people in the world are infected with whipworm (Hotez, 2009).

The eggs are passed out through stool of an infected person and develop into a two-cell, followed by the advanced cleavage stage before becoming an embryo in the soil. The embryonic eggs become infective in 15 to 30 days. Upon ingestion, the eggs hatch and migrate into the small intestine wall, where the larvae develop and reach adulthood. The slender anterior end burrows into the large intestine and the thicker posterior end hangs into the lumen and mates with nearby worms. The females begin to lay eggs 60 to 70 days after infection and shed about 3,000 to 20,000 eggs per day. Adults can live

about 1 to 3 years, and females can grow up to 50 mm (2 inches) long (Figure 1.9) (DPDx, 2013).

Table 1.10: Scientific classification of *Trichuris trichiura*.

Kingdom	Animalia
Phylum	Nematoda
Class	Enoplea
Order	Trichocephalida
Family	Trichuridae
Genus	*Trichuris*
Species	*Trichuris trichiura*

Source: DPDx, 2013

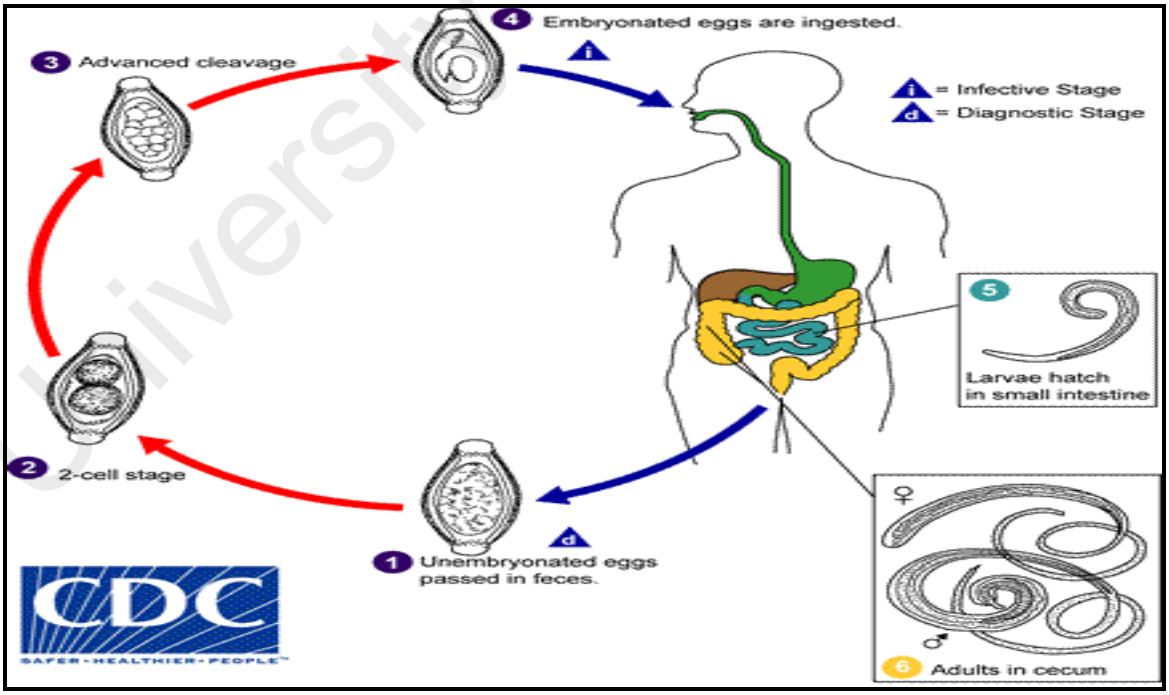

Figure 1.9: Life cycle of *Trichuris trichiura* (Source: DPDx, 2013).

Trichuriasis is common in tropical countries and poor sanitation practices. Light infections are normally asymptomatic while heavier infections include frequent, painful passage of stool that contains a mixture of mucus, water, and blood. In children, heavy infections can lead to growth retardation. Similarly, diagnosis is through microscopic examination for the feces for eggs. Mebendazole and albendazole are currently the drugs of choice for treatment of adult worms.

1.4.1.4 *Strongyloides stercoralis*

Strongyloides stercoralis (Table 1.11) is a threadworm and known to exist in all continents except for Antarctica, but is most common in the tropics, subtropics, and in warm temperate regions. The estimate global prevalence of strongyloidiasis is between 30–100 million worldwide (Olsen *et al*., 2009).

The life cycle involves a free-living cycle and parasitic cycle. The free-living rhabditiform larva passed out through the stool can either become the infective filariform larvae (direct development) or free-living adult male or female that mates and produces eggs. The filariform larvae penetrate the human skin and migrate into the small intestine to initiate the parasitic cycle. The L3 larvae are transported via the bloodstream to the lungs, where they are eventually coughed up and swallowed. In the small intestine, the larvae molt twice before becoming adult female worms. The females live threaded in the epithelium of the small intestine and through parthenogenesis produce eggs, which yield rhabditiform larvae. The rhabditiform larvae can either be passed in the stool or can cause autoinfection. In autoinfection, the rhabditiform larvae become infective and penetrate either the intestinal mucosa (internal autoinfection) or

the skin of the perianal area (external autoinfection). The filariform larvae also may disseminate throughout the body (Figure 1.10).

Table 1.11: Scientific classification of *Strongyloides stercoralis.*

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Rhabditida
Family	Strongyloididae
Genus	*Strongyloides*
Species	*Strongyloides stercoralis*

Source: DPDx, 2015

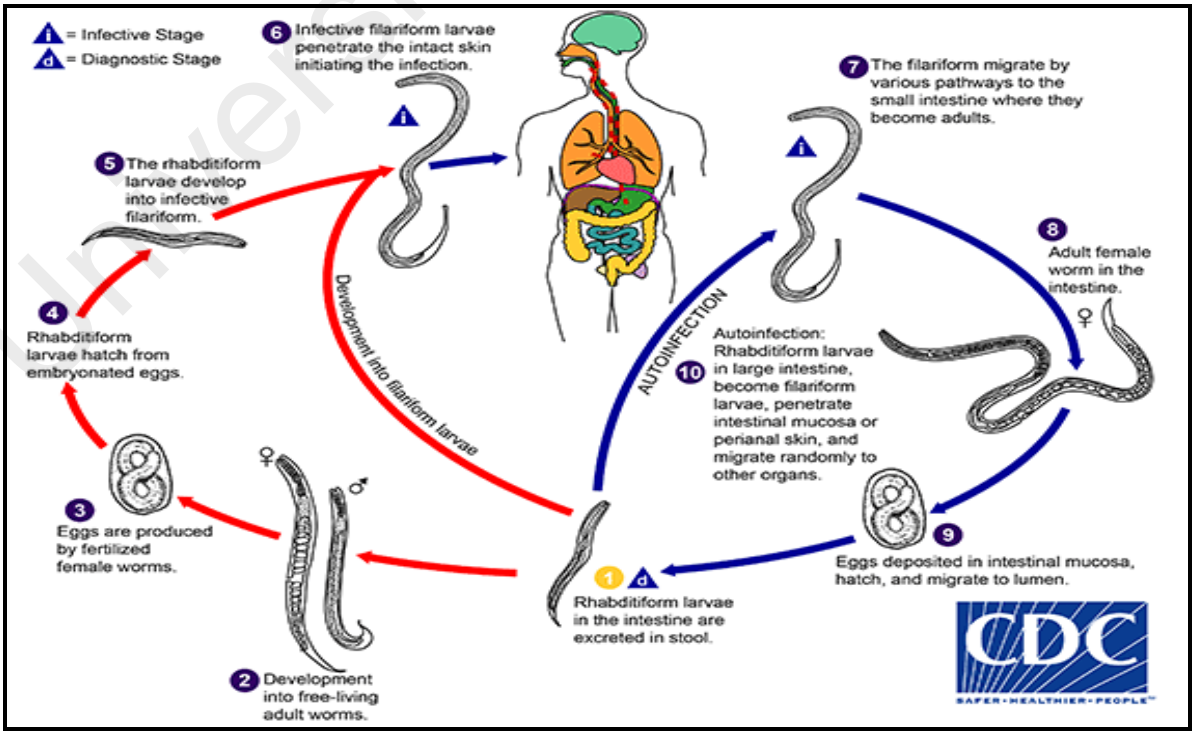

Figure 1.10: Life cycle of *Strongyloides stercoralis* (Source: DPDx, 2015).

Those infected normally showed no symptom however, in severe infections, infected persons may experience stomach ache, bloating, heartburn, intermittent episodes of diarrhea and constipation, nausea and loss of appetite, dry cough, throat irritation, itch, red rash that occurs when the worm enter the skin and recurrent raised red rash typically along the thighs and buttocks. Results of treatment for strongyloidiasis with albendazole and mebendazole vary while, ivermectin has been shown to be more effective than albendazole.

1.4.1.5 *Enterobius vermicularis*

Enterobius vermicularis (Table 1.12) or also known as pinworm is a common human parasite. The worms are small, white, and thread-like, with larger females ranging between 8-13 mm x 0.3-0.5 mm and males ranging between 2-5 mm x 0.1-0.2 mm in length. Females also possess a long, pin-shaped at the posterior end from which the parasite's name is derived. Pinworm infection occurs worldwide and affects all ages and socioeconomic background especially school-aged children and household members or caretakers with pinworm infection.

The worm is spread via fecal-oral route i.e.by the transfer of infective pinworm eggs from the anus to someone’s mouth, either directly by hand or indirectly through contaminated clothing, bedding, food or other articles. Following ingestion of infective eggs, the larvae hatches in the small intestine before maturing as adults in the colon. The time interval from ingestion to oviposition of an adult female is about 1 month. The life span of the adults is about 2 months. Gravid females migrate nocturnally outside the anus and oviposit on the skin of the perianal area. The larvae contained inside the eggs

develop (the eggs become infective) in 4 to 6 hours under optimal conditions (Figure 1.11).

Table 1.12: Scientific classification of *Enterobius vermicularis.*

Kingdom	Animalia
Phylum	Nematoda
Class	Secernentea
Order	Oxyurida
Family	Oxyuridae
Genus	*Enterobius*
Species	*Enterobius vermicularis*

Source: DPDx, 2013

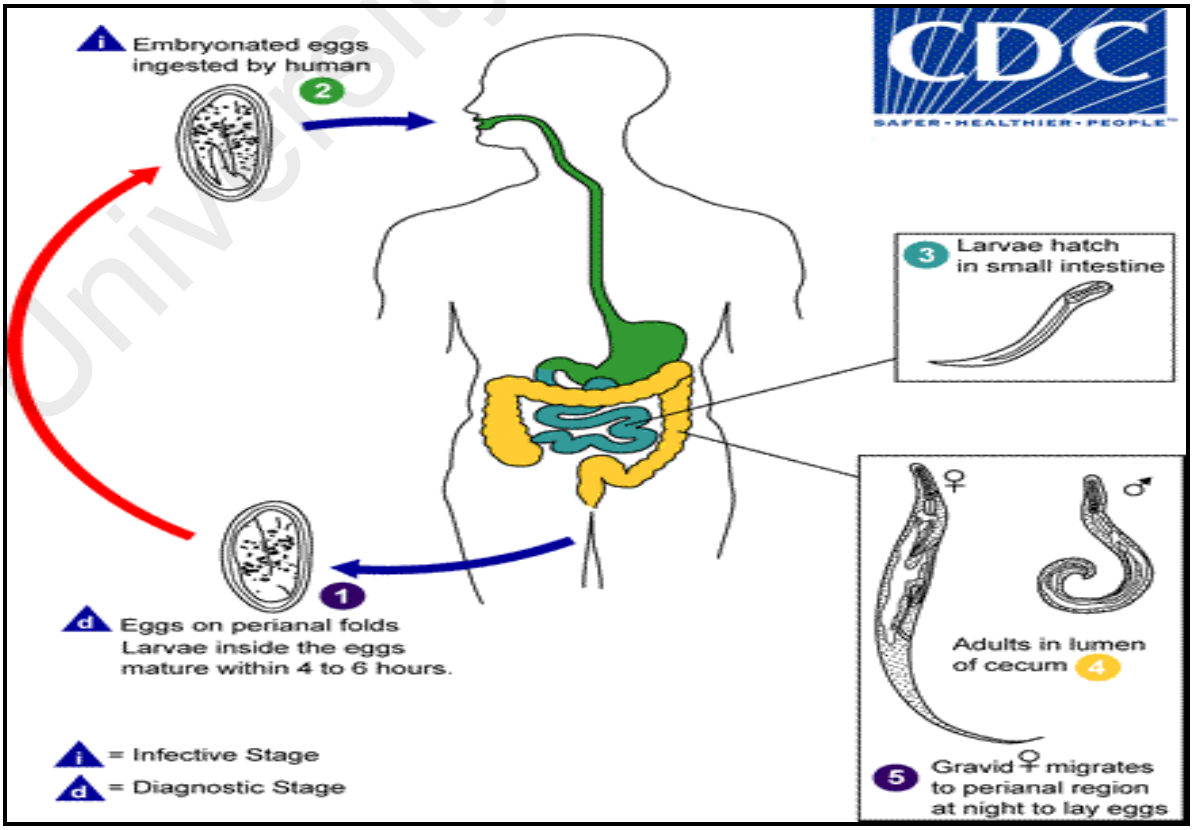

Figure 1.11: Life cycle of *Enterobius vermicularis* (Source: DPDx, 2013).

The most common method in diagnosing pinworm infection is via the "Scotch tape" test, where a clear adhesive cellulose tape is applied to the anal area early in the morning before bathing or defecation. This is then observed under a microscope for the presence of pinworm eggs. Treatment of pinworm includes mebendazole, pyrantel pamoate and albendazole. In all cases, treatment of the entire household is strongly recommended, with or without the presence of symptoms due to the fact that pinworms are easily transmitted among members of the household.

1.4.1.6 *Hymenolepis nana*

Hymenolepis nana (Table 1.13) is a small worm (adults are only 15–40 mm long) found worldwide especially in children, in persons living in institutional settings and areas where sanitation and personal hygiene is inadequate.

Infection is through accidental ingestion of contaminated foods or water, by touching mouth with contaminated fingers, ingesting contaminated soil or ingestion of an infected arthropod (intermediate host, such as a small beetle or mealworm). Eggs become infective immediately after passing out through the stool and cannot survive more than 10 days in the external environment. Upon ingestion by an intermediate arthropod host, the eggs develop into cysticercoids. Humans or rodents become infected upon ingestion of the arthropod host and develop into adults in the small intestine. Upon ingestion of eggs (in contaminated food or water or from hands contaminated with feces), the oncospheres contained in the eggs are released. The oncospheres (hexacanth larvae) penetrate the intestinal villus and develop into cysticercoid larvae. Upon rupture of the villus, the cysticercoids return to the intestinal lumen, evaginate their scoleces, attach to the intestinal mucosa and develop into adults in the ileal of the small intestine

producing gravid proglottids. Eggs are passed from proglottids in the stool through its genital atrium or when proglottids disintegrate in the small intestine. An alternate mode of infection consists of internal autoinfection, where the eggs release their hexacanth embryo, which penetrates the villus continuing the infective cycle without passage through the external environment. The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years. The life cycle is described in Figure 1.12.

Table 1.13: Scientific classification of *Hymenolepis nana*.

Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Cyclophyllidea
Family	Hymenolepididae
Genus	*Hymenolepis*
Species	*Hymenolepis nana*

Source: DPDx, 2013

Similarly, infections are asymptomatic although some may experience nausea, weakness, loss of appetite, diarrhea and abdominal pain. In patients with heavy infection may develop headache, itchy bottom or difficulty in sleeping. Diagnosis is via identification of eggs in stool. Treatment is with a prescription drug called praziquantel that causes the tapeworm (both adults and larvae) to dissolve.

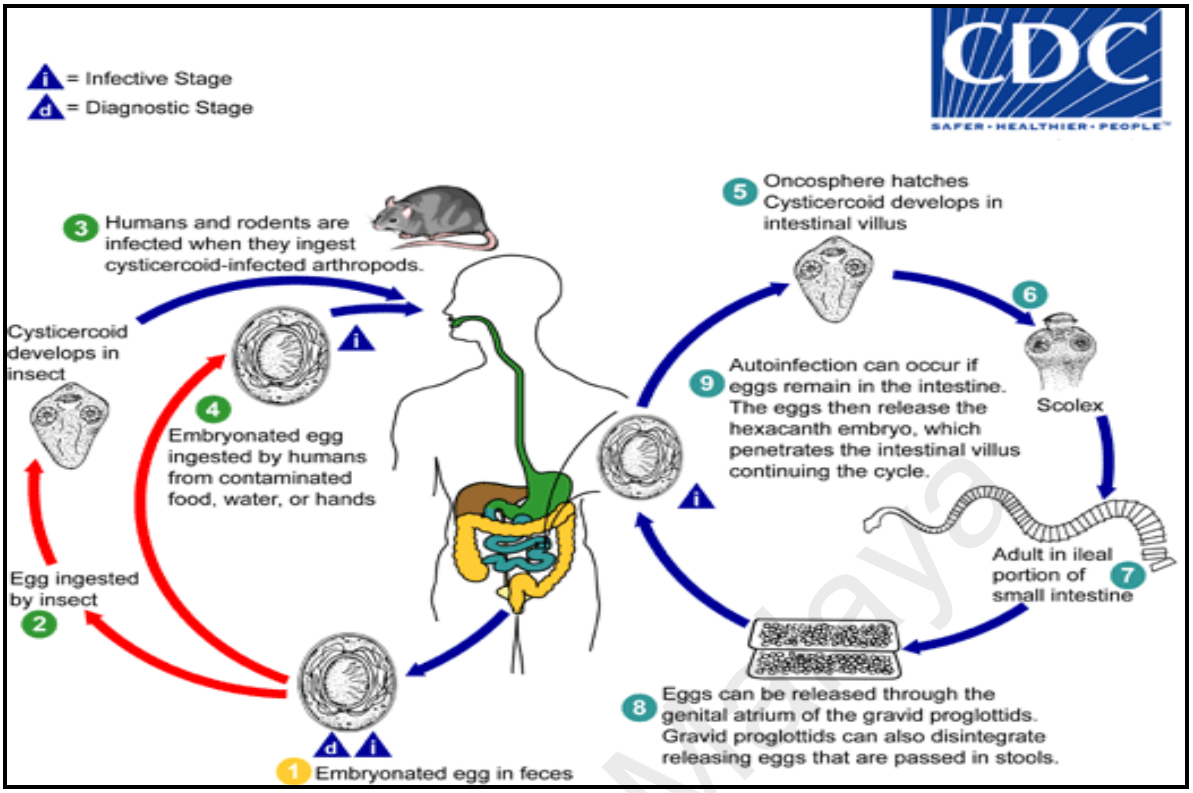

Figure 1.12: Life cycle of *Hymenolepis nana* (Source: DPDx, 2013).

1.4.2 Protozoa

Most protozoan species are free living and but the small numbers of parasitic species infect higher animals with one or more species. Protozoa are microscopic unicellular eukaryotes with a relatively complex internal structure and carry out complex metabolic activities. Some have structures for propulsion or other modes for movement (Yaeger, 1996). Infection ranges from asymptomatic to life threatening and dependent on the species, strain and resistance of the host.

The pathogenic species include the amoeba, *Entamoeba histolytica* and the flagellate, *Giardia duodenalis*. Although many other species are known to cause intestinal disease throughout the world, such as *Cryptosporidium* parvum, *Cyclospora*

cayetanensis and *Balantidium coli*, the importance of these organisms remains unclear as most are considered non-pathogenic.

1.4.2.1 *Entamoeba* spp.

Species in the genus *Entamoeba* (Table 1.14) colonizes humans, but not all are associated with disease. Six human intestinal species *Entamoeba* spp. include *Entamoeba histolytica, E. dispar, E. moshkovskii, E. coli, E. hartmanni* and *E. polecki*. Amoebiasis is a global health problem caused by the protozoan *E. histolytica* and is well recognized as a pathogenic amoeba associated with intestinal and extraintestinal infections. *E histolytica* is a pseudopod-forming, non-flagellated protozoal parasite that causes proteolysis and tissue lysis and can induce host-cell apoptosis. Worldwide, approximately 50 million cases of invasive *E histolytica* disease occur each year with high incidences in developing countries (Stauffer *et al*., 2006) resulting in as many as 100,000 deaths with only 10%-20% infected individuals become symptomatic (Valenzuela *et al*., 2007; Van Hal *et al*., 2007; Ximenez *et al*., 2009).

Entamoeba is transmitted via ingestion of the cystic form (infective stage). Cysts and trophozoites are passed in feces with cysts typically found in formed stool, whereas trophozoites in diarrheal stool. Infection occurs by ingestion of mature cysts in feces contaminated food, water, or hands. Excystation occurs in the small intestine releasing trophozoites that migrate to the large intestine. Trophozoites multiply by binary fission and produce cysts, and both stages are passed out through the feces. The cysts can survive days to weeks in the external environment due to the protection conferred by their walls, and are responsible for transmission. Trophozoites are rapidly destroyed once outside the body, and if ingested, would not survive exposure to the gastric

environment. In many cases, the trophozoites remain confined to the intestinal lumen (noninvasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool (Figure 1.13).

Table 1.14: Scientific classification of *Entamoeba* spp.

Kingdom	Protista
Phylum	Amoebozoa
Class	Archamoebae
Order	Amoebida
Family	Endamoebidae
Genus	*Entamoeba*
Species	*Entamoeba histolytica* *Entamoeba dispar* *Entamoeba coli*

Source: DPDx, 2013

Amoebiasis exhibits a wide spectrum symptom. Invasive extraintestinal amebiasis cause liver abscess, peritonitis, pleuropulmonary abscess, cutaneous and genital amebic lesions. Diagnosis is commonly through employment of immunologic techniques in addition to standard blood tests and other laboratory studies (microscopy, culture, serologic testing, and polymerase chain reaction (PCR) assay). Treatment of amebiasis includes pharmacologic therapy, surgical intervention, and preventive measures.

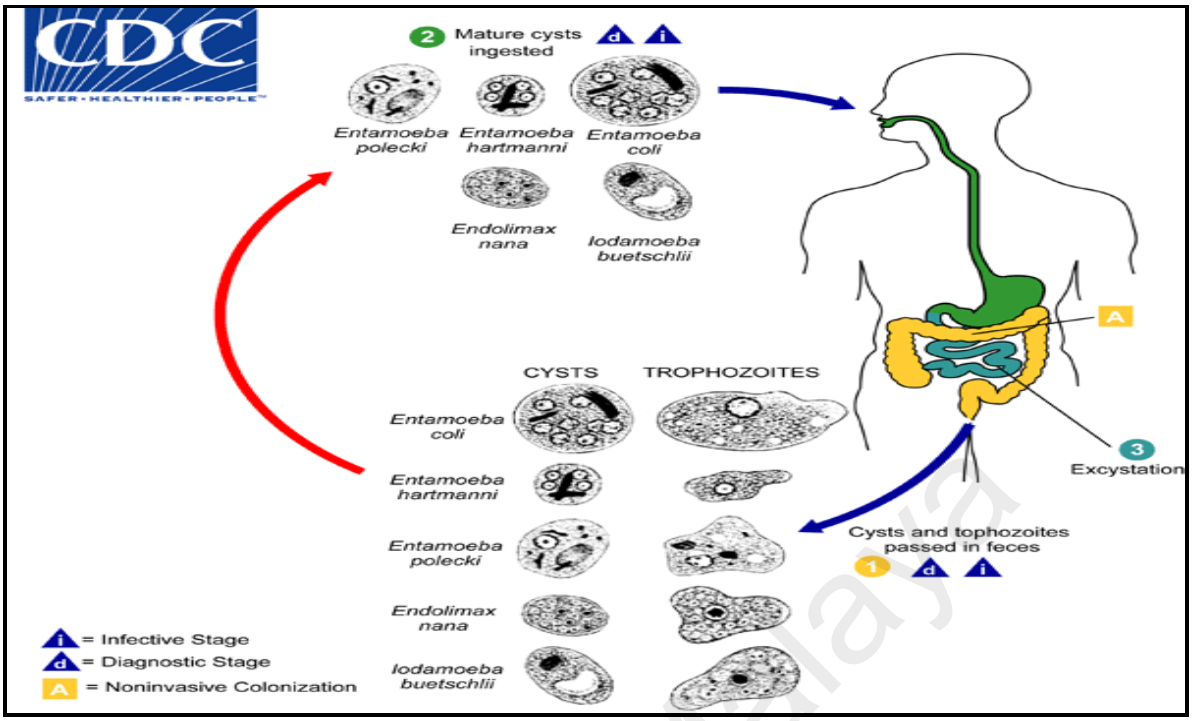

Figure 1.13: Life cycle of *Entamoeba* spp. (Source: DPDx, 2013).

1.4.2.2 *Giardia* sp.

Giardia (Table 1.15) is one of the most common microscopic parasitic gastrointestinal diseases that cause diarrheal illness known as giardiasis. It is estimated that 20,000 cases occur each year in the U.S. alone and prevalence of infection ranging from 20% to 30% globally (CDC, 2010). There are two forms; the active form or trophozoite, and the inactive form called a cyst. The active trophozoite attaches to the lining of the small intestine with an attachment sucker causing the signs and symptoms of giardiasis.

The infection is spread from person to person by contamination of food with feces, or by direct fecal-oral contamination. Cysts are resistant and responsible for the transmission of giardiasis. Both forms are present in the feces (diagnostic stages). The cysts are hardy and can survive several months in cold water. In the small intestine, the

excystation of cysts release trophozoites (each cyst produces two trophozoites). The trophozoites multiply through longitudinal binary fission and can remain in the lumen of the proximal small bowel either freely or attached to the mucosa via a ventral sucking disk. The encystation process occurs as the parasites transit toward the colon. The cyst is most common found in non-diarrheal feces (Figure 1.14).

Table 1.15: Scientific classification of *Giardia* sp.

Kingdom	Protista
Phylum	Sarcomastigophora
Class	Zoomastigophora
Order	Diplomonadida
Family	Hexamitidae
Genus	*Giardia*
Species	*Giardia duodenalis*

Source: DPDx, 2013

Symptoms vary greatly from asymptomatic to diarrhea, gas or flatulence, greasy stool that float, stomach or abdominal cramps, upset stomach or nausea, dehydration and weight loss. Diagnosis is through the identification of cyst in stool. Effective treatments include administration of metronidazole, tinidazole and nitazoxanide or alternatively, paromomycin, quinacrine, and furazolidone (Escobedo & Cimerman, 2007).

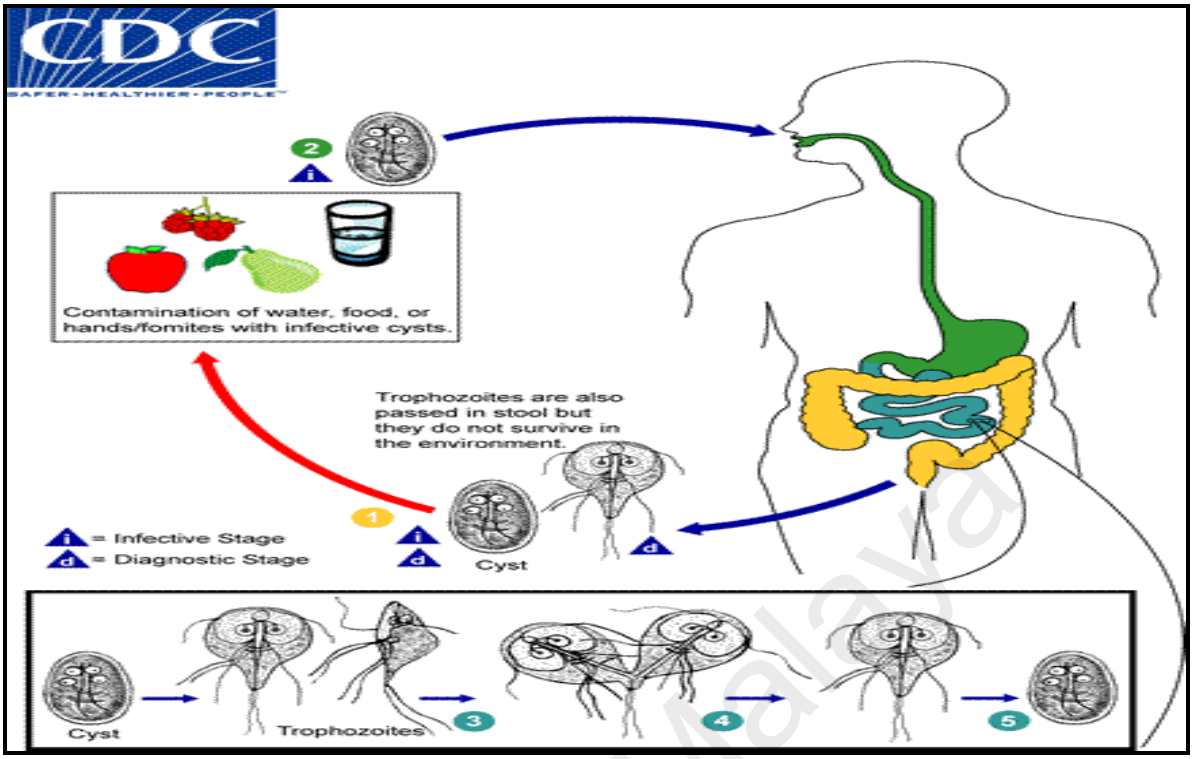

Figure 1.14: Life cycle of *Giardia* sp. (Source: DPDx, 2013).

1.4.2.3 *Cryptosporidium* spp.

Cryptosporidium (Table 1.16) is a microscopic parasite affecting an estimated 748,000 persons each year (Scallan *et al*., 2011).

Sporulated oocysts, containing 4 sporozoites are excreted through feces and possibly other routes such as respiratory secretions. However, transmission of *Cryptosporidium parvum* and *C. hominis* occur mainly through contact with contaminated water (e.g., drinking or recreational water). Following ingestion (and possibly inhalation) of cysts, excystation release sporozoites which subsequently parasitize epithelial cells of the gastrointestinal tract or other tissues such as the respiratory tract. Within these cells, the organism undergoes firstly asexual multiplication (schizogony or merogony) before sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts

(female). Upon fertilization, the oocysts sporulate in the infected host into two types; a thick-walled commonly excreted out from the host, or a thin-walled oocyst primarily involved in autoinfection. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission (Figure 1.15).

Table 1.16: Scientific classification of *Cryptosporidium* spp.

Kingdom	Protista
Phylum	Sarcomastigophora
Class	Conoidasida
Order	Eucoccidiorida
Family	Cryptosporidiidae
Genus	*Cryptosporidium*
Species	*Cryptosporidium parvum* *Cryptosporidium hominis*

Source: DPDx, 2013

Symptoms generally appear 2 to 10 days (average 7 days) after infection with watery diarrhea, stomach cramps or pain, dehydration, nausea, vomiting, fever and weight loss. Diagnosis of cryptosporidiosis is via examination of stool samples using several techniques (e.g., acid-fast staining, direct fluorescent antibody [DFA], and/or enzyme immunoassays for the detection of *Cryptosporidium* sp. antigens) and molecular methods. Those with healthy immune systems will recover without treatment. Diarrhea can be managed by drinking plenty of fluids to prevent dehydration.

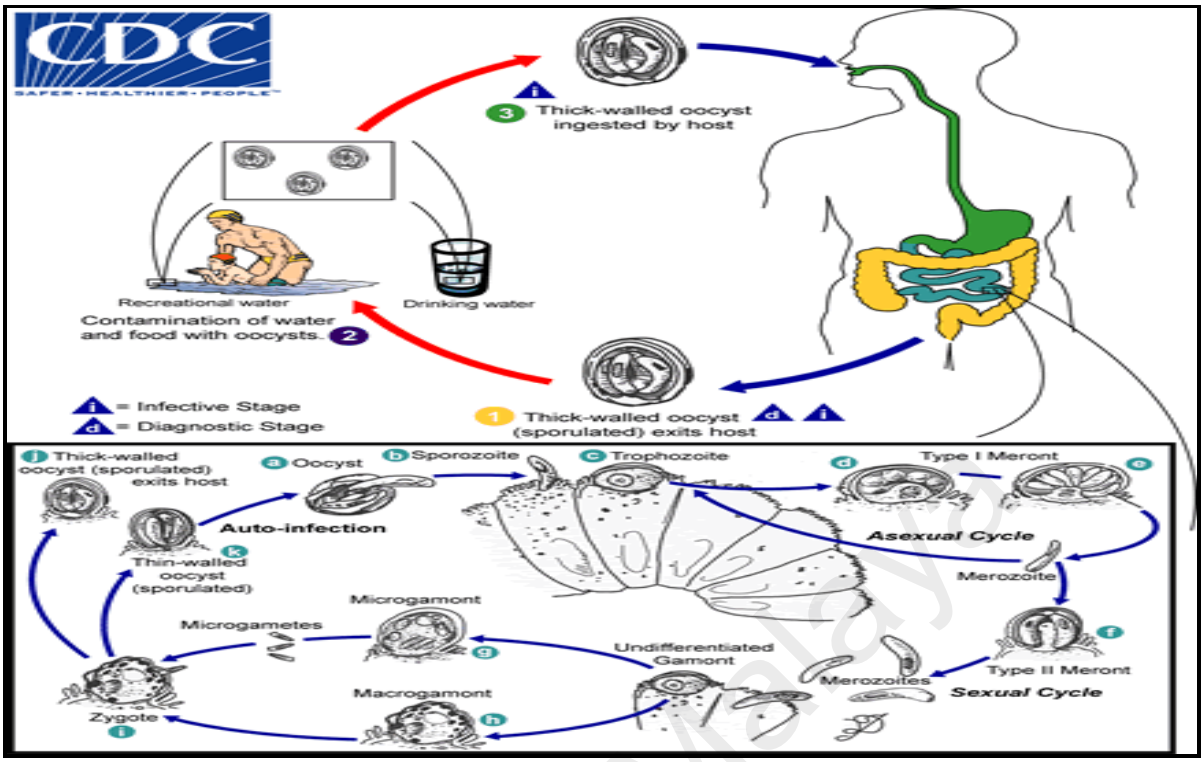

Figure 1.15: Life cycle of *Cryptosporidium* spp. (Source: DPDx, 2013).

1.4.2.4 *Toxoplasma gondii*

Toxoplasma gondii (Table 1.17) is a microscopic protozoan infecting more than 60 million people in the United States (Feldman, 1974; Montoya & Liesenfeld, 2004; Hill *et al*., 2005). Those with healthy immune response often do not have symptoms however, 10–20% of patients develop sore lymph nodes, muscle pains and other minor symptoms that last for several weeks (acute toxoplasmosis). The parasites remain in the body as tissue cysts (bradyzoites) and reactivate, if the person becomes immunosuppressed by other diseases or by immunosuppressive drugs.

The only known definitive hosts for *Toxoplasma gondii* are members of the family Felidae (domestic cats and their relatives). Unsporulated oocysts shed in the cat's feces take 1 to 5 days to sporulate in the environment to become infective. Intermediate hosts

(including birds and rodents) become infected after ingesting contaminated soil, water or plant material with oocysts. Oocysts transform into tachyzoites shortly after ingestion and localize in neural and muscle tissue and further develop into tissue cyst bradyzoites. Cats become infected after consuming intermediate hosts harboring tissue cysts or by direct ingestion of sporulated oocysts. Humans become infected either through consumption of undercooked meat harboring tissue cysts, food or water contaminated with cat feces, blood transfusion or organ transplantation and transplacental transmission from mother to fetus. In the human host, the parasites form tissue cysts most commonly in skeletal muscle, myocardium, brain, and eyes and the cysts may remain throughout the life of the host (Figure 1.16).

Table 1.17: Scientific classification of *Toxoplasma gondii*.

Kingdom	Protista
Phylum	Apicomplexa
Class	Sporozoasida
Order	Eucoccidiorida
Family	Sarcocystidae
Genus	*Toxoplasma*
Species	*Toxoplasma gondii*

Source: DPDx, 2015

Diagnosis is usually achieved through serology test, although tissue cysts may be observed in stained biopsy specimens. Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods. A

number of drug therapies are available for treatment of acute toxoplasmosis. The recommended treatment for acute human toxoplasmosis is a combination of the anti-malarial medication pyrimethamine and the antibiotic sulfadiazine (Guerina *et al*., 1994). Cysts respond to treatments with atovaquone and clindamycin (Djurkovic-Djakovic *et al*., 2002).

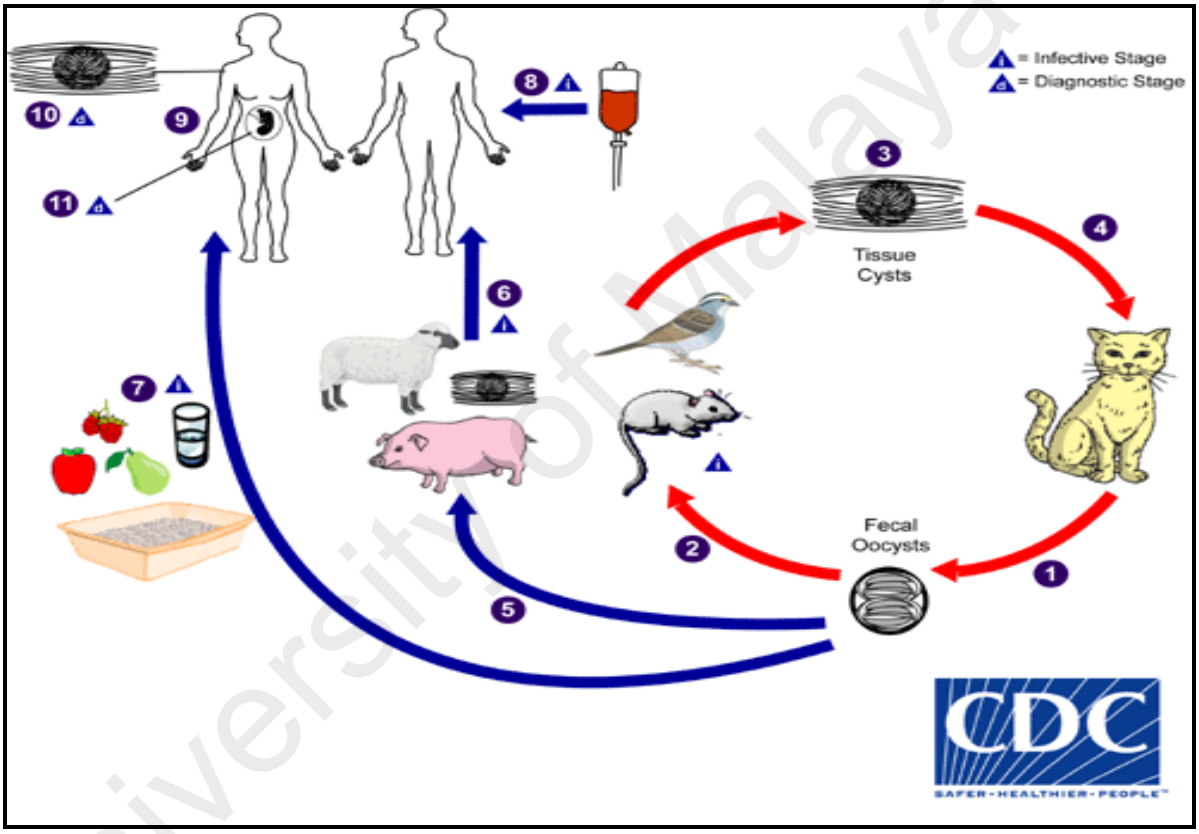

Figure 1.16: Life cycle of *Toxoplasma gondii*. (Source: DPDx, 2015).

1.5 Studies on the status of parasitic infections amongst migrant workers

Many health status studies of parasitic infections amongst migrant workers have been conducted worldwide, particularly in Asia (Thailand (Nuchprayoon *et al*., 2009; Ngrenngarmlert *et al*., 2012), Taiwan (Lo & Lee, 1996; Wang, 1998; 2004; Lu & Sung, 2008; Hsieh *et al*., 2011), Taipei (Cheng & Shieh, 2000)) and in the Middle East primarily in the Kingdom of Saudi Arabia (Abha district (Al-Madani & Mahfouz, 1995), Riyadh (Kalantan, 2001), Al-Khobar (Abahussain, 2005), Makkah (Wakid *et al*., 2009), Al-Baha (Mohammad & Koshak, 2011) and Medina (Taha *et al*., 2013)). Meanwhile in Qatar, Abu-Madi *et al.* (2008; 2010; 2011) extensively studied the parasitic infections in migrant workers. In Malaysia, a large study scope was conducted more than a decade ago (Zaini *et al*., 2002) that included physical examination and basic haematological findings (Chia, 2002), microbiological analysis (Ngeow *et al*., 2002), health status of woman respondent (Siti Norazah, 2002a), sexual health (Siti Norazah, 2002b), oral health problem (Ishak, 2002), lifestyle habit and risk behaviors (Wong, 2002) and psychiatric morbidity (Mohd Hussain, 2002). However, limited studies were conducted on parasitic infections (Suresh et al., 2002; Kamarulzaman & Khairul Anuar, 2002; Zurainee *et al*., 2002; Khairul Anuar *et al*., 2002; Zurainee, 2002 & Rajah *et al*, 2002).

1.5.1 Studies in Asia

In Thailand, most studies focused among workers from Myanmar. A cross-sectional survey showed that the overall prevalence of intestinal parasitic infections was low (13.6%) primarily with helminth infections (10.3%) being more common than protozoa (8.5%) particularly with *E. histolytica/dispar* (3.8%), followed by *A. lumbricoides* (3.3%), and *T. trichiura* (2.3%) (Ngrenngarmlert *et al*., 2012). Another study focused

on Myanmar workers in the food industry reported with more than half screened were infected (62.3%) with *Blastocystis hominis* (41.5%) followed by *T. trichiura* (22.2%), *G. lamblia* (14.1%) and *A. lumbricoides* (1.8%) (Nuchprayoon *et al*., 2009).

In Taiwan, 20.2% (188/932) immigrants were reported infected with *B. hominis* (Lu & Sung, 2008). While another study in Taiwan showed low infection (7.4%, 214/2875) among foreigners, with hookworm (2.92%) being the highest followed by *T. trichiura* (1.18%), and *B. hominis* (1.14%) (Hsieh *et al*., 2011). In Taipei, of the 302 Thailand laborers examined, 196 (64.9%) were infected with *Opisthorchis viverrini* (43.0%) followed by hookworm (38.4%) and *S. stercoralis* (13.9%) (Cheng & Shieh, 2000).

In Saudi Arabia, many studies were conducted throughout the kingdom, Abha district (Al-Madani & Mahfouz, 1995), Riyadh (Kalantan, 2001), Al-Khobar (Abahussain, 2005), Makkah (Wakid *et al*., 2009), Al-Baha (Mohammad & Koshak, 2011), and Medina (Taha *et al*., 2013). More recently, in Medina (Taha *et al*., 2013) of the 2732 stool samples collected and screened, 407 (14.9%) stool samples were positive with intestinal parasites particularly among farmers, food handlers and shepherds and those who originated from Pakistan (23.2%). Prevalence of *G. lamblia* (21.9%) infections was the highest, followed by *E. histolytica/ E. dispar* (17.8%), *T. trichiura* (16.2%), *A, lumbricoides* (15.8%), hookworm (13%), *H. nana* (8.9%), *S. sterocoralis* (3.5%), *Schistosoma mansoni* (2.2%) and *E. vermicularis* (0.43%) (Taha *et al*., 2013). Infections were lower among expatriate workers in cities, such as Al-Khobar (31.4%) (Abahussain, 2005). In Al-Khobar, Abahussain (2005) reported that *A. lumbricoides* infection was common among Indonesians, *T. trichiura* and *E. histolytica* were common among Philippines and hookworms among Sri Lankan workers. Another study by Al-

Madani & Mahfouz (1995) reported 46.5% of Asian female domestic workers were positive with parasitic infection. In Makkah, 31.9% of food handlers working in the hospitals were positive with parasitic infections (Wakid *et al*., 2009). Similar to the report by Taha *et al*. (2013), in Makkah (Wakid *et al*., 2009), *G. lamblia* was the most prevalent infection encountered in the examined populations.

In Qatar, Abu-Madi *et al*. reported the occurrence of parasitic infections among migrant workers in three subsequent years; 33.9% in 2008, 10.2% in 2009 and 21.5% in 2011. In 2008, seven species of intestinal parasitic infections (IPI) amongst food handlers and housemaids from different geographical regions were recorded with three nematode species (13.6%) i.e. *T. trichiura*, hookworms and *A. lumbricoides* and four protozoans (24.8%) (*E. histolytica/dispar*, non-pathogenic *Entamoeba*, *Blastocystis hominis* and *G. duodenalis*). In 2009, Abu-Madi *et al*. looked into the trends of IPI among long-term-residents and settled immigrants after introduction of routine albendazole treatment as a condition of entry, residence and issuance of a work permit. Results reported low infection rate (10.2%) with at least one species of intestinal parasite (2.6% with helminths and 8.0% with protozoan species). Infections were primarily found in 20-30 year old male subjects from Nepal with hookworms (69%). While in 2011, Abu-Madi *et al*. compared IPI between newly arrived and resident workers in Qatar and results showed that 21.5% of the subjects were infected with at least one of the species recorded (8 helminthes and 4 protozoan species) with the high infection being hookworms (8.3%). Most helminth infections declined in subjects that acquired residency status in Qatar, especially among female subjects, however one male Nepalese worker continued to harbor helminth infections (notably hookworms) after he became resident.

1.5.2 Migrant health status studies in Malaysia

Health status of foreign workers was conducted more than a decade ago (Zaini *et al*., 2002) that included physical examination and basic haematological (Chia, 2002), parasitic infection (Suresh *et al*., 2002; Kamarulzaman & Khairul Anuar, 2002; Zurainee *et al*., 2002; Khairul Anuar *et al*., 2002; Zurainee, 2002 & Rajah *et al*, 2002), microbiological (Ngeow *et al*., 2002), health status of women (Siti Norazah, 2002), male sexual health (Siti Norazah, 2002), oral health problem (Ishak, 2002), lifestyle habit and risk behaviors (Wong, 2002) and psychiatric morbidity (Mohd Hussain, 2002).

Among the results reported were 36% from 173 stool samples were infected with *Blastocystis hominis* (Suresh *et al*., 2002) with smaller forms (3-5 µm in size) of *B. hominis* in Indonesian immigrants compared to other nationalities (10-15 µm) (Rajah *et al*., 2002). One case of visceral leishmaniasis (Kala-azar) in a 28 years old Bangladesh migrant worker was also reported (Kamarulzaman & Khairul Anuar, 2002).

Eight serological tests were performed on 698 foreign workers including amoebiasis, echinococcosis, filariasis (*Brugia malayi* and *Wuchereria bancrofti*), leishmaniasis, malaria, schistosomiasis and trypanosomiasis (Zurainee, 2002; Zurainee *et al*., 2002) with 38.1% positive with at least one parasitic infection. One Bangladeshi was found positive for *Wuchereria bancrofti* and an Indonesian positive for malaria (*Plasmodium falciparum*) from the 241 blood samples screened (Khairul Anuar *et al*., 2002).

1.6 Justification of the study

To date, the only study to determine the health status among foreign workers in Malaysia was conducted by Zaini *et al.* (2002) more than a decade ago. This study provided useful methodological enquiries however, was not designed for policy recommendations. Over the last decade, the number of migrant workers has grown exponentially. Despite compulsory medical screening for workers prior to entering the Malaysian workforce, screening for parasitic infections is grossly inadequate or lacking. Therefore, there is an acute need for more accurate and up-to-date information on the parasitic infections in this particular group of workers and an understanding of the factors associated with transmission of these infections, especially as they are likely to impact significantly upon the local community through close contact, lost productivity and the heighten cost of healthcare. This study therefore is timely in adopting a scientific approach to address an important public health problem and to provide conclusions that can inform the design of effective public health policies.

1.7 Objectives

The main objective of this study is to analyze the current parasitic infections status among migrant workers in Malaysia and to evaluate the health dangers posed by the migrant workers to the local population.

The specific objectives of the present study are as follows:

- To analyze the socio-demographic profile of the respondents in the present study in Malaysia.
- To determine the intestinal parasitic infections from stool samples among migrant workers.
- To determine the status of parasitic infections among migrant workers by using serological tests.
- To associate the prevalence of infections in the migrant populations to factors such as host age, gender, geographical origin and employment sector with a view to provide a descriptive epidemiological assessment.
- To characterize parasite using molecular techniques where appropriate.

CHAPTER 2: MIGRANT WORKERS IN MALAYSIA: SOCIO-DEMOGRAPHY BACKGROUND

2.1 Introduction

Mass migration from less developed to more developed country has created a shift in the global population. Urbanization and extensive industrialization of developing nations have resulted in millions of migrants travelling to major urban cities around the globe to join the expanding workforces. The International Labour Organization (ILO) estimates that there are approximately 232 million international migrant workers worldwide. Globalization, demographic shifts, conflicts, income inequalities and climate change are some of the influences that drive workers and their families to cross borders in search of better employment and security (International Labour Organization, 2015). In Malaysia, the robust economic growth of the different sectors has led to the mushrooming of small to large enterprises requiring high demand of a low-skilled workforce primarily in sectors such as construction, domestic, food services, manufacturing and plantation. This has attracted many to flock to the country both legally and illegally (Ministry of Human Resources, 2015; Bardan, 2014) from South East Asian (Indonesia, Cambodia, Vietnam, the Philippines and Myanmar) and South Asian countries (Nepal, India and Bangladesh) (Ministry of Human Resources, 2015; Bardan, 2014) where endemic infections are very much prevalent and most likely to pose public health problems to the local community (Abu-Madi *et al*., 2008; 2010; 2011; Taha *et al*., 2013).

Malaysia is a middle-income country whose economy has transformed into an emerging multi-sector economy and since the 1970s it has been facilitated largely by

imported migrant workers. Malaysia has a higher standard of living compared with other neighbouring countries in the South East Asian and South Asian region. A total of 74.7% of the population in Malaysia has undergone urbanization with 2.66% annual rate of change (2010 - 2015) (Central Intelligence Agency, 2016). Access to sanitation facilities in Malaysia has improved also in both urban and rural areas up to 96.0% of the population. Meanwhile drinking water sources have improved up to 98.2% of the population (Central Intelligence Agency, 2016). The percentage of the population in Malaysia still living below the poverty line is 3.8%, considerably lower than that of other nationalities recruited in the present study including Myanmar (32.7%), Bangladesh (31.5%), India (29.8%), Nepal (25.2%), Vietnam (11.3%) and Indonesia (11.3%). The push factors for migration include poor remuneration and slim employment opportunities in their home country. Meanwhile the main factors for choosing Malaysia as a destination country are perceived abundant opportunities, high wage levels and attractive job offers (Abdul-Aziz, 2001).

In Malaysia, Zaini *et al*. (2002) conducted a study to determine the health status of migrant worker more than a decade ago involving clinical subjects from University Malaya Medical Centre (UMMC) and Peremba Group of Companies (PEREMBA), which provided useful methodological enquiries. In the last decade, there has been an influx in number of migrant workers from 1.06 in 2002 to 2.07 million in 2014 to Malaysia in the seeking for better job opportunities. This has lead to a change in the demographic background of the working forces in this country. Therefore, the objective of this chapter is to determine the socio-demographic profile of the migrant respondent in the different working sectors in Malaysia.

2.2 Materials and Methods

2.2.1 Subjects

Migrant workers in Malaysia primarily employed in the low skilled and semi-skilled of five working sectors only, namely manufacturing, food services sector, agriculture and plantation, construction and domestic work were selected as the main study subjects in this study. Workers who voluntarily participated in this study were recruited from September 2014 to August 2015 from agencies and companies in Peninsular Malaysia. A minimum sample size using Leedy and Ormrod (2001) was calculated based on the last study of intestinal parasitic infections in migrant workers (36%) in Malaysia (Zaini *et al*., 2002). Using this formula, a minimum of 355 subjects were required however, a total of 610 migrant workers were successfully recruited.

2.2.2 Questionnaire

Questionnaires were distributed to gather relevant information related to the study to all respondents. An individual clinical interview with questionnaire was performed in order to collect information pertaining socio-demographic (nationality, sex, age, religion, marital status, educational level and employment sector), migration history (area in country origin, years of residence in Malaysia, mode of entry, working history), environmental health (current residential area, type of accommodation, amenities), life-style habits (smoker, consumption of alcohol and illegal drugs), access to healthcare and episodes of illness (health care utilization, mode of payment, health history) and occupational health and safety (safety hazard, personal protective equipment). The interview process was performed through an interpreter in situations where the respondents had difficulty in understanding Malay or English. All

participants were fully informed of the nature of the study to enable maximum co-operation and completed the consent forms (Appendix B).

2.2.3 Ethical considerations

An 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 respondents were adults and made aware of the nature of the study and provided written consent to participate in the study.

2.2.4 Data analysis

Data were statistically analyzed using SPSS software statistic program version 22 to determine values prevalence of the set factors. Figures and tables were constructed using the Descriptive Statistics of the software. Factors were analyzed with each other to calculate prevalence and presented in tables and figures.

2.3 Results

2.3.1 Socio-demographic profile

A total of 610 migrant workers from five working sectors in Malaysia were successfully recruited. The highest number of volunteers came from the domestic (n=148; 24.3%) workers, followed by construction (n=139; 22.8%), food service (n=128; 21.0%), plantation (n=102; 16.7%) and manufacturing sector (n=93; 15.2%). Six nationalities were recruited with a majority originating from Indonesia (n=302, 49.5%), followed by Bangladesh (n=117, 19.2%), Nepal (n=100, 16.4%), India (n=64, 10.5%), Myanmar (n=26, 4.3%) and Vietnam (n=1; 0.2%). The majority of the volunteers were Muslims (74.9%) with more than half married (67.4%). The socio-demographic description of volunteers (sex, age, educational level, religion and marital status) is shown in Table 2.1.

Analysis of the employment sectors by educational level showed most workers had basic primary level education. Workers from the manufacturing and food service sector had most workers with higher education with 88.2% and 63.3%, respectively. Meanwhile, the plantation sector were dominated by workers with primary education (72.5%) and with no formal education (27.5%) (Figure 2.1). Those involved in the domestic sector were primarily Indonesian (144/148, 97.3%). Indonesian workers also dominated the construction sector (54.0%) followed by Bangladeshi (34.5%), Burmese (7.9%) and Indian (3.6%). In manufacturing sector, Nepalese (76/93; 81.7%) were mostly employed (Figure 2.2).

Table 2.1: Demographic profile of migrant workers according to sex, age, employment sector, nationality, educational level, religion and marital status. (N=610)

Factors		Total samples	Percentage (%)
Sex	**Male**	474	77.7
	Female	136	22.3
Age	**<25**	168	27.5
	25-34	239	39.2
	35-44	148	24.3
	45-54	40	6.6
	>55	15	2.5
Employment Sector	**Construction**	139	22.8
	Manufacturing	93	15.2
	Plantation	102	16.7
	Food Service	128	21.0
	Domestic	148	24.3
Nationality	**Indonesia**	302	49.5
	Bangladesh	117	19.2
	Myanmar	26	4.3
	Vietnam	1	0.2
	India	64	10.5
	Nepal	100	16.4
Educational Level	**Primary**	297	48.7
	Secondary	227	37.2
	University	12	2.0
	No formal schooling	74	12.1
Religion	**Muslim**	457	74.9
	Buddhist	22	3.6
	Hindu	121	19.8
	Christian	10	1.6
Marital status	**Married**	411	67.4
	Divorced	8	1.3
	Single	191	31.3

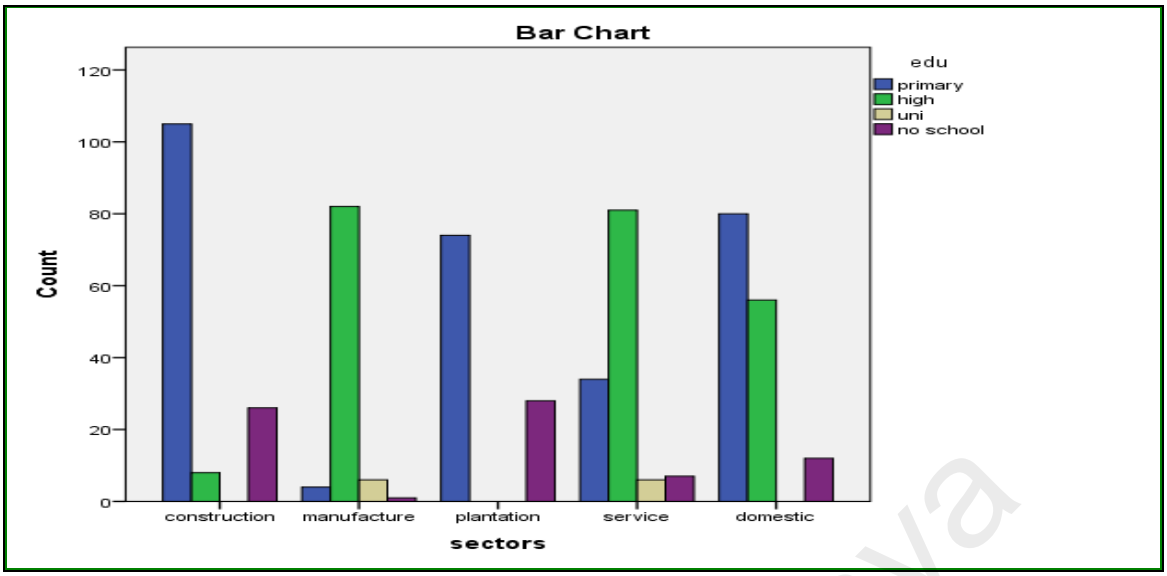

Figure 2.1: Education profile of migrant workers according to employment sectors.

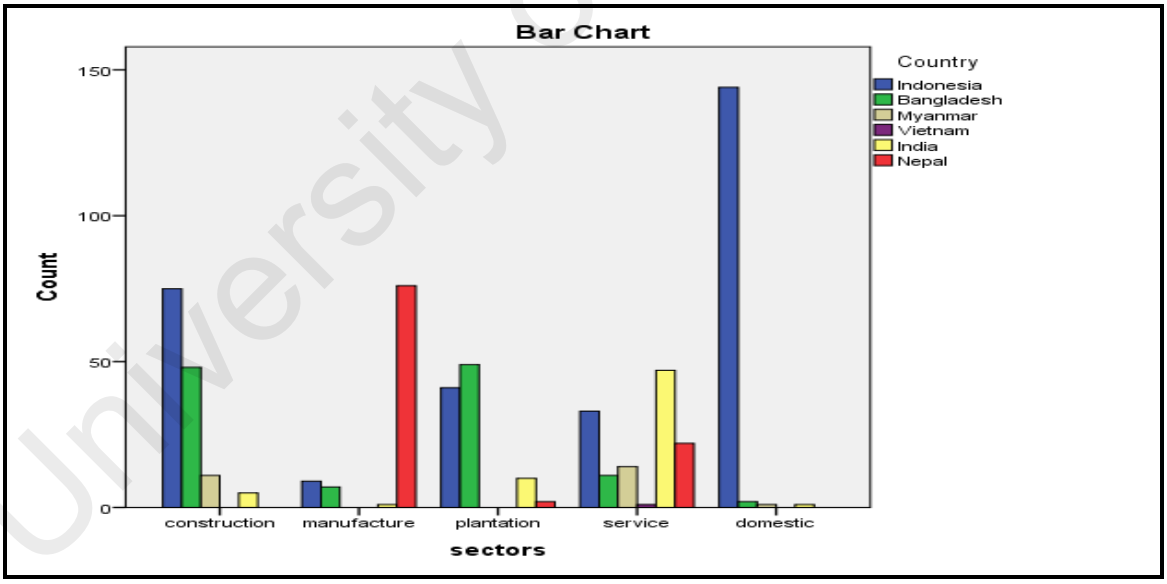

Figure 2.2: Worker's nationality profile in relation to employment sectors

2.3.2 Migration history

Most workers in Malaysia originated from less developed countries with majority (n=564/610; 92.5%) from rural areas, followed by workers who came from the city (n=42; 6.9%) and from the inland area (n=4; 0.7%) (Figure 2.3). Of the total, three quarters traveled by air (n=458; 75.1%) especially those from Indonesia (n= 157; 34.3%), followed by Bangladesh (n=117; 25.5%), Myanmar (n=20; 4.4%), India (n=64; 14%) and Nepal (n=100; 21.8%). The rest travelled to this country by sea (n=152; 24.9%) [Indonesia (n=145; 95.4%), Myanmar (n=6; 3.9%) and Vietnam (n=1; 0.7%)].

Most participants in this study were newly arrived workers to Malaysia with length of stay of less than a year (n=198; 32.5%). Two workers settled the longest in Malaysia of approximately 38 years. Figure 2.4 showed the duration of stay in Malaysia of all participants with a mean value of 5.34 years. Majority (n=609; 99.8%) reported Malaysia was their first country of migration.

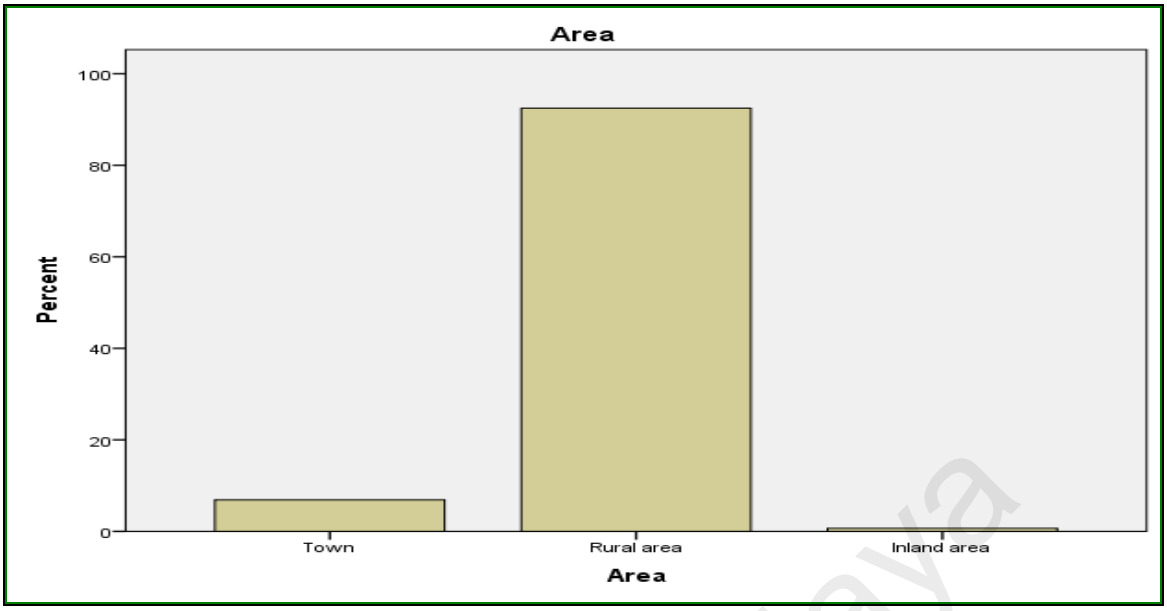

Figure 2.3: Home setting distribution in country origin of migrant workers.

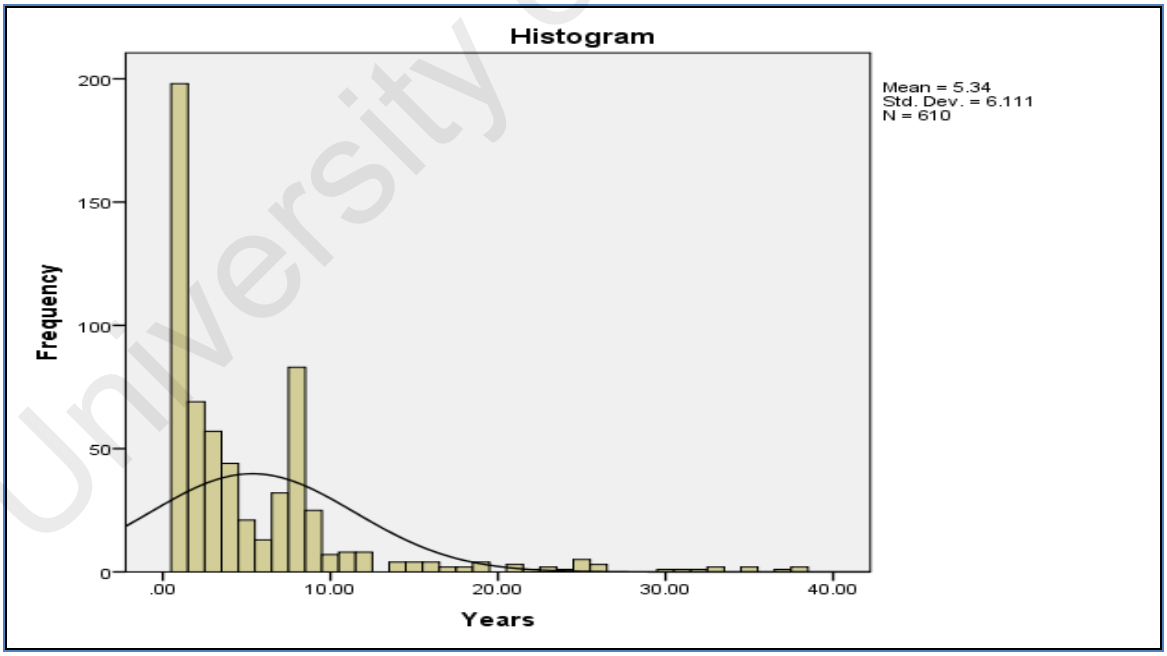

Figure 2.4: Distribution of migrant workers according to length of stay in Malaysia

2.3.3 Environmental health

The majority of respondents were provided housing by their employers. The types of accommodation provided were mostly hostel (n=374; 61.3%), followed by work-site accommodation (for construction workers) (n=56; 9.2%) and employer's residence (n=3; 0.5%). A total of 173 (28.4%) owned or rented accommodation and only 4 (0.7%) workers lived in a squatter home (Table 2.2). Majority of the workers lived together with their fellow workers (n=340; 55.7%) and only 84 workers (13.8%) lived with family.

Most of the workers were provided better amenities such as piped water and sanitary toilets in their present accommodation, compared to their home countries. We were also given a site visit to confirm their claim and were provided with suitable accommodation, equipped with clean water system, proper sewage toilet and efficient waste disposal system despite being overcrowded with too many workers to a room.

Table 2.2: Types of accommodation provided and living companion of migrant workers in Malaysia.

Types of accommodation		Living companion			Total
		Family	Friends	Fellow employees	
Squatter home	**Count**	1	3	0	4
	(%)	0.2	0.5	0.0	0.7
Employer's residence	**Count**	0	3	0	3
	(%)	0.0	0.5	0.0	0.5
Hostel	**Count**	1	90	283	374
	(%)	0.2	14.8	46.4	61.3
Work-site accommodation (construction)	**Count**	0	0	56	56
	(%)	0.0	0.0	9.2	9.2
Own/rented accommodation	**Count**	82	90	1	173
	(%)	13.4	14.8	0.2	28.4
Total	**Count**	84	186	340	610
	(%)	13.8	30.5	55.7	100.0

2.3.4 Life-style habits

Analysis on lifestyle habits of migrant workers showed the majority did not engage in risk behavior such as smoking, consumption of alcohol and illegal drugs. However, slightly more than a third (n=216; 35.4%) were active smokers, and a small minority quit smoking (n=14; 2.3%). Only 1 female (0.7%) was a smoker and the rest were males (45.4%). A total of 5.6% (n=34) consumed alcohol and all were males. Most of the workers (n=608; 99.7%) reported never consumed illegal drugs. One male from Nepal was found currently involved with all three risk behaviors (Table 2.3).

Table 2.3: Risk behavior lifestyle profile of migrant workers

	Smoking		Alcohol		Illegal drug	
	Frequency	(%)	Frequency	(%)	Frequency	(%)
Yes	216	35.4	34	5.6	1	0.2
Previously	14	2.3	7	1.1	1	0.2
Never	380	62.3	569	93.3	608	99.7
Total	610	100.0	610	100.0	610	100.0

2.3.5 Medical history and recent illness

Most workers had access to medical treatment either from government hospitals/ clinics (n= 183; 30%) or private hospitals/ clinics (n= 421; 69%) with most receiving treatment fully covered by the employer (n= 525; 86.1%). Only 81 (13.3%) workers paid their own medical treatment (Table 2.4). The workers also were questioned on symptoms of parasitic infections over the past year such as fever, blood/mucus in the stool, diarrhea, abdominal discomfort, fatigue/ lethargy and stomach pain. Less than 10% (2.0% – 8.5%) admitted to having symptoms (Table 2.5).

Table 2.4: Migrant workers access to medical treatment and mode of payment.

Access to treatment		Payment mode			
		Self-paying	Employer	Others	Don't know
Government hospital/clinic	Count	30	153	0	0
	%	4.9	25.1	0.0	0.0
Private hospital/ clinic	Count	49	372	0	0
	%	8.0	61.0	0.0	0.0
Self medication	Count	1	0	0	0
	%	0.2	0.0	0.0	0.0
No treatment	Count	1	0	1	3
	%	0.2	0.0	0.2	0.5
Total	Count	81	525	1	3
	%	13.3	86.1	0.2	0.5

Table 2.5: Migrant workers with symptoms of parasitic infection in the past year.

Symptoms	Yes N (%)	No N (%)
Fever	50 (8.2)	560 (91.8)
Blood and/ or mucus in the stool	12 (2.0)	598 (98.0)
Diarrhea	40 (6.6)	570 (93.4)
Abdominal discomfort	32 (5.2)	578 (94.8)
Fatigue / Lethargy	51 (8.4)	559 (91.6)
Stomach pain	52 (8.5)	558 (91.5)

2.3.6 Occupational Health and Safety

The majority of workers were given occupational health and safety briefing (n=588; 96.4%) and were provided with Personal Protective Equipment (PPE) (n=588; 96.4%) (Table 2.6). A total of 519 (85.1%) had awareness on protection by using the PPE all the times at their work place. Only 2 workers (0.3%) never wore their PPE during working (Figure 2.5).

Table 2.6: Number of migrant worker had given occupational health & safety briefing and provision with personal protective equipment (PPE).

	Health safety briefing		Provision with PPE	
	Frequency	**(%)**	**Frequency**	**(%)**
Yes	588	96.4	588	96.4
No	22	3.6	22	3.6
Total	**610**	**100.0**	**610**	**100.0**

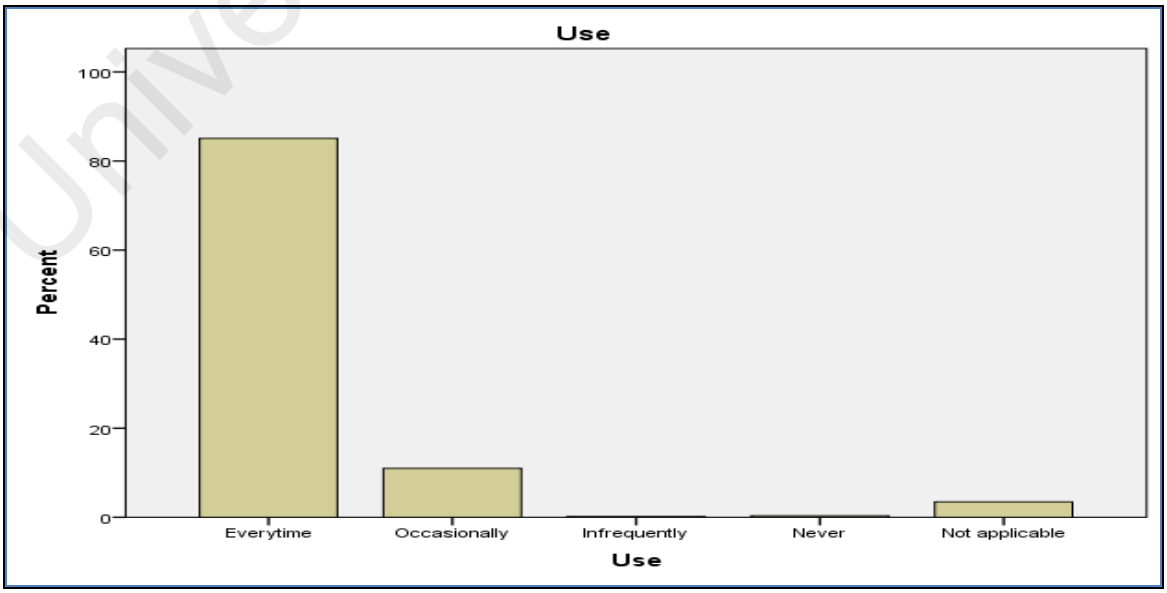

Figure 2.5: Percentage of usage of personal protective equipment (PPE) among migrant workers.

2.4 Discussion

The demand for low and semi-skilled workers in plantation/agriculture, construction, factories and domestic service in Malaysia saw a dramatic rise in the number of workers from 1.06 million in 2002 to 2.07 million in 2014 (Bardan, 2014) from countries including Indonesia, Bangladesh, the Philippines, Nepal, Myanmar, and other Asian countries labor. This study reports the socio-demographic profile of 610 migrant workers successfully recruited from the five main working sectors (construction, manufacturing, plantation, food service and domestic service) compared to the previous study by Zaini *et al.,* (2002) with only 250 respondents from three working sectors (construction, service and domestic). Recruitment were on a voluntary basis at their work sites compared to Zaini *et al*., (2002) which focused mainly on clinical data from University Malaya Medical Centre (UMMC) and PEREMBA group. Six nationalities (Indonesia, Bangladesh, Myanmar, Vietnam, India and Nepal) participated compared to only three (Indonesia, The Philippines and Bangladesh) previously (Zaini *et.al*., 2002) with most workers particularly from Indonesia and Bangladesh were Muslims.

Most workers originated from less developed countries of Southeast Asia with a majority from rural locations (92.5%) in search for better remuneration and security. Malaysia is a country rich in natural resources such as palm oil, rubber, tin, petroleum, natural gas and timber. In addition, its robust economic development is unable to meet the labor demand in the different sectors. Hence, Malaysia opens its borders to allow workers from neighboring country to engage in employment. According to the Central Intelligence Agency (2016), only 3.8% of population in Malaysia is living below the poverty line compared to other nationalities. Myanmar is reported to have the largest

proportion of the population living under the poverty line (32.7%), followed by Bangladesh (31.5%), India (29.8%), Nepal (25.2%), Vietnam (11.3%) and Indonesia (11.3%). The push factor for migration for most workers were the poor remuneration and slim employment opportunities in their home country. Among factors Malaysia was chosen as a popular destination for those seeking employment were due to the perceived abundant job opportunities, the high wage levels and attractive job offers (Abdul-Aziz, 2001). Most of the workers travel by low-cost air flights (75.1%) to Malaysia although other options such as sea routes were also opted particularly for workers from Indonesia and Myanmar. Abdul-Aziz (2001) also reported travel preferences among Bangladeshi workers to Malaysia were firstly by air (71.6%), followed by land (14.9%) and sea (13.5%). The option for travel to Malaysia is normally managed by an employment agency. Generally, almost all of the participants in this study have some form of travel documents, either the passport, work permit or both and they do not have any trouble to travel to Malaysia.

The majority of workers were provided with hostel accommodation by their employers (61.3%), sharing with other fellow employees. Only a small minority (13.8%) mainly Indonesians, lived on their own with their families. The provision of accommodation to the migrant workers is part of an agreement in a Memoranda of Understanding (MOU) between the Malaysian government and the worker's government stipulating the obligation for employers to provide accommodation in addition to free access to water and electricity as well as transportation to their working places. This was further confirmed as better amenities were provided to all workers compared to their own home country where majority came from low socio-economic locations in their home country which lacked basic utilities such as proper piped water, electricity and toilet facilities. In general, sanitation facilities in both urban and rural

area in this country were available almost to all (96%) of the population compared to other countries; Vietnam (78%), Myanmar (77.4%), Indonesia (60.8%), Bangladesh (60.6%), Nepal (45.8%) and India (39.6%) (Central Intelligence Agency, 2016).

More than a third of the workers were active smokers (35.4%). World Health Organization (2016) also reported similar global prevalence values of active smokers aged 15 years old with more males (36.1%) engaging in this behavior than females (6.8%). This values were also similar between nationalities in this region [i.e., Indonesia (39.7%), Bangladesh (36.8%), Myanmar (34.6%), India (32.8%) and Nepal (22.0%)] with more active male smokers (32.1%) compared to females (2.6%) (World Health Organization, 2016). With regards to alcohol consumption, only a small minority (6.7%) engaged in this activity similar to global values with more males involved (7.4%) compared to females (1.4%). Alcohol consumption was among the lifestyle activities evaluated among the workers. This habit may impact the surrounding people in many ways as it can harm family members, friends and co-workers when the drinkers are intoxicated. The risks can be determined by volume of alcohol consumed, patterns of drinking and quality of alcohol consumed (World Health Organization, 2016). Monitoring and surveillance of alcohol intake can reduce the negative health and social implications.

In the present study, almost all workers (99.7%) have access to modern healthcare services in this country. Majority had history of treatment fully supported by their employer either at private hospitals/clinics (61.0%) or government hospitals/clinics (25.1%). Zaini *et al*. (2002) also reported similar results that indicated good accessibility of the healthcare provisions in this country to migrant workers.

Personal protective equipment (PPE) as defined by the Occupational Safety and Health Administration (OSHA) is the "specialized clothing or equipment, worn by an employee for protection against infectious materials". Results also indicate that most workers were briefed on occupational health and safety (96.4%), provided with Personal Protective Equipment (PPE) (96.4%) and adhered to the wearing of PPE at all times during work (85.1%). Awareness of occupational safety and health and the used of PPE plays a crucial role to make the workplace safe and in the prevention of occupational injuries and diseases (Lugah *et al*., 2010). In the latest report of the occupational accidents by the Department of Occupational Safety and Health, Ministry of Human Resources Malaysia in 2015, the manufacturing (58.5%) sector accounts the highest incidents followed by agriculture and plantation (13.6%) and construction (5.8%) sector (Department of Occupational Safety and Health, 2015). The enactment of the Occupational Safety and Health Act (OSHA), 1994 implemented various programs by different agencies to increase awareness and knowledge of OSH in the workplace including providing instructions, procedures, training and supervision to encourage people to work safely and responsibly**.**

2.5 Conclusion

The majority of migrant worker in this country were provided with suitable living accommodations, clean water system, proper sewage toilets, and efficient waste disposal system. Majority of the workers did not engage in risk behavior such as smoking, consumption of alcohol or illegal drugs. More than 80% of the migrant workers also were fully covered for medical treatment with the accessibility to private or government hospitals/clinics. The workers have all been briefed on the occupational health and safety and were provided with personal protective equipment (PPE) and adhered to wearing the gear at work at all times.

CHAPTER 3: CURRENT IMPLICATIONS OF SOCIO-DEMOGRAPHIC AND ENVIRONMENTAL CHARACTERISTICS IN THE TRANSMISSION OF INTESTINAL PARASITIC INFECTIONS (IPIS)

3.1 Introduction

Neglected intestinal parasitic infections (IPIs) such as soil-transmitted helminths (STH) have been recognized as one of the main causes of illnesses especially among disadvantaged communities (Ngui *et al*., 2011a; Sinniah *et al*., 2014). According to the World Health Organization (WHO), STH have been identified as one of 17 neglected tropical diseases, with more than 1.5 billion people or 24% of the world's population infected (World Health Organization, 2015) with roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*) primarily through soil contaminated by human faeces. These infections can cause anaemia, vitamin A deficiency, stunted growth, malnutrition, intestinal obstruction and impaired development (Hotez *et al*., 2007). It is estimated that currently up to 800 million people are infected with *A. lumbricoides*, 600 million people with *T. trichiura* and 600 million with hookworms (Norhayati *et al*., 2003; Hotez, 2009; Ngui *et al*., 2011a).

In addition, common human intestinal protozoan infections such as *Entamoeba histolytica/ dispar*, *Giardia duodenalis* and *Cryptosporidium* spp. (Ngui *et al*., 2011a; Norhayati *et al*., 2003) are also widespread. It is estimated that there are 50 million cases of invasive *E. histolytica* disease each year, resulting in as many as 100,000 deaths. In several parts of the world, *Entamoeba* infection affects 50% of the population especially in areas of Central and South America, Africa, and Asia (Tengku *et al*.,

2011). Whilst *G. duodenalis,* a parasite that is frequently associated with cases of diarrheal disease throughout the world, affects approximately 200 million people worldwide (Flanagan, 1992; Mineno & Avery, 2003). On the other hand, *Cryptosporidium* spp. infection has been reported in every region of the United States (Scallan *et al*., 2011) and throughout the world, with approximately 4% of people in developed countries infected (Davies & Chalmers, 2009). Intestinal protozoan infections are spread by the faecal-oral route, so infections are widespread particularly in areas with inadequate sanitation and water treatment (Ngui *et al*., 2011a; Norhayati *et al*., 2003; Bertrand *et al*., 2004; Yoder & Beach, 2007).

There is continuous migration of populations from rural to urban areas as well as mass influx of immigrants from neighbouring countries to big cities. This sudden influx of people has contributed to the mushrooming of numerous mega urban slums where the environment is conducive for the transmission of intestinal pathogens (Sinniah *et al*., 2014). Studies on parasitic infections amongst migrant workers have been conducted worldwide particularly in Asia, for example in Thailand (Nuchprayoon *et al*., 2009; Ngrenngarmlert *et al*., 2012), Taiwan (Lo & Lee, 1996; Wang, 1998; 2004; Lu & Sung, 2008; Hsieh *et al*., 2011), Taipei (Cheng & Shieh, 2000)) and in the Middle East primarily in the Kingdom of Saudi Arabia (Abha district (Al-Madani & Mahfouz, 1995), Riyadh (Kalantan, 2001), Al-Khobar (Abahussain, 2005), Makkah (Wakid *et al*., 2009), Al-Baha (Mohammad & Koshak, 2011) and Medina (Taha *et al*., 2013). In Qatar, Abu-Madi *et al*. (2008; 2010; 2011) have also extensively studied the parasitic infections in migrant workers. In Malaysia, Suresh *et al.* (2002) conducted a similar study more than a decade ago among migrant workers however, the study only involved clinically ill subjects from University Malaya Medical Centre (UMMC). The findings of this study provided useful data but the study was not robustly designed to identify

priorities for policy recommendations to the health and political authorities. Studies on intestinal parasitic infections have been conducted also among the Malaysian population and infections continue to be a public health problem especially among the poverty-stricken communities. Studies analyzing parasitic infections among various communities in Malaysia include; the Orang Asli (indigenous group) (Dunn, 1972; Dissanaike *et al*., 1977; Al-Mekhlafi *et al*., 2005a; 2006; Ngui *et al*., 2011a; Nasr *et al*., 2013; Sinniah *et al*., 2014), plantation and rural communities (Bisseru & Aziz, 1970; Lo *et al*., 1979; Al-Mekhlafi *et al*., 2007; 2008; Sinniah *et al*., 2011), slum dwellers (Chia *et al*., 1978; Sinniah *et al*., 2014), fishing communities (Heyneman *et al*., 1967; Balasingam *et al*., 1969; Nawalinski & Roundy, 1978; Anuar *et al*., 1978; Sinniah *et al*., 1988) and flat dwellers (Kan, 1983; Che Ghani *et al*., 1989; Sinniah *et al*., 2002; 2014).

The current study is timely as in the past decade, the number of migrant workers has grown exponentially with a percentage increase of 49% between 2002 (1.06 million) and 2014 (2.07 million) (Bardan, 2014). Despite compulsory medical screening for workers prior to entering the Malaysian workforce, screening for parasitic infections is grossly inadequate or lacking. Therefore, there is an acute need for more accurate and up-to-date information on the parasitic infections in this particular group of workers and an understanding of the factors associated with transmission of these infections, especially as they are likely to impact significantly upon the local community through close contact, lost productivity and the heightened cost of healthcare.

3.2 Materials and Methods

3.2.1 Study population and sample collection

A total of 388 from 610 volunteers of migrant workers in Malaysia agreed to provide stool sample. Questionnaires were distributed to the participants to gather relevant information related to the study. All participants were fully informed of the nature of the study for their maximum co-operation and completion of consent forms. Details of the participants, composition of the questionnaire and ethical clearance of this study are as described in Chapter 2.

After consent was obtained and the questionnaire completed, each individual was provided with a plastic container marked with a specific identification number and the name of the participant. The participant was 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.

3.2.2 Analysis of faecal sample

For the formalin ether concentration technique, approximately 1 to 2g of sample were mixed with 7 ml of formalin and 3 ml ethyl acetate and centrifuged for 5 minutes at 2500 rpm. After centrifugation, 4 layers were seen, 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 (Ngui *et al*., 2011a). The slide was examined under

a light microscope at 10x and 40x magnification for helminths and protozoa, respectively.

For *Cryptosporidium* sp., modified Ziehl-Neelsen staining technique was conducted. A smear was made on a glass slide and allowed to dry. Then the smear was fixed with methanol for about 5 minutes and afterwards flooded with cold strong neat carbol fuchsin for 5 to 10 minutes. The slide was washed in tap water and differentiated in 1% acid alcohol until colour ceased to leach. The smear was next rinsed under tap water, again followed by counter staining with malachite green for 30 seconds. Slides were blotted dry and examined using 1000x oil immersion objective (Ngui *et al*., 2011a). Three slides per sample were examined and further confirmed by supervisors in the Department of Parasitology, Faculty of Medicine in the University of Malaya.

3.2.3 Statistical analysis

Prevalence data (percentage of subjects infected) are shown with 95% confidence limits (CL_{95}), as described by Rohlf & Sokal (1995) using bespoke software. Prevalences were analyzed using maximum likelihood techniques based on log linear analysis of contingency tables using the software package SPSS (Version 22). Analysis was conducted in two phases. In the first phase, full factorial models were fitted with the intrinsic factors sex (2 levels, males and females), age (5 age classes comprising those <25 years old, 25-34 years old, 35-44 years old, 45-54 years old and those >54 years) and nationality (5 countries, Indonesia, Bangladesh, Myanmar, India and Nepal). Infection was considered as a binary factor (presence/absence of parasites). These explanatory factors were fitted initially to all models that were evaluated. For each level of analysis in turn, beginning with the most complex model, all possible main effects

and interactions were investigated and those combinations that did not contribute significantly to explaining variation in the data were eliminated in a stepwise fashion beginning with the highest-level interaction (backward selection procedure). A minimum sufficient model was then obtained, for which the likelihood ratio of $\chi 2$ was not significant, indicating that the model was sufficient in explaining the data. The importance of each term (i.e. interactions involving infection) in the final model was assessed by the probability that its exclusion would alter the model significantly and these values relating to interactions including presence/absence of infection are given in the text. The remaining terms in the final model that did not include presence/absence of infections are not given but can be made available by the authors upon request.

In the second phase, models were fitted with four environmental factors (employment sector [Construction, manufacturing, plantation workers, food services and domestic services], educational level [no formal education, primary education only, education to high school level and to university level], accommodation [hostel/employer provided or own/rented] and years of residency [less than one year or more than 1 year] and presence/absence of infections). The most significant of the intrinsic factors detected in the first phase of the analysis was also included and the model re-run as explained above.

3.3 Results

3.3.1 Socio-demographic characteristics

A total of 388 volunteers of migrant workers provided stool specimens. The socio-demographic profile of this subset comprised 304 males (78.4%) and 84 females. Among the males, 37.4% were between 25 to 34 years old (n=145), 29.4% were younger than 25 (n=114) and 23.2% older (n=90 for 35 to 44 years). Most respondents were from Indonesia (n=167, 43%) followed by Nepal (n=81, 20.9%), Bangladesh (n=70, 18%), India (n=47, 12.1%) and Myanmar (n=23, 5.9%). The majority were involved in the domestic sector (n=105, 27.1%), followed closely by the food service sector (n=104, 26.8%), while, only a small proportion were from among those working on plantations (n=71, 18.3%), manufacturing (n=61, 15.7%) and construction (n=47, 12.1%) sectors.

3.3.2 Prevalence of intestinal parasitic infections (IPIs)

The stool screening showed a high proportion of the workers positive (n=244, 62.9%) for intestinal parasitic infections (IPIs) with more than 50% infected in each working sector except for domestic sector (48.6%). Higher infections were recovered from workers primarily in the manufacturing (77%) and food service sector (74%). A total of 8 species were recovered consisting of 5 helminthes [*Ascaris lumbricoides* (43.3%), hookworm (13.1%), *Trichuris trichiura* (9.5%), *Hymenolepis nana* (1.8%) and *Enterobius vermicularis* (0.5%)] and 3 protozoans [*Entamoeba* spp. (11.6%), *Giardia* spp. (10.8%) and *Cryptosporodium* spp. (3.1%)] (Figure 3.1 – 3.8). The workers were primarily infected with intestinal helminth (68.3%) compared to intestinal protozoa (25.5%) infections (Table 3.1). Of which, *A. lumbricoides* (43.3%) was the most

common helminth recovered, while *Entamoeba* spp. infection (11.6%) was the most predominant intestinal protozoa. *A. lumbricoides* infection was the most common according to each working sector. With regards to the protozoa infections, *Entamoeba* spp. and *Giardia* spp. were also found to be common in all working sectors.

Single species (37.9%) infection was most common followed by infections with two species (19.3%), three species (5.4%) and four parasite species 0.3%). *A. lumbricoides* (24.6%) showed the highest prevalence for the single parasite infection. Among the mixed infections, combination of helminth and protozoa, namely *A. lumbricoides* and *Entamoeba* sp. showed the highest prevalence (4.6%) in the two parasite species infections, meanwhile in the three parasite species infections, combination of three soil-transmitted helminthes (STH); *A. lumbricoides*, *T. trichiura* and hookworm were the most common with 1.3% (Table 3.2).

3.3.3 Intrinsic effects on prevalence of intestinal parasitic infections

3.3.3.1 Higher taxa

Stool screening revealed a high proportion of workers positive for intestinal helminths and protozoan infections (both helminths and protozoa combined = 62.9% [56.87-68.55]). There was no significant effect of age or sex, but a highly significant effect of nationality was found (χ^2_4=38.1, P<0.001). Prevalence was higher among the Nepalese and Indians (Table 3.3) compared with Indonesians, Bangladeshi and Myanmar. Analyses of combined helminth infections yielded a similar outcome, with again only nationality showing a significant effect on prevalence (χ^2_4=47.4, P<0.001). The highest prevalence was also among the Nepalese and Indians with lower values

among the remaining three national groups (Table 3.3). In contrast to the above, none of the main effects (sex, age or nationality; see Table 3.3 for nationality) were significant in the case of combined protozoan infections, but there was a weak interaction between sex, age and infection (χ^2_4=10.3, *P*=0.036). This arose primarily through relatively small differences in prevalence in age class 3 (males =8.8%, n=57; females =24.2%, n=33), age class 4 (males =28.6%, n=21; females =0%, n=8), and age class 5 (males =0.0%, n=4; females = 33.3%, n=6), but sample sizes in some subsets were small.

3.3.3.2 Individual helminth species

A. lumbricoides. This was the most common species with an overall prevalence of 43.3% [37.45-49.32]. Prevalence was almost twice as high among males (47.7% [42.39-53.00]) compared with females (27.4% [17.28-39.89]). This was a significant difference when fitted only with infection (χ^2_1=11.5, *P*=0.001), but when nationality was taken into account (χ^2_4=68.5, *P*<0.001), the effect of host sex disappeared. Prevalence did not differ significantly between different age classes.

Hookworms. The overall prevalence of hookworms was 13.1% [9.56-17.78]. There was no difference between prevalence in male and female subjects (males = 13.2% [9.95-17.19] and females 13.1% [6.51-24.04]), but there was a significant effect of age (χ^2_4=18.8, *P*=0.001). Prevalence was highest in the youngest age class and none of the ten subjects in the oldest age class was infected (for age classes 1-5, prevalence = 23.7%, 8.3%, 12.2%, 3.4% and 0% respectively). Prevalence did not differ significantly between the 5 nationality classes.

T. trichiura. This was the rarest of the 3 major intestinal nematode species with a prevalence of 9.5% [6.50-13.72]. There was a marked difference between sexes with prevalence among males (11.5% [8.48-15.35]) being more than 4 times that among females (2.4% [0.33-10.10]), a difference that was highly significant (χ^2_1=11.5, *P*=0.001). Prevalence also varied significantly between age classes (χ^2_4=13.2, *P*=0.010), with the highest prevalence among the youngest individuals and no infection recorded among the oldest (for age classes 1-5, prevalence = 17.5%, 8.3%, 3.3%, 6.9% and 0% respectively). With both age and sex taken into account, prevalence also varied significantly between the different nationalities (Table 3.4, (χ^2_4=13.2, *P*=0.010)). Prevalence was highest among those from Myanmar and lowest among subjects from India.

H. nana. This species was recorded in just 7 subjects (1.8% [0.72-4.31]) and therefore statistical analysis was not robust. Four subjects were from Nepal, two from Bangladesh, and one from India (Table 3.4) and no infections were detected among the Indonesians or subjects from Myanmar. All seven infected subjects were male.

E. vermicularis. Only two cases of *E. vermicularis* were detected (0.5% [0.14-2.22], both among male subjects, one from Indonesia and the other from Bangladesh.

3.3.3.3 Individual protozoan species

Three species of intestinal protozoans were recorded.

E. histolytica/dispar. This was the most common protozoan infecting 45 subjects (11.6% [8.19-16.02]. Prevalence did not vary significantly between age classes or nationalities, but there was a significant difference between the sexes (χ^2_1=5.2, *P*=0.022). Prevalence was twice as high among female subjects (19.0% [10.83-31.00] compared with males (9.5% [6.81-13.16]).

Giardia sp. The overall prevalence of *Giardia* was 10.8% [7.51-15.18]. Prevalence was not affected by host age or nationality, although a marginal significance was found with host age classes (Table 3.5, (χ^2_4=9.9, *P*=0.042; for age classes 1-5, prevalence = 14.0%, 13.8%, 5.6%, 3.4% and 0% respectively).

Cryptosporidium spp. This species was detected in 12 subjects (3.1% [1.55-5.95), and none of the intrinsic factors significantly affected prevalence.

3.3.4 Extrinsic (environmental) effects on intestinal parasitic infections

3.3.4.1 Higher taxa

With nationality taken into account, the prevalence of all parasitic infections differed between subjects who had resided in Malaysia for less than a year and those who have been there for longer (more than one year; Table 3.3 ; χ^2_1=10.7, *P*=0.001). Prevalence also differed between subjects from different employment sectors (Table 3.3; χ^2_4=38.1, *P*<0.001), with the highest prevalence among workers in manufacturing

and the food service sector and the least in those working in domestic employment, but there were no significant effects of education or accommodation.

Analysis of combined helminth infections by 1-way tests fitting only individual factors with infection in turn (see table 3.3 for prevalence values for all factors and levels) showed that there were highly significant effects of employment sector (χ^2_4=33.0, P<0.001; highest among those in manufacturing and least among those employed in the domestic industry), accommodation type (χ^2_1=23.2, P<0.001; higher in those living in hostels or employer provided residences) and years of residence (χ^2_1=21.7, P<0.001; higher among those with less than 1 year residence) but not of education level (χ^2_3=4.9, P=0.2). Fitting a full factorial model resulted in a more complex outcome with 4 significant interactions affecting prevalence of combined helminth infections. The strongest interaction was between education, employment and infection (χ^2_{12}=25.8, P=0.012). The highest prevalence was among workers in manufacturing and the food service industry and the least in those working in domestic employment (Table 3.3). However, there were exceptions among the 4 education classes. Thus, for those employed in the food service industries, prevalence was highest if the subjects had no formal education (66.7% [27.14-93.71]), only primary education (82.6% [61.13-93.83]) or university education (80.0% [34.26-98.97]), but among those with high school education, although high for those employed in the food service industry (62.9% [51.26-73.27]), prevalence was higher among those working in manufacturing (81.8% [72.5-88.66]). In contrast, there were no cases of helminth infections among those in manufacturing if they had not experienced any formal education, or just primary education, but not surprisingly, sample sizes were very small in these latter categories. The other three interactions were between accommodation type and education (χ^2_3=9.3, P=0.025), nationality and employment sector (χ^2_{16}=28.6,

P=0.026), and years of residence and employment sector (χ^2_4=10.2, *P*=0.037), but all these were weaker than the former and we did not explore these further.

Analysis of combined protozoan infections by tests fitting first just each of the environmental factors with infection in turn as above, (see Table 3.3 for values for all factors and levels), showed that there were only relatively weak effects, of which the strongest was employment sector (χ^2_4=16.6, *P*=0.002). Worryingly, prevalence was highest among those in the food service industry and lowest among the plantation workers (Table 3.3). Prevalence also varied significantly with education (χ^2_3=10.6, *P*=0.014; highest among those with high school education and lowest among the 8 university graduates) and years of residence in Malaysia (χ^2_1=4.2, *P*=0.041; higher among those with less than a year of residence in Malaysia). Prevalence did not vary significantly in relation to the accommodation categories. However, when a full factorial model was fitted, thereby controlling for each of the 4 factors above and nationality, these effects were no longer significant and the only term which emerged as significant was an interaction between accommodation, education and infection (χ^2_3=13.8, *P*=0.003). The principal source of this interaction (Figure 3.9) was the contrast between subjects whose education ended at either primary or high school levels, among which residence had little effect, and the huge difference in prevalence among those who had no formal education. Among this latter group, those living on their own or rented accommodation (*n*=10) showed considerably higher prevalence of protozoan infections than those who relied upon their employers to provide accommodation or who lived in hostels (*n*=44), although the sample size for the former group was low and this needs to be taken into consideration in interpreting the overall significance of this finding.

3.3.4.2 Individual helminth species

A. lumbricoides. With nationality taken into account, prevalence varied significantly in relation to each of the 4 environmental factors examined (Table 3.4; years of residence, χ^2_1=18.5, P<0.001; Educational level, χ^2_3=14.9, P=0.002; accommodation, χ^2_1=15.3, P<0.001; Employment sector, χ^2_4=54.0, P<0.001). The highest prevalence was among subjects in manufacturing, those living in hostels, residents under one year and perhaps surprisingly among those with a university education, although in the latter case, the sample size was small. There were also several relatively weak more complex interactions with infection (Accommodation x Nationality χ^2_4=9.5, P=0.049, Years resident x Employment sector χ^2_4=10.8, P=0.029, Educational level x Nationality χ^2_{12}=28.3, P=0.005 and Educational level x Employment sector χ^2_{12}=24.5, P=0.017) which we did not explore further.

Hookworms. With host age taken into account, none of the environmental factors affected prevalence of hookworms significantly (Table 3.4).

T. trichiura. With sex taken into account, the only environmental factors that significantly affected prevalence were employment sector (χ^2_4=19.7, P=0.001) and years of residency (χ^2_1=8.1, P=0.004). Prevalence was highest among those employed in construction and lowest among those in the manufacturing industries (Table 3.4), and higher among subjects with less than a year's residency relative to those with more than year's residency. Accommodation type was marginally significant when fitted on its own with infection (χ^2_1=4.1, P=0.044) but not when other factors were also part of the model.

H. nana. With just 7 subjects (1.8% [0.72-4.31]) infected by *H. nana* statistical analysis was not robust, values for prevalence in each of the different levels of the four environmental factors considered are shown in Table 3.4.

E. vermicularis. With only two cases of *E. vermicularis* recorded, further analysis was not reliable. Both subjects were registered as not having any formal education, living in hostel accommodation and both with residency exceeding one year.

3.3.4.3 Individual protozoan species

E. histolytica/dispar. When the four environmental factors were fitted along with host sex and infection, the only significant term was the interaction between sex, education and infection (Figure 3.10; χ^2_3=18.8, P<0.001); education x infection alone was not a significant term in the model. Prevalence also varied with employment sector when employment sector and infection were fitted alone (χ^2_4=23.2, P<0.001) but not when the other factors were included in the model.

Giardia sp. With host age taken into account, the only environmental factor affecting prevalence was duration of residency (χ^2_1=6.3, P=0.012). As Table 3.5 shows prevalence of *Giardia* was markedly higher among those with less than one year of residency compared with prevalence among those that have lived locally for more than a year.

Cryptosporidium spp. This species was detected in 12 subjects (3.1% [1.55-5.95). We fitted a model with nationality, the 4 environmental factors and infection. The only significant factor to emerge from this analysis was employment sector (Table 3.5; χ^2_4=12.8, *P*=0.012). Prevalence was clearly highest among food service workers, and much lower among those in other types of employment, with no infections at all detected among those in the domestic sector.

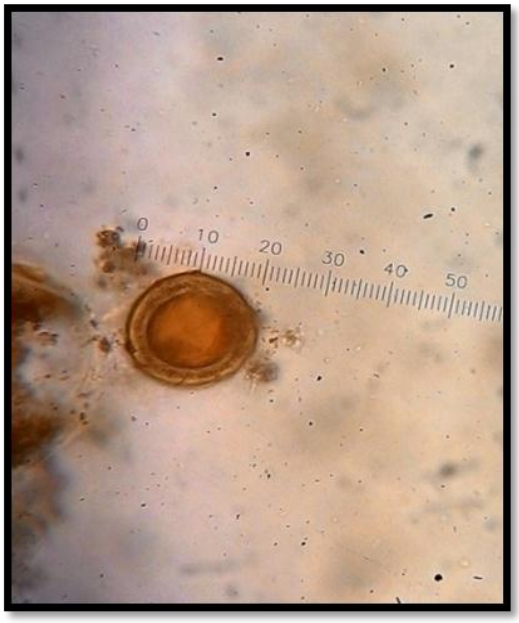

Figure 3.1: ***Ascaris lumbricoides***

Magnification: 400x

1 scale = 2.5 micrometres

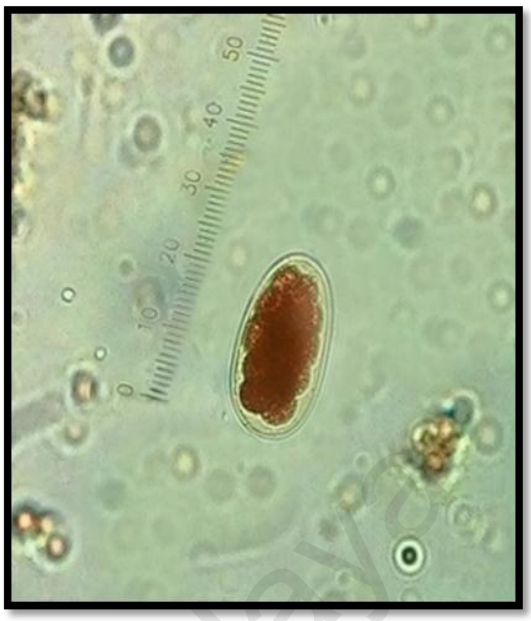

Figure 3.2: Hookworm

Magnification: 400x

1 scale = 2.5 micrometres

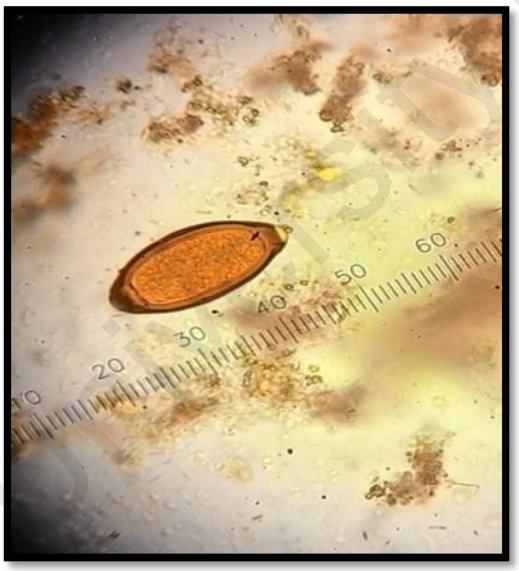

Figure 3.3: ***Trichuris trichiura***

Magnification: 400x

1 scale = 2.5 micrometres

Figure 3.4: ***Enterobius vermicularis***

Maginification: 400x

1 scale = 2.5 micrometres

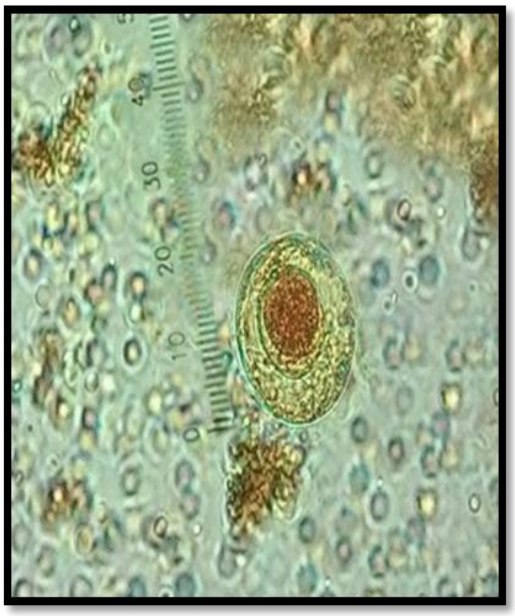

Figure 3.5: ***Hymenolepis nana***

Magnification: 400x

1 scale = 2.5 micrometres

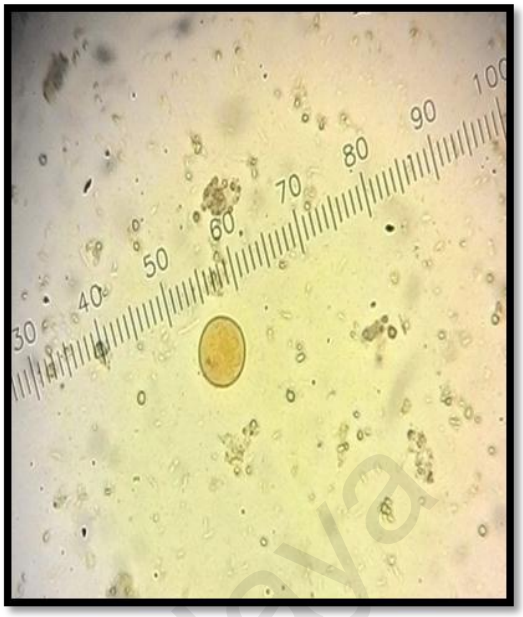

Figure 3.6: ***Entamoeba*** **spp.**

Magnification: 400x

1 scale = 2.5 micrometres

Figure 3.7: ***Giardia*** **sp.**

Magnification: 400x

1 scale = 2.5 micrometres

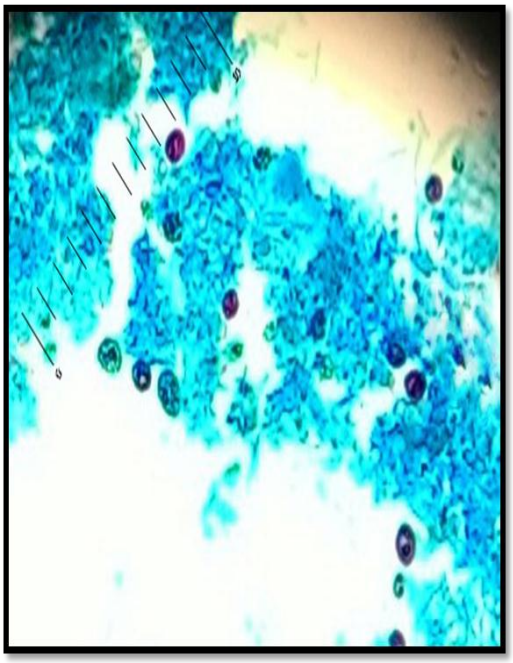

Figure 3.8: ***Cryptosporidium*** **spp**.

Magnification: 1000x

1 scale = 1 micrometre

Table 3.1: Species of intestinal parasitic infections recovered from migrant workers in Peninsular Malaysia.

Parasites	**No. of positive (n=388)**	**Percentage (%)**
Helminth		
Ascaris lumbricoides	168	**43.3**
Trichuris trichiura	37	**9.5**
Hookworm	51	**13.1**
Enterobius vermicularis	2	**0.5**
Hymenolepis nana	7	**1.8**
Total	**265**	**68.3**
Protozoa		
Entamoeba spp.	45	**11.6**
Giardia spp.	42	**10.8**
Cryptosporidium spp.	12	**3.1**
Total	**99**	**25.5**

Table 3.2: Multiplicity of intestinal parasitic infections amongst migrant workers infected.

No. of intestinal parasite infection	No. positive (n=388)	Percentage (%)
Single		
Ascaris lumbricoides	94	24.2
Trichuris trichiura	10	2.6
Hookworm	16	4.1
Hymenolepis nana	1	0.3
Enterobius vermicularis	1	0.3
Entamoeba spp.	8	2.1
Giardia spp.	13	3.4
Cryptosporidium spp.	4	1.0
Total single parasitic infection	**147**	**37.9**
Two parasites		
A.lumbricoides + *T.trichiura*	6	1.5
A.lumbricoides+ Hookworm	9	2.3
A.lumbricoides + *E.vermicularis*	1	0.3
A.lumbricoides + *H.nana*	3	0.8
A.lumbricoides + *Entamoeba*spp.	18	4.6
A.lumbricoides + *Giardia* spp.	13	3.4
A.lumbricoides + *Cryptosporidium* spp.	5	1.3
T.trichiura + Hookworm	6	1.5
T.trichiura + *Entamoeba*spp.	1	0.3
T.trichiura + *Giardia* spp.	4	1.0
Hookworm + *Entamoeba* spp.	5	1.3
Hookworm + *Giardia* spp.	1	0.3
*Entamoeba*spp. + *Giardia* spp.	3	0.8
Total two parasitic infections	**75**	**19.3**
Three parasites		
A.lumbricoides + *T.trichiura* + Hookworm	5	1.3
A.lumbricoides + *T.trichiura* + *Entamoeba*spp.	1	0.3
A.lumbricoides + *T.trichiura* + *Giardia* spp.	1	0.3
A.lumbricoides + Hookworm + *Entamoeba* spp.	4	1.0
A.lumbricoides + Hookworm + *Giardia* spp.	1	0.3
A.lumbricoides + Hookworm + *Cryptosporidium* spp.	1	0.3
A.lumbricoides + *H.nana* + *Entamoeba*spp.	1	0.3
A.lumbricoides + *H.nana* + *Giardia* spp.	1	0.3
A.lumbricoides + *H.nana* + *Cryptosporidium* spp.	1	0.3

A.lumbricoides + *Entamoeba* spp. + *Giardia* spp.	1	0.3
A.lumbricoides + *Entamoeba* spp. + *Cryptosporidium* spp.	1	0.3
T.trichiura + Hookworm + *Giardia* spp.	1	0.3
T.trichiura + *Entamoeba* spp. + *Giardia* spp.	1	0.3
Hookworm + *Entamoeba* spp. + *Giardia* spp.	1	0.3
Total three parasitic infections	**21**	**5.4**
Four parasites		
A.lumbricoides + *T.trichiura* + Hookworm + *Giardia* spp.	**1**	**0.3**
Total all migrant workers infected	**244**	**62.9**

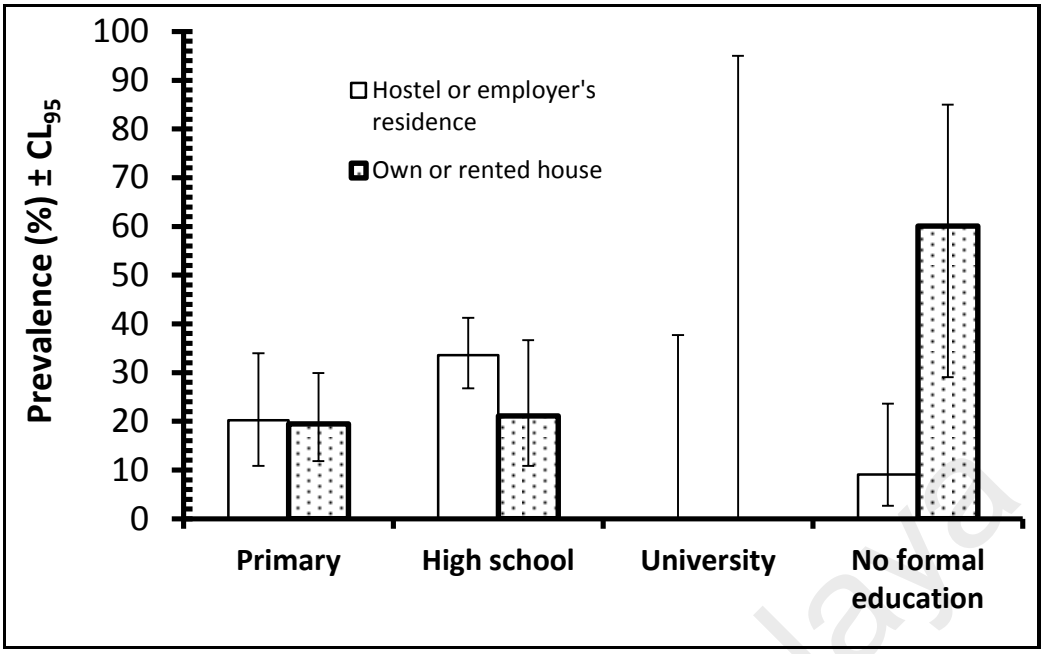

Figure 3.9: Prevalence of combined protozoan infections in the host population in relation to levels of education and types of residences

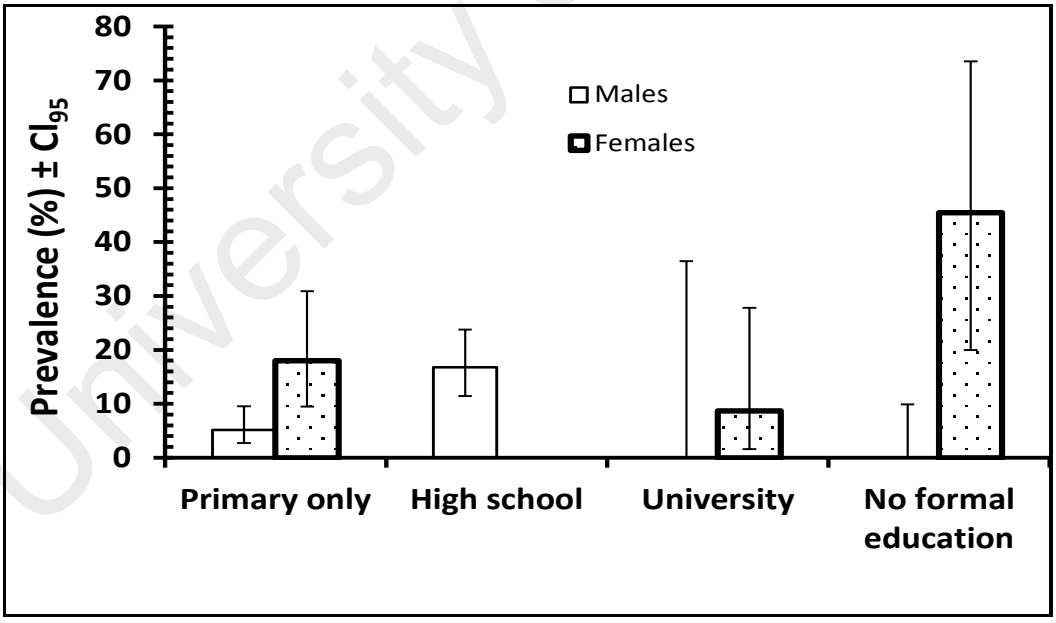

Figure 3.10: Prevalence of *Entamoeba* in relation to the host-sex and levels of education

Table 3.3: Prevalence of intestinal parasitic infections amongst migrant workers according to nationality, employment sector, education, accommodation type and years of residence in Malaysia.

			Prevalence (%) ± 95% confidence limits		
Factor	Level	N	All parasites	Combined helminthes	Combined protozoa
Nationality					
	Indonesia	167	52.1 [43.07-61.00]	43.1 [34.48-52.13]	21.6 [15.04-29.78]
	Bangladesh	70	52.9 [41.21-64.15]	45.7 [34.67-57.35]	14.3 [7.85-24.44]
	Myanmar	23	56.5 [36.02-75.34]	43.5 [24.66-63.98]	21.7 [8.99-43.34]
	India	47	83.0 [65.67-92.73]	78.7 [61.18-89.84]	31.9 [17.57-49.59]
	Nepal	81	84.0 [72.78-91.56]	80.2 [68.34-88.64]	32.1 [21.46-44.55]
Employment Sector					
	Construction	47	59.6 [41.80-75.54]	53.2 [35.33-69.61]	12.8 [4.59-28.56]
	Manufacturing	61	77.0 [66.64-85.19]	75.4 [64.99-83.62]	27.9 [19.08-38.37]
	Plantation workers	71	53.5 [41.80-64.75]	49.3 [37.55-61.04]	11.3 [5.61-20.79]
	Food services	104	74.0 [70.34-82.50]	68.3 [61.30-74.59]	33.7 [27.28-40.75]
	Domestic services	105	48.6 [41.41-55.72]	37.1 [30.49-44.28]	24.8 [19.12-31.39]
Educational Level					
	Primary only	166	56.0 [47.04-64.63]	50.0 [41.00-59.00]	19.9 [13.50-27.88]
	High school	160	69.4 [60.71-76.95]	61.9 [53.09-69.92]	30.6 [23.05-39.29]
	University	8	62.5 [28.93-88.88]	62.5 [28.93-88.88]	0 [0-36.46]
	No formal education	54	64.8 [54.51-74.15]	53.7 [43.36-63.57]	18.5 [11.72-27.77]
Accommodation					
	Hostel/ Employer	272	69.5 [64.69-73.91]	63.6 [58.63-68.32]	23.9 [19.83-28.46]
	Own/rented house	116	47.4 [39.97-54.90]	37.1 [30.13-44.54]	23.3 [17.43-30.21]
Years of residence					
	Less than 1 year	134	79.1 [71.97-85.02]	71.6 [63.91-78.30]	29.9 [22.97-37.67]
	More than 1 year	254	54.3 [49.47-59.19]	47.2 [42.37-52.11]	20.5 [16.78-24.69]

Table 3.4: Prevalence of individual helminth species amongst migrant workers according to nationality, employment sector, education, accommodation type and years of residence in Malaysia

			Prevalence (%) ± 95% confidence limits			
Factor	Level	n	Hookworms	*A. lumbricoides*	*T. trichiura*	*H. nana*
Nationality						
	Indonesia	167	15.0 [9.44-22.47]	26.3 [19.08-34.84]	9.6 [5.39-16.16]	0 [0-3.13]
	Bangladesh	70	4.3 [1.28-11.81]	41.4 [30.42-53.04]	8.6 [3.80-17.41]	2.9 [0.61-9.70]
	Myanmar	23	17.4 [6.17-38.87]	17.4 [6.17-38.87]	26.1 [12.03-47.78]	0 [0-14.51]
	India	47	12.8 [4.59-28.56]	68.1 [50.41-82.43]	2.1 [0.12-14.67]	2.1 [0.12-14.67]
	Nepal	81	16.0 [8.44-27.22]	72.8 [60.60-82.71]	9.9 [4.44-19.94]	4.9 [1.47-13.63]
Employment Sector						
	Construction	47	10.6 [3.35-26.30]	36.2 [21.28-53.89]	21.3 [10.16-38.82]	0 [0-10.60]
	Manufacturing	61	13.1[7.12-22.05]	72.1 [61.63-80.92]	4.9 [1.77-12.06]	4.9 [1.77-12.06]
	Plantation workers	71	14.1 [7.72-24.32]	25.4 [16.51-36.67]	15.5 [8.50-25.76]	2.8 [0.58-9.74]
	Food services	104	14.4 [10.01-20.09]	58.7 [51.54-65.54]	7.7 [4.65-12.40]	1.9 [0.64-5.11]
	Domestic services	105	12.4 [8.33-17.80]	26.7 [20.76-33.44]	4.8 [2.46-8.75]	0 [0-1.96]
Educational Level						
	Primary only	166	10.8 [6.22-17.74]	36.1 [27.93-45.11]	12.0 [7.30-19.03]	1.2 [0.18-5.12]
	High school	160	13.1 [8.06-20.08]	53.8 [44.94-62.29]	5.6 [2.70-11.08]	2.5 [0.76-7.00]
	University	8	25.0 [4.64-63.53]	62.5 [28.93-88.88]	12.5 [0.64-50.00]	0 [0-36.46]
	No formal education	54	18.5 [11.72-27.77]	31.5 [22.64-41.77]	13.0 [7.16-21.22]	1.9 [0.30-7.26]
Accommodation						
	Hostel/ Employer	272	14.0 [10.79-17.84]	49.6 [44.61-54.66]	11.4 [8.52-15.01]	2.6 [1.37-4.72]
	Own/rented house	116	11.2 [7.14-16.82]	28.4 [22.16-35.65]	5.2 [2.69-9.53]	0 [0-2.17]
Years of residence						
	Less than 1 year	134	17.9 [12.49-24.87]	58.2 [50.20-65.93]	12.7 [8.15-18.91]	1.5 [0.36-5.03]
	More than 1 year	254	10.6 [7.92-14.03]	35.4 [30.90-40.21]	7.9 [5.60-10.96]	2.0 [0.98-3.88]

Table 3.5: Prevalence of individual protozoan species amongst migrant workers according to nationality, employment sector, education, accommodation type and years of residence in Malaysia

			Prevalence (%) ± 95% confidence limits		
Factor	Level	N	*Entamoeba*	*Giardia*	*Cryptosporodium*
Nationality					
	Indonesia	167	13.8 [8.58-21.12]	8.4 [4.46-14.88]	1.2 [0.17-5.13]
	Bangladesh	70	2.9 [0.61-9.70]	10.0 [4.82-19.14]	2.9 [0.61-9.70]
	Myanmar	23	13.0 [3.66-32.35]	8.7 [1.57-27.81]	4.3 [0.23-21.25]
	India	47	12.8 [4.59-28.56]	10.6 [3.35-26.30]	10.6 [3.35-26.30]
	Nepal	81	13.6 [7.00-24.50]	17.3 [9.56-28.64]	2.5 [0.38-10.00]
Employment Sector					
	Construction	47	2.1 [0.12-14.67]	8.5 [2.23-23.41]	2.1 [0.12-14.67]
	Manufacturing	61	11.5 [6.06-20.22]	14.8 [8.38-24.12]	3.3 [0.88-9.62]
	Plantation workers	71	1.4 [0.14-7.63]	8.5 [3.69-17.34]	1.4 [0.14-7.63]
	Food services	104	15.4 [10.86-21.19]	13.5 [9.22-19.01]	7.7 [4.65-12.40]
	Domestic services	105	19.0 [13.90-25.38]	8.6 [5.31-13.52]	0 [0-1.96]
Educational Level					
	Primary only	166	9.0 [4.99-15.57]	9.0 [4.99-15.57]	3.0 [1.00-7.71]
	High school	160	15.6 [10.15-22.95]	13.8 [8.66-20.89]	3.1 [1.10-7.76]
	University	8	0 [0-36.46]	0 [0-36.46]	0 [0-36.46]
	No formal education	54	9.3 [4.69-16.96]	9.3 [4.69-16.96]	3.7 [1.17-9.72]
Accommodation					
	Hostel/ Employer	272	9.6 [6.95-12.97]	12.1 [9.18-15.81]	3.7 [2.16-6.06]
	Own/rented house	116	16.4 [11.47-22.62]	7.8 [4.57-12.79]	1.7 [0.51-5.05]
Years of residence					
	Less than 1 year	134	13.4 [8.75-19.79]	16.4 [11.22-23.18]	2.2 [0.72-6.15]
	More than 1 year	254	10.6 [7.92-14.03]	7.9 [5.60-10.96]	3.5 [2.10-5.81]

3.4 Discussion

The demand for low and semi-skilled workers in several sectors in Malaysia has seen a dramatic rise in the number of workers entering the country from 1.06 million in 2002 to 2.07 million in 2014 (Bardan, 2014). The presence of such a substantial foreign work force originating from countries where parasitic infections are endemic is a major concern especially as this community is highly dynamic, and the emerging and re-emerging infectious diseases that they may carry are a great concern. For the present study we successfully recruited 388 migrant workers from their workplace who provided stool specimens compared to 173 stool specimens of clinically ill subjects from the University Malaya Medical Centre (UMMC) in the previous study (Suresh *et al*., 2002). Recruiting migrant workers to participate in the present study was very difficult since most workers and employers in Malaysia refused to participate. The main reason given by employers was that this procedure was not compulsory by the FOMEMA (agency involved in the implementation, management and supervision of a nationwide mandatory health screening programme for all legal migrant workers in Malaysia), Ministry of Health and Immigration Department of Ministry of Home Affairs Malaysia upon entry / residing in Malaysia. Other reasons often given by the workers and their employers included lack of interest in participating, disgust with faeces and preoccupation with matters related to work and achieving important deadlines.

Our study identified a two-fold increase of IPIs (62.9%) among workers compared to a decade ago (36.0%) (Suresh *et al*., 2002). Studies reporting analyses of parasitic infections among various communities in Malaysia have been conducted also among the Orang Asli (44.33%-99.2%) (Dunn, 1972; Dissanaike *et al*., 1977; Al-

Mekhlafi *et al*., 2005a; 2006; Ngui *et al*., 2011a; Nasr *et al*., 2013; Sinniah *et al*., 2014), plantation and rural communities (32.3%-70.0%) (Bisseru & Aziz, 1970; Lo *et al*., 1979; Al-Mekhlafi *et al*., 2007; 2008; Sinniah *et al*., 2011), slum dwellers (20.6%-90.9%) (Chia *et al*., 1978; Sinniah *et al*., 2014), fishing communities (54.2%-98.0%) (Heyneman *et al*., 1967; Balasingam *et al*., 1969; Nawalinski & Roundy, 1978; Anuar *et al*., 1978; Sinniah *et al*., 1988) and flat dwellers (5.1%-57.0%) (Kan, 1983; Che Ghani *et al*., 1989; Sinniah *et al*., 2002; 2014). Our findings based on migrant workers are in agreement with other studies on poverty- stricken communities in Malaysia although some studies have reported fluctuations in prevalence values especially among the slum dwellers (90.9 % in 1978 to 20.6% in 2014) (Chia *et al*., 1978; Sinniah *et al*., 2014), flat dwellers (57% in 1983 to 5.5% in 2014) (Kan, 1983; Sinniah *et al*., 2014) and rural communities (90.0% in 1970 to 32.3% in 2014) (Bisseru & Aziz, 1970; Sinniah *et al*., 2014). A total of 8 species of parasites were identified (*A. lumbricoides*, *T. trichiura*, hookworm, *E. vermicularis, H. nana*, *Entamoeba* sp., *Giardia* sp. and *Cryptosporidium* spp.), compared to only 6 species recorded previously (*A. lumbricoides*, *T. trichiura*, hookworm, *H. nana*, *Giardia* sp. and *Blastocystis* sp.) among migrant workers (Suresh *et al*., 2002). This outcome is not surprising as it reflects the government's failure to include mandatory STH screening as part of the requirement for working in this country.

Soil-transmitted helminth (STH) (68.3%) infections were more prevalent compared to protozoan infections (25.5%). Of the three common intestinal nematodes, *A. lumbricoides* (43.3%) infections were the most frequently identified, followed by hookworm (13.1%) and *T. trichiura* (9.5%). In contrast, a study more than a decade ago highlighted hookworm infections as the most prevalent (Suresh *et al*., 2002). However, our result concurs with global findings highlighting *A. lumbricoides* infections as the

most common helminth among the underprivileged communities (World Health Organization, 2015). A high presence of *A. lumbricoides* eggs contaminating public parks in Peninsular Malaysia has also been reported recently (Rahman *et al*., 2015).

The demographic profiles of respondents comprised predominantly volunteers from rural areas in their respective countries of origin where IPIs are still very much prevalent and a major concern among the poor and in deprived communities, particularly among workers from India and Nepal where prevalence can exceed 80%. The latest study in the low socio-economic areas of South Chennai documented a prevalence of 75.7% with IPI (Dhanabal *et al*., 2014), especially in children from rural and urban locations among whom prevalence with *A. lumbricoides* ranged between 60 to 91% (Fernandez *et al*., 2002). This was the most common helminth infection in this community (52.8%). Both studies suggest that inadequate sanitation and poor drainage is likely to have contributed to disease prevalence. Similarly, parasitic infections in Nepal have also been reported as being linked to rapid, unplanned urbanization, open defaecation and other unhygienic habits, as well as a lack of health awareness (Singh *et al*., 2013; Rabindranath *et al*., 2006; Uga *et al*., 2004).

Among the significant explanatory factors associated with the high prevalence of parasitic infections in this country were two main factors i.e, the number of working years in Malaysia and anthelmintic treatment. Workers with an employment history of less than a year or newly arrive workers in Malaysia were those who were most likely to be infected. In addition, they were also most likely to have no history of taking any anthelmintic drugs in the last 12 months. This is not surprising as the mandatory medical screening procedure upon entry to this country excludes examination for IPIs

and does not require administration of anthelmintic drugs to newly arrived workers (FOMEMA, 2015). Therefore our findings call for an improvement in health screening in future to include screening for parasitic infections and compulsory administration of anthelmintic drugs to workers upon entering Malaysia for employment. Such requirement is already implemented in some countries, that depend on an immigrant workforce, as for example in Qatar where currently prospective workers are required to undergo health checks at approved health clinics in their country of origin and if infection with helminths is detected, are routinely given albendazole prior to arrival as a condition for entry, residence and issuance of a work permit (Abu-Madi *et al*., 2010; 2011). Moreover those working in the food service industry have to undergo subsequent annual compulsory examinations by the Medical Commission as a condition of the continuation of their work permits.

Transmission of intestinal nematode infections within the community is predominantly dependent on human behaviour, particularly during eating and defaecation, personal hygiene, and cleanliness. The high prevalence of parasitic infections among the immigrant community sampled in this study provides an insight into the conditions under which the subjects live, and reflects the availability of environmental sanitation as well as the socioeconomic status of this sector of the population in Malaysia (World Health Organization, 2015).

3.5 Conclusion

High intestinal parasitic infections amongst migrant workers of all five working sectors in Malaysia was reported in this study and highlight the urgent need to refine the current health polices for workers entering the country for employment to include mandatory screening for parasitic infections. It is also recommended that workers be exposed to health education campaigns and programs aimed at increasing in the community awareness of the importance of personal hygiene, sanitation, cleanliness and healthy behaviors in controlling parasitic infections.

CHAPTER 4: SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTIONS AMONG MIGRANT WORKERS IN MALAYSIA

4.1 Introduction

Toxoplasma gondii is one of the most common protozoan parasites affecting up to one-third of the world's population (Feldman, 1974; Montoya & Liesenfeld, 2004; Hill *et al*., 2005). Human infection may occur via ingestion of food or water contaminated with oocysts shed in faeces of infected cats, consumption of undercooked or raw meat of venison, rabbits, raw oysters, clams, or mussels containing tissue cysts (Jacobs, 1974; Abu Bakar *et al*., 1992; Kolbekova´ *et al*., 2007; Ferguson, 2009; Jones *et al*., 2009), exposure to contaminated soil through activities such as gardening or children playing in sandpits (Petersen *et al*., 2010), blood transfusion or organ transplantation from infected donors (Siegel *et al*., 1971; Derouin & Pelloux, 2008) and vertical transmission from mother to fetus (Husni *et al*., 1994; Remington & Klein, 2001). Damage to the brain, eyes, or other organs can occur from a severe acute infection or through reactivation of past infection. Infections acquired during pregnancy may cause severe damage to the fetus (Remington & Klein, 2001). In immunocompromised patients, reactivation of latent disease can cause life-threatening encephalitis (Hanifa *et al*., 1996; Montoya & Liesenfeld, 2004). The standard method of diagnosis of this infection is through serological testing, based on the detection of Toxoplasma-specific immunoglobulin IgG and IgM antibodies in serum and is routinely practiced in many parts of the world (Alvarado-Esquivel *et al*., 2006; Binnicker *et al*., 2010; Mwambe *et al*., 2013).

Over the years, the number of migrant workers in Malaysia has grown exponentially. The recruitment of workers to Malaysia from neighboring countries has raised concerns that diseases endemic to their countries may be inadvertently brought into the country (Chan *et al*., 2009a). Despite compulsory medical screening prior to entering the workforce, parasitic infections screening including for *T. gondi* is lacking. In Malaysia, the previous reports on seroprevalence of *T. gondii* used indirect hemagglutination (IHA) test, Sabin Feldman dye test, indirect fluorescent antibody test (Yahaya, 1991) and the enzyme-linked immunosorbent assay (ELISA) (Nissapatorn *et al*., 2002).

To date, several studies among migrant workers in Malaysia highlighted the high presence of *T. gondi* infection, up to 42% (138/336) with positive IgG while twenty workers (6%) were positive with IgM particularly among Indonesian plantation workers and workers in detention camps (Chan *et al*., 2009a). Prior to that, Chan *et al*. in 2008 recorded the highest infection rate among Nepalese workers (46.2%) compared to other ethnic groups. Similarly, another serological study showed that just over a third (34.1%, 171/501) of migrant plantation workers and individuals in detention camps were IgG positive and 5.2% (26/501) were IgM positive (Chan *et al*., 2009b). Subsequently, Chan *et al.* (2009b) also noted that high numbers of local workers (n=89, 44.9%) were IgG positive in plantations as compared to migrants (n=171, 34.1%). However, workers with raised IgM were lower among locals (n=17, 8.6%), although not significantly, compared to migrants (n=26, 5.2%). On the other hand, Amal *et al.* (2008) noted lower rate of raised specific IgG among workers (n=16, 18.8%) from the Indian subcontinent from the same plantation and detention camp compared to locals (n=89, 44.9%).

There is a need to determine the health status among migrant workers with regard to *T. gondii* infection as most workers originate from countries with low socioeconomic backgrounds and live in deprived environments with poor sanitation and low hygiene practices (Norhayati *et al*., 2003). Therefore, this study was undertaken to determine the seroprevalence of *T. gondii* in the migrant workers and factors associated with the infection.

4.2 Materials and Methods

4.2.1 Study population and sample collection

A total of 484 from 610 volunteers of migrant workers in Malaysia agreed to participate in blood sample collection. Questionnaires were distributed to the participants to gather relevant information related to the study. All participants were fully informed of the nature of the study for their maximum co-operation and completion of consent forms. Details of the participants, composition of the questionnaire and ethical clearance of this study are as described in Chapter 2.

After consent was obtained and questionnaire answered, approximately 5ml of venous blood were drawn by trained medical assistants and nurses using disposable syringes and needles into a plain tube (without anticoagulant) from the participants. The bloods were transported back to the Parasitology Lab, Institute of Biological Science, Faculty of Science, University of Malaya. Blood samples were spun at 1,500 rpm for 10 minutes and the sera were kept in -20°C until use.

4.2.2 Detection of immunoglobulin G and M antibodies to *T. gondii*

Toxoplasmosis was screened using enzyme-linked immunosorbent assay (ELISA) commercial kit for immunoglobulin G (IgG) and M (IgM) (The Trinity Biotech Captia^{TM}, New York) in accordance to the manufacturer's instructions. The process was performed at the Biohealth Laboratory, Institute of Biological Science, Faculty of Science, University of Malaya.

All reagents were removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). All samples and controls were vortexed before use. 50 mL of the 20X Wash Buffer Type I were diluted to 1 L with distilled and/or deionized H20. The desired numbers of strips were placed into a microwell frame. Four (4) calibrator were determined (one Negative Control, two Calibrators and one Positive Control) per run. A reagent blank (RB) was ran on each assay.

For the IgG assays, test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) were diluted in Serum Diluent Plus. 100 µL of diluted patient sera, Calibrator and Control sera were added to individual wells. 100µL of Serum Diluent Plus was added to the reagent blank well. Each well was incubated at room temperature (21° to 25° C) for 25 minutes +/- 5 minutes. Liquid was aspirated or shake out from all wells. Using semi-automated or automated washing equipment, 250-300 µL of diluted Wash Buffer was added to each well. The wash procedure was repeated two times (for a total of three washes) for semi-automated equipment or four times (for a total of five washes) for automated equipment. After the final wash, the plate was blotted dry on paper toweling to remove all liquid from the wells. 100 µL Conjugate was added to each well, including the reagent blank well. Each well was incubated 25 minutes +/- 5 minutes at room temperature (21° to 25° C). Washing procedure was repeated as described above. 100 µL Chromogen/Substrate (TMB) solutions was added to each well, including reagent blank well, maintaining a constant rate of addition across the plate. Each well was incubated at room temperature (21° to 25° C) for 10-15 minutes. 100 µL of Stop Solution (1N H2SO4) was added to stop the reaction following the same order of Chromogen/Substrate addition, including reagent blank well. The plate was tap gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading. The developed color should be

read on an ELISA plate reader equipped with a 450 nm filter. The instrument was blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank was > 0.150, the run was repeated. Positive results were defined as >51 IU/ml, indicating latent or pre-existing *Toxoplasma* infection.

For IgM assays, test sera, Calibrator and Control sera 1:81 (e.g., 10 µL + 800 µL) were diluted in Serum Diluent Plus. 100 µL of diluted patient sera, Calibrator and Control sera were added to individual wells. 100µL of Serum Diluent Plus was added to the reagent blank well. Each well was incubated at room temperature (21° to 25° C) for 30 minutes +/- 2 minutes. Liquid was aspirated or shake out from all wells. Using semi-automated or automated washing equipment, 250-300 µL of diluted Wash Buffer was added to each well. The wash procedure was repeated two times (for a total of three washes) for semi-automated equipment or four times (for a total of five washes) for automated equipment. After the final wash, the plate was blotted dry on paper toweling to remove all liquid from the wells. 100 µL Conjugate was added to each well, including the reagent blank well. Each well was incubated 30 minutes +/- 2 minutes at room temperature (21° to 25° C). Washing procedure was repeated as described above. 100 µL Chromogen/Substrate (TMB) solutions was added to each well, including reagent blank well, maintaining a constant rate of addition across the plate. Each well was incubated at room temperature (21° to 25° C) for 15 minutes +/- 2 minutes. 100 µL of Stop Solution (1N H2SO4) was added to stop the reaction following the same order of Chromogen/Substrate addition, including reagent blank well. The plate was tap gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. The instrument was blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the

reagent blank was > 0.150, the run was repeated. Positive results for IgM assays were defined as >51 IU/ml, indicating recently acquired *Toxoplasma* infection.

In addition, all samples that were both IgG-positive and IgM-positive were tested using the IgG avidity assay (IgG; NovaLisa, Dietzenbach, Germany) according to manufacturer's instruction. All samples were diluted 1+100 with IgG Sample Diluent. 10µl sample and 1ml IgG Sample Diluent were dispensed into tubes to obtain a 1+100 dilution and thoroughly mixed with a Vortex. Wells were covered with the foil supplied in the kit and incubated for 1 hour ± 5 min at 37±1°C. Then, the content off the wells were aspirated and washed three times with 300µl of washing solution. 100µl Toxoplasma anti-IgG Conjugate was dispensed into all wells except for the blank well, covered with foil and incubated for 30 min at room temperature (20 to 25°C). Washing procedure was repeated. 100µl TMB Substrate Solution was dispensed into all wells and incubated for exactly 15 min at room temperature (20 to 25°C) in the dark. 100µl Stop Solution was dispensed into all wells in the same order and at the same rate as for the TMB Substrate Solution. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution. *Toxoplasma* antibodies with high avidity (>40%) indicates past infection, meanwhile *Toxoplasma* antibodies with low avidity (≤40) indicates acute or recent infections.

4.2.3 Data analysis

Prevalence data (percentage of subjects infected) are shown with 95% confidence limits (CL_{95}), as described by Rohlf & Sokal (1995) using bespoke software. Prevalences were analyzed using maximum likelihood techniques based on log linear analysis of contingency tables using the software package SPSS (Version 22). Analysis

was conducted with the intrinsic factors sex (2 levels, males and females), age (5 age classes comprising those <25 years old, 25-34 years old, 35-44 years old, 45-54 years old and those >54 years) and nationality (5 countries, Indonesia, Bangladesh, Myanmar, India and Nepal). Infection was considered as a binary factor (presence/absence of parasites). For extrinsic factors including 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 (3 types: hostel provided by the employer, construction site and own/rent house) and education (4 levels: primary school, secondary school, university and no formal schooling).

4.3 Results

4.3.1 Sociodemographic characteristics

A total of 484 migrant workers in Malaysia originated from rural areas in neighboring countries including; Indonesia (n=247, 51.0%), Nepal (n=99, 20.5%), Bangladesh (n=72, 14.9%), India (n=52, 10.7%) and Myanmar (n=14, 2.9%) were included in this study. Out of the 485 participants, just slightly over three quarters (n=375, 77.5%) were males, and the rest (n=109, 22.5%) females. Most were between the ages of 25 to 34 years old (n=183, 37.8%), followed by less than 25 years (n=142, 29.3%), 35 to 44 years (n=111, 22.9%), 45 to 54 years (n=35, 7.2%) and more than 55 years (n=13, 2.7%). According to the working sectors, the majority of volunteers were from the food service sector (n=115, 23.8%), followed by domestic (n=106, 21.9%), plantation (n=102, 21.1%), manufacturing (n=93, 19.2%) and construction (n=68, 14.0%) sectors. Most participants had at least a primary level education (n=228, 47.1%) followed by high school (n=201, 41.5%) and university level (n=10, 2.1%), and 45 participants (9.3%) did not receive any formal education.

4.3.2 Seroprevalence of *Toxoplasma gondii* infections

The overall *T. gondii* seroprevalence among 484 migrant workers was 57.4% (n= 278; CI = 52.7-61.8%) with 52.9% (n= 256; CI= 48.4-57.2%) being seropositive for anti-*Toxoplasma* IgG only, 0.8% (n= 4; CI = 0.2-1.7%) seropositive for anti-*Toxoplasma* IgM only and 3.7% (n= 18; CI = 2.1-5.4%) seropositive with both IgG and IgM antibodies (Table 4.1). All 18 positive samples with both IgG and IgM antibodies showed high avidity (> 40%), suggesting chronic infection.

Infection with *T. gondii* was found almost similar between males (213/375; 56.8%) and female workers (65/109; 59.6%). Based on age factor, highest prevalence was found among workers age more than 55 years old (10/13; 76.9%), followed by 45 to 54 years old (26/35; 74.3%), less than 25 years old (84/142; 59.2%), 35 to 44 years old (61/111; 55.0%) and 25 to 34 years old (97/183; 53.0%). Meanwhile, according to country of origin, the highest prevalence were among Nepalese (77.8%), followed by Indonesian (58.3%), Bangladeshi (45.8%), Indian (38.5%) and Myanmaris (28.6%). The highest seroprevalence according to working sector were from the manufacturing (76.3%) sector followed by construction (61.8%), domestic (57.5%), food service (50.4%), and plantation (45.1%). A total of 66.1% of the infected workers have been in Malaysia for less than a year compared to those has been living in Malaysia for more than a year (52.3%). Based on the accommodation of the workers, prevalence of infection was almost similar among the workers living in the construction site (62.3%), hostel by the employer (56.4%) and own/rent house (57.9%).

4.3.2.1 Intrinsic effects on seroprevalence of IgG and IgM antibodies to *T. gondii* infections

The seropositivity of *T. gondii* was examined in relation to sociodemographic factors using maximum likelihood techniques based on log linear analysis. The analysis showed that two factors were found associated with seropositivity of anti-*Toxoplasma* IgG including age (X^2_4 = 9.761, P = 0.045) and nationality (X^2_4 = 30.046, P = <0.001) (Table 4.2). Meanwhile, based on seropositivity of anti-*Toxoplasma* IgM and seropositivity with both IgG and IgM antibodies, none of the factors were found significant (Table 4.2).

4.3.2.2 Extrinsic effects on seroprevalence of IgG and IgM antibodies to *T. gondii* infections

The analysis of four extrinsic factors (employment sectors, years of residence in Malaysia, accommodation and education) showed that two factors were found associated with seropositivity of anti-*Toxoplasma* IgG including employment sector (X^2_4 = 21.306, P = <0.001) and years of residence in Malaysia (X^2_1 = 8.294, P = 0.004) (Table 4.2). Similarly, none of the factors were found significant based on seropositivity of anti-*Toxoplasma* IgM and seropositivity with both IgG and IgM antibodies (Table 4.2).

Table 4.1: Seroprevalence of IgG and IgM antibodies to *T. gondii* among 485 migrant workers using ELISA: CI=95%- confidence intervals

Antibodies	*T.gondii* seropositive		
	No. of seropositive	Seropositive (%)	CI
IgG+	256	52.9	48.4-57.2
IgM+	4	0.8	0.2-1.7
IgG+IgM+	18	3.7	2.1-5.4
Total	**278**	**57.4**	**52.7-61.8**

Table 4.2: **Seroprevalence of IgG and IgM antibodies to *T. gondii* infections among migrant workers in Malaysia according to sex, age, sex, nationality, employment sector, years of residence, accommodation and education; *significant at p<0.01**

Factors		IgG +		IgM+		IgG+ IgM+	
		% (95% CI)	*P* - value	% (95% CI)	*P* - value	% (95% CI)	*P* - value
Intrinsic Factor							
Sex	**Male (n=375)**	55.7 [50.5-60.8]	0.469	4.8 [2.9-7.5]	0.610	3.7 [2.1-6.2]	0.975
	Female (n=109)	59.6 [49.8-68.9]		3.7 [1.0-9.1]		3.7 [1.0-9.1]	
Age*	**<25 (n=142)**	59.2 [50.6-67.3]	**0.045**	3.5 [1.2-8.0]	0.732	3.5 [1.2-8.0]	0.853
	25-34 (n=183)	51.4 [43.9-58.8]		5.5 [2.7-9.8]		3.8 [1.6-7.7]	
	35-44 (n=111)	54.1 [44.3-63.6]		4.5 [1.5-10.2]		3.6 [1.0-9.0]	
	45-54 (n=35)	74.3 [56.7-87.5]		5.7 [0.7-19.2]		5.7 [0.7-19.2]	
	>55 (n=13)	76.9 [46.2-95.0]		0.0 [0.0-24.7]		0.0 [0.0-24.7]	
Nationality*	**Indonesia (n=247)**	58.3 [51.9-64.5]	**<0.001**	5.3 [2.8-8.8]	0.448	5.3 [2.8-8.8]	0.325
	Bangladesh (n=72)	44.4 [32.7-56.6]		2.8 [0.3-9.7]		1.4 [0.0-7.5]	
	Myanmar (n=14)	28.6 [8.4-58.1]		0.0 [0.0-23.2]		0.0 [0.0-23.2]	
	India (n=52)	38.5 [25.3-53.0]		1.9 [0.0-10.3]		1.9 [0.0-10.3]	
	Nepal (n=99)	74.7 [65.0-82.9]		6.1 [2.3-12.7]		3.0 [0.6-8.6]	

Extrinsic Factor							
Employment Sector*	**Construction (n= 68)**	61.8 [49.2-73.3]	**<0.001**	5.9 [1.6-14.4]	0.417	5.9 [1.6-14.4]	0.400
	Manufacturing (n= 93)	74.2 [64.1-82.7]		4.3 [1.2-10.6]		2.2 [0.3-7.6]	
	Plantation (n=102)	44.1 [34.3-54.3]		4.9 [1.6-11.1]		3.9 [1.1-9.7]	
	Food service (n=115)	50.4 [41.0-59.9]		1.7 [0.2-6.1]		1.7 [0.2-6.1]	
	Domestic (n=106)	56.6 [46.6-66.2]		6.6 [2.7-13.1]		5.7 [2.1-11.9]	
Years of Residence*	**< than 1 year (n=180)**	65.0 [57.6-71.9]	**0.004**	6.7 [3.5-11.4]	0.091	5.6 [2.7-10.0]	0.107
	> than 1 year (304)	51.6 [45.9-57.4]		3.3 [1.6-6.0]		2.6 [1.1-5.1]	
Accommodation	**Own/rent house (n=133)**	57.1 [48.3-65.7]	0.638	5.3 [2.1-10.5]	0.447	4.5 [1.7-9.6]	0.236
	Construction site (n=53)	62.3 [47.9-75.2]		7.5 [2.1-18.2]		7.5 [2.1-18.2]	
	Hostel by employer (n=298)	55.4 [49.5-61.1]		3.7 [1.9-6.5]		2.7 [1.2-5.2]	
Education	**Primary (228)**	54.4 [47.7-61.0]	0.114	4.8 [2.4-8.5]	0.674	3.9 [1.8-7.4]	0.572
	Secondary (n=201)	62.7 [55.6-69.4]		4.0 [1.7-7.7]		3.0 [1.1-6.4]	
	University (n=10)	50.0 [18.7-81.3]		0.0 [0.0-30.8]		0.0 [0.0-30.8]	
	No formal schooling (n=45)	42.2 [27.7-57.8]		6.7 [1.4-18.3]		6.7 [1.4-18.3]	

4.4 Discussion

This study investigated *T. gondii* infection among migrant workers in Malaysia using standard commercial kits that detect anti-*Toxoplasma* IgG and IgM antibodies in order to determine the seroprevalence and factors that may contribute to the infection. The results showed that more than half of the workers had latent infection (53.0%), an indication of previous exposure to *T. gondi.* Four males (0.8%) were positive for specific IgM but negative for IgG antibodies, indicating possible acute infection. All respondents in this study possessed valid working permits and were provided with proper housing, equipped with clean water, toilets and efficient waste disposal systems. The workers were also provided with full medical benefits and personal protective equipment (PPE) whilst at work. Thus the high prevalence of latent *T. gondii* infection among these workers probably indicated previous infection acquired from their home countries where the infections are prevalent (Gandahusada, 1991; Rai *et al*., 1994; 1999).

This is the first study to determine the seroprevalence of *T.gondii* infections in migrant workers from multi-sectors. Previous studies have only reported amongst workers involved in plantation sector (Chan *et al*., 2008; 2009a; 2009b; Amal *et al*., 2008) and presently, *T. gondii* seroprevalence was slightly higher (57.4%) compared to previous reports of 34.1% to 54.4% (Chan *et al*., 2008; 2009a; 2009b; Amal *et al*., 2008). This is not surprising as human infection is widely prevalent, with nearly one-third of the world population are exposed to this parasite (Dubey & Beattie, 1988; Montoya & Liesenfeld, 2004; Dubey, 2010). This disease is not exclusive to migrants only but has been reported in healthy persons (13.9%-30.2%) (Tan & Zaman, 1973; Thamas *et al*., 1980; Sinniah *et al*., 1984), pregnant women (23.0%-31.6%) (Cheah *et*

al., 1975; Tan *et al*., 1976; Khairul Anuar *et al*., 1991; Ravichandran *et al*., 1998), HIV patients (21.0%-41.2%) (Nissapatorn *et al*., 2002; 2003a; 2003b; 2003c; 2003d), newborn babies (2.0%) (Tan & Mak, 1985) and the indigenous communities (10.6%-37.0%) (Lokman *et al*., 1994; Ngui *et al*., 2011b) in Malaysia. In Southeast Asia, *T. gondii* seroprevalence varied from < 2% up to 70% (Nissapatorn, 2007). In developed countries such as the United States and United Kingdom, it was estimated that 10–40% of people are infected (Bhatia *et al*., 1974; Dubey, 1994; Sukthana, 2006), whereas infections in Central and South America and continental Europe ranged from 50 to 80 % (Jones *et al*., 2007).

In the present study, two internal factors showed significant association with *T. gondii* infection. The first variable was age group ($\square^2_4$= 15.99, p=0.003) where infections were higher in workers above 45 years old (74.3-76.9%) compared with those below this age group (53.0%-59.2%). Higher infection with age was also in agreement with previous studies (Tenter *et al*., 2000; Nissapatorn & Khairul Anuar, 2004; Sobral *et al*., 2005; Ngui *et al*., 2011b). Most infections were acquired early in age and increased with age (Tan & Zaman 1973; Nissapatorn *et al*., 2003a). As the host aged, the probability of other *Toxoplasma* transmission mechanisms are also increased (Apt *et al*., 1973; Amendoeira *et al*., 1999; Ngui *et al*., 2011b). Similarly, a study among the indigenous communities (Orang Asli) showed that the seroprevalence was comparatively higher in participants above 12 years old compared to those below (Ngui *et al*., 2011b). Nevertheless, no significant difference was noted between infection rate and host-sex. Probable recently acquired infections (positive anti-IgM, negative anti-IgG) in 4 males (3 Nepalese, 1 Bangladeshi) were possibly due to personal hygiene and dietary habits.

According to nationality, the infections were highest among workers from Nepal (77.8%), followed by Indonesia (58.3%), Bangladesh (45.8%), India (38.5%) and Myanmar (28.6%). All nationalities examined for *Toxoplasma* antibodies were seropositive which is also in agreement with the previous study in 2008 from Malaysia (Chan *et al*., 2008). The analysis also revealed that country of origin (χ^2_4= 30.046, p = <0.001) was a significant factor for seropositivity. Variations in prevalence rates among migrants from different nationality are most likely due to differences in dietary habits, behavioral risks, environmental conditions, socioeconomic status and hygiene (Chan *et al*., 2008). The high infection among Nepalese could be due to the habitual ingestion of minced raw meat or insufficiently cooked meat by ethnic groups as reported in the past with positive rates of 57.9% and 65.3% (Rai *et al*., 1994; 1999). *T. gondi* infection is also one of the most frequently observed food-borne diseases reported in Indonesia. Gandahusada (1991) reported that *Toxoplasma* antibodies correlated with the presence of cats and with eating raw or partly cooked meat. This parasite is widespread, with seroprevalence rates of 2-63% in humans, 35-73% in cats, 75% in dogs, 11-36% in pigs, 11-61% in goats, and less than 10% in cows (Gandahusada, 1991). In the present study, 100% of workers (n=485) originated from rural areas in their respective countries where parasitic infections are still very much prevalent and a major concern among the poor and deprived communities. Significant correlations between consumption of unboiled water and the *T. gondii* seropositivity also have been noted in many studies, particularly among disadvantaged and indigenous communities living in rural and remote areas (de Moura *et al*., 2006; Sroka *et al*., 2006; 2010a; 2010b).

According to the working sector, there were significant differences (X^2_4 = 21.306, P = <0.001) with infections higher among workers from the manufacturing (76.3%) compared with other sectors. The nature of one's occupation increases the risks

acquring *T. gondii* infection as Rai *et al* (1999) described high prevalence among those engaged in agricultural activities. However, the present results showed the lowest prevalence (45.1%) amongst plantation workers compared to other sectors. The current results may be biased since most (91.1%) working sectors were dominated by a particular nationality. Most Nepalese dominated the manufacturing sector (81.7%) and this corroborated with high infections amongst workers in the manufacturing sector (76.3%) were Nepalese (77.8%).

Workers with an employment history of less than a year or newly arrive workers (X^2_1 = 8.294, P = 0.004) in Malaysia were those who were mostly infected with *T. gondii*. The differences in dietary habits, behavioral risks, environmental condition, socioeconomic status and lack of hygiene were more likely to be the risk factors among these workers that probably indicated previous infection acquired from their home countries where the infections are prevalent (Gandahusada, 1991; Rai *et al*., 1994; 1999).

4.5 Conclusion

High seroprevalence of *T. gondii* latent infection, an indication of previous exposure to *T. gondi* was recorded among migrant workers of all five working sectors in Malaysia with significant association with host-age, nationality, working sector and period of residence. This indicate that infections were acquired from their home countries where the infections are prevalent. This calls for public health authorities to include health education program on transmission of this disease; however not only among migrant workers but also the general public in an effort to prevent the infection.

CHAPTER 5: SEROPREVALENCE OF *STRONGYLOIDES STERCORALIS* INFECTIONS AMONG MIGRANT WORKERS IN MALAYSIA

5.1 Introduction

Strongyloides stercoralis is one of four known soil-transmitted helminth infections categorized as neglected tropical diseases (NTD) which infects an estimated 30-100 million people worldwide (Olsen *et al*., 2009). Infection is through penetration of the infective larvae into the intact skin or through auto-infection by the rhabditiform larvae. Infected individuals may be symptomatic or asymptomatic depending on the host immune response and number of larvae. Respiratory or gastrointestinal symptoms may develop include diarrhea, pneumonia, gastrointestinal bleeding and hemorrhagic pneumonitis (Montes *et al*., 2010; Ahmad *et al*., 2013).

Strongyloidiasis is prevalent in socioeconomic deprived communities where hygiene and sanitation is lacking. In Malaysia, the limited number of studies to detect the presence of *S. stercoralis* was previously reported among the rural communities of the *Orang Asli* (Rahmah *et al*., 1997; Ahmad *et al*., 2013). Rahmah and colleagues conducted a study among the aborigine children in 6 villages in Post Brooke, Kelantan using formal-ether concentration technique and detected only 1.2% of the children infected (Rahmah *et al*., 1997). While Ahmad *et al*. (2013) reported no presence of *S. stercoralis* larvae from 54 stool samples screened, however, serological examination of the same individuals revealed a prevalence of 31.5%. Subsequently, a nested PCR confirmed that only 3 (5.6%) samples were positive (Ahmad *et al*., 2013). Another study among patients reporting gastrointestinal symptoms from a hospital in Sarawak recorded prevalence of 39% using pentaplex-PCR (Basuni *et al*., 2011).

Similar studies were also conducted among refugees and immigrants from developed countries with infection rates varying substantially up to 75% dependent on the refugees' country of origin (Schar *et al*., 2013). In Canada, a prevalence of 11.8% was recorded among Vietnamese and 76.6% among Cambodian refugees (Gyorkos *et al*., 1990). Other worldwide studies conducted among refugees and immigrants included Australia (22.3% - 28.5%) (Martin & Mak, 2006; Rice *et al*., 2003; Fisher *et al*., 1993; de Silva *et al*., 2002; Caruana *et al*., 2006; Gibney *et al*., 2009), Canada (61.3% - 73.5%) (Gyorkos *et al*., 1989; 1990; 1992), China (15.2% - 19.2%) (Peng *et al*., 1993; Cheng & Shieh, 2000; Wang, 1998), France (5.6%) (Lamour *et al*., 1994), Israel (27.0% - 35.1%) (Nahmias *et al*., 1991; Berger *et al*., 1989), Italy (3.3%) (Gualdieri *et al*., 2011), Libya (1.1%) (Al Kilani *et al*., 2008), Saudi Arabia (5.5% - 9.0%) (Al-Madani & Mahfouz, 1995; Mohammad & Koshak, 2011), Spain (2.8% - 6.1%) (Diaz *et al*., 2002; Martin *et al*., 2004; Vilalta *et al*., 1995), Sudan (98.9%) (Marnell *et al*., 1992), Sweden (1.0%) (Persson & Rombo, 1994) and United States of America (37.8% - 43.0%) (Lurio *et al*., 1991; Buchwald *et al*., 1995; Ciesielski *et al*., 1992; Garg *et al*., 2005; Geltman *et al*., 2005; Lifson *et al*., 2002; Miller *et al*., 2000; Seybolt *et al*., 2006; Posey *et al*., 2007; Brodine *et al*., 2009; Hochberg *et al*., 2011).

Study of this infection among migrant workers was reported among several female Asian nationalities working as domestic helpers in Saudi Arabia. In this study, Al-Madani and Mahfouz (1995) recorded low prevalence (0.6%) (0.4% in Filipinos, 0.5% in Indonesians, 1.5% in Sri Lankans, 2.6% in Indians and 3.4% in Thais).

Most workers to Malaysia come from the neighbouring countries where this infection is endemic (Hall *et al*., 1994; Hoge *et al*. in 1995; Lanjewar *et al*., 1996; Singh

et al., 2004; Singh *et al*., 1993; Kang *et al*., 1998; Joshi *et al*., 2002; Bangs *et al*., 1996; Widjana & Sutisna, 2000; Hasegawa *et al*., 1992; Mangali *et al*., 1993; 1994; Toma *et al*. 1999). The last record on parasitic infections among migrant workers in Malaysia was conducted more than a decade ago however this study excluded screening for *S. stercoralis* infections (Suresh *et al.*, 2002). Despite compulsory medical screening for workers prior to entering the workforce, parasitic infections screening including detection of *S. stercoralis* is not carried out. Therefore, this study is necessary to determine the seroepidemiology of *S. stercoralis* infection among migrant workers to this country as it could possibly have public health implications and to identify factors associated to this infection.

5.2 Materials and Methods

5.2.1 Study population and sample collection

A total of 306 from 610 volunteers of migrant workers in Malaysia consented to blood collection for the detection of *Strongyloides stercoralis*. Questionnaires were distributed to the participants to gather relevant information related to the study. All participants were fully informed of the nature of the study for their maximum co-operation and completion of consent forms. Details of the participants, composition of the questionnaire and ethical clearance of this study are described in Chapter 2.

After consent was obtained and questionnaire answered, approximately 5ml of venous blood were drawn by trained medical assistants and nurses using disposable syringes and needles into a plain tube (without anticoagulant) from each volunteer. The blood samples were transported back to the Parasitology Lab, Institute of Biological Science, Faculty of Science, University of Malaya. Blood samples were spun at 1,500 rpm for 10 minutes and the sera were kept in -20°C until further use.

5.2.2 Detection of immunoglobulin G to *Strongyloides stercoralis* infection

Strongyloidiasis was screened using enzyme-linked immunosorbent assay (ELISA) commercial kit for immunoglobulin G (IgG) (Scimedx Corporation, NJ, USA) in accordance to the manufacturer's instructions. The process was performed at the Biohealth Laboratory, Institute of Biological Science, Faculty of Science, University of Malaya.

All reagents were removed from refrigeration and left at room temperature before use (15° to 25° C). All samples and controls were vortexed before use. Aliquot of 25 mL of the 20X wash concentrate was diluted to 500 mL with distilled and/or deionized H_20. The desired numbers of strips were placed into a microwell frame. Three calibrators were determined; one negative control, one positive control and a reagent blank (RB), was run on each assay. Patient sera were diluted 1:64 in dilution buffer (5µl sera and 315 µl dilution buffer). 100µl (or two drops) of the negative control was added to the well number 1, 100µl of the positive control was added to well number 2 and 100µl of the diluted test samples were added to the remaining wells. Wells were incubated at room temperature (15 to 25˚C) for 10 minutes, and then washed. Washing procedure consisted of vigorously filling each well to overflow and decanting contents for three separate times. The wells were slapped on a clean absorbent towel to remove excess wash buffer. Two drops (100µl) of enzyme conjugate were added to each well. The wells were incubated at room temperature for 5 minutes and then washed. Two drops (100µl) of the chromogen were added to every well. The wells were incubated at room temperature for 5 minutes. Two drops (100µl) of the stop solution were added to each well. The wells were mixed by gently tapping the side of the strip holder with index finger for approximately 15 seconds.

The wells were read at 450/ 620-650nm by the ELISA Reader. Positive results were defined when absorbance reading greater than or equal to 0.2 OD units that indicated possible *Strongyloides* infection. Negative results were defined when absorbance reading was less than 0.2 OD units indicating no detectable level of antibodies due to no infection or poor immune response.

5.2.3 Statistical analysis

Prevalence data (percentage of subjects infected) are shown with 95% confidence limits (CL_{95}), as described by Rohlf & Sokal (1995) using bespoke software. Prevalences were analyzed using maximum likelihood techniques based on log linear analysis of contingency tables using the software package SPSS (Version 22). Analysis was conducted with the intrinsic factors sex (2 levels, males and females), age (5 age classes comprising those <25 years old, 25-34 years old, 35-44 years old, 45-54 years old and those >54 years) and nationality (5 countries, Indonesia, Bangladesh, Myanmar, India and Nepal). Infection was considered as a binary factor (presence/absence of parasites). For extrinsic factors including 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 (3 types: hostel provided by the employer, construction site and own/rent house) and education (4 levels: primary school, secondary school, university and no formal schooling).

5.3 Results

5.3.1 Socio-demographic characteristics

All 306 migrant workers recruited in this study originated from rural areas in their country of origin. Majority were from Indonesia (n=124, 40.5%), followed by Nepal (n=76, 24.8%), Bangladesh (n=53, 17.3%), India (n=41, 13.4%) and Myanmar (n=12, 3.9%) Slightly more than three quarters were males (male: n=243, 79.4%; female: n=63, 20.6%). A third were within the age range of 25 to 34 years old (n=110, 35.9%), followed by less than 25 (n=93, 30.4%), 35 to 44 (n=70, 22.9%), 45 to 54 (n=25, 8.2%) and more than 55 years old (n=8, 2.6%). According to the employment sectors, a majority were employed in the food service sector (n=94, 30.7%), followed by plantation (n=71, 23.3%), domestic (n=70, 22.9%), manufacturing (n=61, 19.9%) and construction (n=10, 3.3%). Most of the workers received secondary education (n=140, 45.8%) followed by primary (n=120, 39.2%) and university (n=7, 2.3%), but 39 participants (12.7%) did not receive any formal education.

5.3.2 Seroprevalence of strongyloidiasis and seropositivity of *S. stercoralis* infection

The overall seroprevalence of strongyloidiasis was 115 (37.6%) among the migrant workers. All workers 100% (n=306) originated from rural areas in their country with no history of taking any anthelminthic drugs for the last 12 months. Based on the intrinsic and extrinsic factors, no significant association was found in relation to *S. stercoralis* infection with any for the socio demographic factors (Table 5.1).

Table 5.1: Prevalence of *S. stercoralis* infection in relation to socio-demographic characteristics (sex, age, nationality, employment sector, years of residence, accommodation and education).

Factors		Total samples	% [(95% CI]	*P*-value
Intrinsic factor				
Sex	**Male**	243	38.3 [32.1-44.7]	0.623
	Female	63	34.9 [23.3-48.0]	
Age	**<25**	93	38.7 [28.8-49.4]	0.934
	25-34	110	35.5 [26.6-45.1]	
	35-44	70	38.6 [27.2-51.0]	
	45-54	25	36.0 [18.0-57.5]	
	>55	8	50.0 [15.7-84.3]	
Nationality	**Indonesia**	124	33.1 [24.9-42.1]	0.246
	Bangladesh	53	35.8 [23.1-50.2]	
	Myanmar	12	58.3 [27.7-84.8]	
	India	41	48.8 [32.9-64.9]	
	Nepal	76	36.8 [26.1-48.7]	
Extrinsic factor				
Employment Sector	**Construction**	10	10.0 [0.3-44.5]	0.055
	Manufacture	61	34.4 [22.7-47.7]	
	Plantation	71	29.6 [19.3-41.6]	
	Food Service	94	45.7 [35.4-56.3]	
	Domestic	70	41.4 [29.8-53.8]	
Years of residence	**< than 1 year**	122	37.7 [29.1-46.9]	0.971
	> than 1 year	184	37.5 [30.5-44.9]	
Accommodation	**Own/rent house**	83	39.8 [29.2-51.1]	0.140
	Construction site	4	0.0 [0.0-60.2]	
	Hostel by employer	219	37.4 [31.0-44.2]	
Education	**Primary**	120	35.0 [26.5-44.2]	0.894
	Secondary	140	39.3 [31.1-47.9]	
	University	7	42.9 [9.9-81.6]	
	No formal schooling	39	38.5 [23.4-55.4]	

5.4 Discussion

Malaysia has a strong economic presence in the region over since the early 1970's and experienced mass migration of workers particularly from rural background for employment from neighbouring countries where *S. stercoralis* infections remain endemic. Presently, screening for strongyloidiasis is not mandatory for employment to this country. Therefore, this is the first study to report the presence of anti-*Strongyloides* antibody in sera among migrant workers using an enzyme-linked immunosorbent assay (ELISA) commercial kit for immunoglobulin G (IgG). Socio-demographic description showed in this study provided evidence that all the workers originated mainly from rural areas in their respective countries where parasitic infections were still very much prevalent among the poor and deprived communities. Results also show an overwhelming over a third of the workers screened were seropositive and was in agreement with global values between 10% and 40% in endemic countries in the tropical and subtropical region (Schar *et al*., 2013). In South-East Asia region, reports have shown that prevalence of *S. stercoralis* infection among the population varied [Cambodia (2.6%-44.7%), Thailand (0.1%-57.0%), Indonesia (0.8%-5.4%), China (0.04%-17.9%), Lao PDR (1.4%-41.0%) and Vietnam (0.1%)] (Schar *et al*., 2016).

Most studies previously conducted in Malaysia focused on other soil-transmitted helminth (STH) ignoring *S. stercoralis* specifically. Other works have used the conventional microscopy technique for the detection of *S. stercoralis* larvae in faecal samples which have low level of sensitivity (Rahmah *et al*., 1997; Ahmad *et al*., 2013). Based on microscopic examination conducted in this study, all of the 306 faecal samples provided were negative for larvae. Siddiqui and Berk (2001) reported that microscopy examination performed to detect *S. stercoralis* larvae using only single

stool sample lacks sensitivity and failed up to 70 % of cases particularly if the intestinal worm load is very low as commonly occurs in most asymptomatic individuals. Although it was suggested that using multiple faecal samples will improve sensitivity by ≥50% (Siddiqui & Berk, 2001), obtaining repeat samples was not possible in this study. Based on the results from the ELISA method, high seropositivity was detected in the targeted population. However, this result was not unequivocally able to prove current active infection and is possible the result from a persisting level of antibody from a past infection (Yori *et al*., 2006; Ahmad *et al*., 2013). The ELISA method has more advantage over the microscopy in which, large-scale screening can be performed simultaneously. In addition, the assay is also easy to perform as it is based on the interpretation of the absorbance reading and can be performed without expertise in morphological identification (Caruana *et al*., 2006; Yori *et al*., 2006; Ahmad *et al*., 2013).

In the present study, infections in male (38.3%) were slightly higher than female workers (34.9%) however not significant. A review on *S. stercoralis* infections in South East Asia recognized gender as one of the risk factors (Schar *et al*., 2016). In rural Lao PDR, Conlan *et al*. (2012) showed that males were at higher risk of infection with an odds ratio (OR) of 2.76. Similar findings were recorded in Cambodia with *S. stercoralis* infection having an OR of 1.4 in males (p = 0.001) (Koga-Kita, 2004) and higher prevalence recorded among males than females and in all age groups (OR: 1.7; $p < 0.001$) in another study (Khieu *et al*., 2014b). In Thailand, infections were significantly different in boys (35.2%) than girls (16.8%) (p = 0.003) (Khampitak *et al*., 2006). While in Northern Ghana, significantly higher infection was recorded in males (12.7%) compared to females (10.6%) (Yelifari *et al*., 2005) as well as in Okinawa, Japan (males: 14.0%; females: 6.8%) (Arakaki *et al*., 1992). Gender played a significant role

particularly among males due to possibly higher involvement in outdoor activities compared to females especially in the plantation/agricultural sector (Ahmad *et al*., 2013). The frequent contact with contaminated soil or water may predispose them to *S. stercoralis* infection, as this parasite was transmitted by penetration of the infective larvae to the intact skin.

Infections from this study also showed workers involved in the food services (45.7%) with the highest seropositivity, followed closely by the domestic (41.4%) sector, manufacturing (34.4%), plantation (29.6%) and finally construction (10.0%). Results were not able to show any significant association to occupation in this study. In contrast, other studies in South Eastern provinces of China (Wang *et al*., 2013) and Yunnan province in the South of China (Steinmann *et al*., 2007; 2008) have found high *S. stercoralis* infections among those involved in agriculture particularly farmers. In Europe and in the United States, infections were also predominantly among those in plantation and mining sectors (Schar *et al*., 2013) and among miners in Germany (Arbeitsmedizin, 2009). The present results suggest that infections were acquired from their home country where infections were more prevalent however, using the current detection method, we were unable to provide evidence to substantiate this statement.

This result highlights public health implications as transmission is basically due to poor personal hygiene and sanitation, in addition to improper method of handling sewage disposal may promote *S. stercoralis* infections (Lim *et al*., 2009, Schar *et al*., 2013). A community based survey of the urban slum community in Bangladesh showed high prevalence of infection (29.8%) (Hall *et al*., 1994) as also recorded in Nepal (22.8%) (Hoge *et al*. in 1995). However, India (Lanjewar *et al*., 1996; Singh *et al*.,

2004; Singh *et al*., 1993; Kang *et al*., 1998; Joshi *et al*., 2002) and Indonesia (Bangs *et al*., 1996; Widjana & Sutisna, 2000; Hasegawa *et al*., 1992; Mangali *et al*., 1993; 1994; Toma *et al*. 1999), recorded lower prevalence of 6.6% and 7.6%, respectively. High infection rates up to 60% can be expected in the resource-poor countries with ecological and socioeconomic conditions that favor the spread of *S. stercoralis* and very low prevalence in societies where faecal contamination is rare especially in urban and developed countries, with the exception of slum areas in the big cities (Schar *et al*., 2013). A study in rural Cambodia found households with latrine were significantly least infected and a reduction of 39% of strongyloidiasis cases in those households with proper latrine used for defecation (Khieu *et al*., 2014a).

Other factors such as behavioral patterns are known to also play a role in the transmission with most acquired infection gotten during childhood and sustained through auto-infection (Schar *et al*., 2013). This is possible due to the parasites' capability to reproduce within a human host (endogenous autoinfection) resulting in long-lasting infection with several studies reporting individuals with *S. stercoralis* infections for more than 75 years (Concha *et al*., 2005; Genta, 1992; Keiser & Nutman, 2004; Vadlamudi *et al*., 2006; Prendki *et al*., 2011; Gill *et al*., 2004).

The current result provided baseline information of the epidemiology of *S. stercoralis* infections among the migrant workers and highlights the importance of using an appropriate technique in the detection of this infection. Similarly, most studies on refugees and migrants worldwide also used copro-diagnostic procedures with low sensitivity (71.8%) compared to copro-diagnostic procedures with moderate sensitivity (7.7%) and serological diagnostic procedures with high sensitivity (20.5%) (Schär *et al*.,

2013). The frequent choice of diagnosis for *S. stercoralis* infections were the Koga Agar culture method and Baermann method, which are considerably time consuming and labour intensive. These methods involve examining stool samples collected over a few days and could mitigate the risk of missing low-intensity infections. Therefore, further analysis needed in order to obtain a clearer picture of the prevalence of this parasite. In addition to serology, further studies such as molecular characterization is necessary to confirm current active infections which will be described in chapter 6.

5.5 Conclusion

High seroprevalence of *S. stercoralis* infections among migrant workers was reported for the first time in Malaysia. This data provided baseline information on epidemiology of *S. stercoralis* infections among the workers and highlights the importance of using an appropriate technique in the detection of this infection. The high infection among these workers calls for health education on transmission and the importance of good personal hygiene and sanitation. Despite failing to differentiate current active or past *S. stercoralis* infection, results suggest that serological method such as ELISA as one of the alternative diagnostic tool for detection.

CHAPTER 6: MOLECULAR CHARACTERIZATION OF HUMAN INTESTINAL PARASITE INFECTIONS

6.1 Introduction

The development of molecular biology especially polymerase chain reaction (PCR) has resulted in a powerful tool to screen, characterize and evaluate genetic diversity (Karp & Edwards, 1997). This method is widely used in many studies to assist identification of organisms particularly those that are morphologically similar. Therefore, the present study was conducted to characterize the genetic makeup of human intestinal parasites recovered in this study namely; *Strongyloides stercoralis*, hookworms, *Entamoeba* spp., *Giardia* spp. and *Cryptosporidium* spp.

Strongyloides stercoralis is commonly found in tropical and subtropical regions of the world including Europe, America and Southeast Asia (Siddiqui & Berk, 2001). The actual prevalence of *S. stercoralis* is often queried due to the lack of standard diagnostic tools (Olsen *et al*., 2009; Verweij *et al*., 2009). Previous chapter showed the importance of using serological analysis (ELISA) as an alternative diagnostic tool for detecting *S. stercoralis* infection however, this method failed to detect current active *S. stercoralis* infection. On the other hand, the conventional microscopic examination of stool specimens is only useful when the larvae load is high, such as acute *S. stercoralis* infection. Therefore, this helminth will be tested further using nested PCR targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene to assist in determining current active infections.

Human hookworm infection is commonly caused by two species of hookworm namely *Necator americanus* and *Ancylostoma duodenale*. The current diagnosis of hookworm infection in human is via the identification of the parasite eggs in the host faeces which is technically simple and low cost. However, this technique is hampered by the morphological similarities between the eggs species of *N. americanus*, *Ancylostoma spp.* and other strongylid nematodes including *Oesophagostoum* spp. and *Trichostrongylus* spp. Therefore, a two-step semi-nested PCR was employed for DNA amplification of internal transcribed spacer 2 and 28S ribosomal RNA region of both *N. americanus* and *Ancylostoma* spp. to determine the hookworm species recovered.

Entamoeba spp. inhabits the human intestine and is commonly comprised of six species including *Entamoeba histolytica, E. dispar, E. moshkovskii, E. coli, E. hartmanni* and *E. polecki*. Amoebiasis is a global health problem caused by the protozoan *Entamoeba histolytica* affecting approximately 40 to 50 million people. Those infected develop colitis or additional intestinal disease causing annual deaths up to 40,000-110,000 (Schmunis & Lopez-Antunano, 2005; Garlington *et al*., 2011). *Entamoeba* infections are normally diagnosed through microscopic examination of faecal samples. However, *E. histolytica* cysts and trophozoites are morphologically undistinguishable from *E. dispar* and *E. moshkovskii*. Therefore, molecular characterization was conducted by nested PCR targeting 16S-like ribosomal RNA gene to genetically characterize *E. histolytica, E. dispar* and *E. moshkovskii* recovered from the human samples obtained.

Giardiasis is a diarrheal disease in a wide range of vertebrate hosts. The taxonomy of *Giardia* at species level is complicated to distinguish due to limited

morphological differences. The genus currently comprises six species namely, *Giardia duodenalis* (commonly found in humans), *G. agilis* (in amphibians), *G. ardeae* and *G. psittaci* (in birds), *G. microti* and *G. muris* (in rodents). Molecular tools have been used recently to characterize the epidemiology of human giardiasis, *G. duodenalis*. Two major groups of *G. duodenalis* have been recognized infecting humans worldwide; assemblage A and B (Maryhofer *et al*., 1995). Therefore, this chapter will describe the development of nested polymerase chain reaction (PCR) to amplify the triosephosphate isomerase (TPI) gene to differentiate *Giardia* parasite at species and genotype levels among the migrant workers.

Cryptosporidium spp. causes cryptosporodiasis, a frequent cause of diarrheal disease in humans. There are more than 27 species of *Cryptosporidium* being considered valid by investigators infecting human and other animals (Fayer, 2010; Traversa, 2010). The main causative agents of human cryptosporidiosis and of greater significance to public health are *C. parvum* and *C. hominis* (Fayer *et al.*, 2000). Identification of *Cryptosporidium* species is usually difficult by simple traditional microscopic measurements and the use of recent advancement in molecular characterization has made it possible to differentiate *Cryptosporidium* spp. oocyst at species, genotype and subgenotype level (Xiao *et al*., 2000). Therefore, present study will perform PCR-RFLP assay to detect the presence of *Cryptosporodium spp*. based on SSU-rRNA sequences.

6.2 Materials and Methods

6.2.1 Samples collection

A total of 388 stool specimens were collected from the workers (as described in Chapter 2 and Chapter 3) meanwhile for *S. stercoralis* screening, 306 stool specimens have been collected. All stool samples were subjected for preliminary screening by microscopy and subsequently subjected to molecular analysis for specific parasite identification up to species level. These parasites include *Strongyloides stercoralis*, hookworm, *Entamoeba* spp., *Giardia* spp. and *Cryptosporidium* spp. (Figure 6.1).

6.2.2 Extraction of genomic DNA

DNA was extracted from microscopically positive faecal samples using NucleoSpin® Soil (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's instructions. Approximately, 250-500 mg fresh sample material was transferred to a NucleoSpin® Bead Tube containing the ceramic beads. Then, a total of 700 µl Buffer SL2 was added to the tube. To adjust lysis conditions, 150 µl Enhancer SX was added to the tube and the cap was closed. To lyse the sample, the NucleoSpin® Bead Tubes was attached horizontally to a vortexer and was vortexed at full speed in room temperature (18-25˚C) for 5 minutes. The tube was then centrifuged for 2 minutes at 11,000x g to eliminate the foam caused by detergent. A volume of 150µl Buffer SL3 was added to the tube and vortexed for 5 seconds. Then the tube was incubated for 5 minutes at 0-4°C and was centrifuged again at 11,000 x g for 1 minute.

To filter the lysate, a Nucleospin® Inhibitor Removal Column (red ring) was placed in a collection tube (2mL, lid). Up to 700µl of clear supernatant was loaded onto the filter and the tube was centrifuged for 1 minute at 11,000x g. The Nucleospin® Inhibitor Removal Column was then discarded and if a pellet was visible in the flow-through, the clear supernatant was transferred to a new collection tube. A total of 250µl Buffer SB was added to adjust binding conditions and then, the tube was vortexed for 5 seconds. To bind the DNA, a Nucleospin® Soil Column (green ring) was placed in a collection tube (2mL). 550µL of samples was loaded onto the column and centrifuged for 1 minute at 11,000 x g. The flow-through was discarded and the column was placed back into the collection tube. The remaining sample was loaded onto the column, centrifuged and flow-through was discarded again. The column was then placed back into the collection tube.

Washing procedure was followed by adding 500µL Buffer SB to the Nucleospin® Soil column and centrifuged for 30 seconds at 11,000 x g. The flow-through was discarded and placed back into the collection tube. Then, 550µL of Buffer SW1 was added to the Nucleospin® Soil column, followed by centrifuged for 30 seconds, flow-through was discarded and placed back into the collection tube. Washing procedure was then continued by adding 700µL Buffer SW2 to the Nucleospin® Soil Column, vortexed for 2 seconds and centrifuged for 30 seconds at 11,000x g. The flow-through was discarded and the column was placed back again into the collection tube. The procedure was repeated again by adding 700µL of Buffer SW2, vortexed, centrifuged, flow-through discarded and column was placed back into the collection tube. The tube was then centrifuged for 2 minutes at 11,000x g to dry the silica membrane. To elute the DNA, the Nucleospin® Soil Column was placed into a new microcentrifuge tube and 40µL of Buffer SE was added to the column. The lid of the

tube was let open and incubated for 1 minute at room temperature (18-25°C). The lid was closed and centrifuged for 30 seconds at 11,000 x g. The extracted DNA was stored at −20°C until required for PCR amplification.

6.2.3 Nested polymerase chain reaction (nested PCR)

6.2.3.1 *Strongyloides stercoralis*

A nested PCR targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene was used to amplify *S. stercoralis* DNA. The assay in this study used two sets of primers and was performed in two separate reactions. The primary reaction consisted of a forward primer SS–FO: 5'- ATC CTT CCA ATC GCT GTT GT -3' and reverse primer SS– RO: 5'- TTT CGT GAT GGG CTA ATT CC -3'. The secondary reaction forward and reverse primers were SS–FI: 5'- GTA ACA AGG TTT TCG TAG GTG A –3' and SS–RI: 5'- ATT TAG TTT CTT TTC CTC CGC TT –3' respectively (Nilforoushan *et al*. 2007). The nested PCR was performed in a Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology, Inc.) in 20µl volume reaction. The reaction contained i-Taq^{TM} DNA polymerase (5U/µl) (2.5U), deoxynucleoside triphosphate (dNTPs) (2.5 mM each), 1X reaction buffer (10x) and gel loading buffer (1x). DNA template (2µl), primers (100 nM each) and distilled water were added to the premix. The conditions for the primary PCR were initial denaturation at 94 °C for 5 minutes and 35 cycles of 94 °C for 45 seconds, 58 °C for 1 minute, 72 °C for 1 minute and a final extension at 72 °C for 5 minutes. Subsequently, 2µl of the primary PCR products were subjected for a secondary PCR performed at 94 °C for 2 minutes and 30 cycles of 94 °C for 45 seconds, 60°C for 45 seconds, 72 °C for 1 minute and a final extension at 72 °C for 5 minutes. PCR products were subjected to electrophoresis

through 2 % (w/v) agarose and visualized in a UV transilluminator after staining with RedSafe^{TM} Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea).

6.2.3.2 Hookworms

A two-step semi-nested PCR was used for DNA amplification of hookworm species. For the first amplification, forward primer NC1 (5'- ACG TCT GGT TCA GGG TTC TT -3') and reverse primer NC2 (5'- TTA GTT TCT TTT CCT CCG CT -3') were used to amplify approximately 310-basepair and 420-basepair regions of internal transcribed spacer 2 and 28S ribosomal RNA region of *N. americanus* and *Ancylostoma* spp. The nested PCR was performed in a Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology, Inc.) in 20µl volume reaction. The reaction contained i-Taq^{TM} DNA polymerase (5U/µl) (2.5U), deoxynucleoside triphosphate (dNTPs) (2.5 mM each), 1X reaction buffer (10x) and gel loading buffer (1x). DNA template (2µl), primers (10 pM each) and distilled water were added to the premix. The conditions for the primary PCR were initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. Samples containing *N. americanus* and *Ancylostoma* spp. genomic DNA (positive control) was included in each PCR. Subsequently, 2µl of the primary PCR products were subjected for a secondary PCR. Amplification was conducted by using forward primer NA (5'- ATG TGC ACG TTA TTC ACT -3') for *N. americanus*, AD1 (5'- CGA CTT TAG AAC GTT TCG GC -3') for *Ancylostoma* spp. and NC2 as a common reverse primer. The secondary amplification reagent concentrations were similar to those of the first round of PCR except that 2 µl of primary PCR product was added instead of DNA. The cycling conditions for the second round of amplification were 94°C for 5 minutes, followed by 35 cycles at 94°C for 1

minute, 55°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 transilluminator after staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea).

6.2.3.3 *Entamoeba* spp.

Nested PCR targeting 16S-like ribosomal RNA gene was used to genetically characterize *E. histolytica*, *E. dispar* and *E. moshkovskii* according to Que and Reed (1991). Primary PCR for the detection of *Entamoeba* genus used forward primer E1 (5'- TAA GAT GCA GAG CGA AA -3') and reverse primer E2 (5'- GTA CAA AGG GCA GGG ACG TA -3'). The nested PCR was performed in a Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology, Inc.) in 20µl volume reaction. The reaction contained i-Taq™ DNA polymerase (5U/µl) (2.5U), deoxynucleoside triphosphate (dNTPs) (2.5 mM each), 1X aeaction buffer (10x) and gel loading buffer (1x). DNA template (2µl), primers (100 pM each) and distilled water were added to the premix. The conditions for the primary PCR were 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, 2µl 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) (Troll *et al*., 1997; Khairnar & Parija, 2007). 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 transilluminator after staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea).

6.2.3.4 *Giardia* spp.

A nested PCR protocol was developed to amplify the triosephosphate isomerase (TPI) fragment from various *Giardia* isolates. For the primary PCR, a PCR product of 605 bp was amplified by using primers AL3543 (5′- AAA TIA TGC CTG CTC GTC G -3′) and AL3546 (5′- CAA ACC TTI TCC GCA AAC C -3′). The 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 (2µl), primers (100 nM each) and distilled water were added to the premix. The conditions for the primary PCR were initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds, and 72°C for 1 minute, and a final extension at 72°C for 10 minutes. For the secondary PCR, a fragment of 530 bp was amplified using 2 µl of primary PCR reaction and primers set AL3544 (5′- CCC TTC ATC GGI GGT AAC TT -3′) and AL3545 (5′- GTG GCC ACC ACI CCC GTG CC -3′). The conditions for the secondary PCR were initial denaturation at 94°C for 5 minutes, followed by 35 cycles at 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were subjected to electrophoresis through 2 % (w/v) agarose

and visualized in a UV transilluminator after staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea).

6.2.3.5 *Cryptosporidium* spp.

A nested PCR protocol was used to identify *Cryptosporidium* spp. using small subunit rRNA as described by Xiao *et al.* (1999). The primary reaction consisted of a forward primer SSU-F2: 5'- TTC TAG AGC TAA TAC ATG CG -3' and reverse primer, SSU-R2: 5'- CCC ATT TCC TTC GAA ACA GGA -3'. The secondary reaction forward and reverse primers were SSU-F3: 5'- GGA AGG GTT GTA TTT ATT AGA TAA AG -3' and SSU-R4: 5'- CTC ATA AGG TGC TGA AGG AGT A -3' respectively. The 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 (2µl), primers (200 nM each) and distilled water were added to the premix. The conditions for both primary and secondary PCR were initial denaturation at 94 °C for 4 minutes and 35 cycles of 94 °C for 45 seconds, 56 °C for 90 seconds, 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 transilluminator after staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea).

6.2.4 Purification of PCR product

The PCR product was purified using MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, 2011, Korea) according to the manufacturer's

protocol. 5 volume of BNL buffer was added to the PCR reaction product, and mixed well by vortexed. A total of 100µl of BNL buffer was added to the PCR tube directly for PCR product that was 20µl. For PCR product <200bp, 1.5 volume of isopropanol was added to the sample and mixed by pipetting several times. One MEGAquick-spin™ column was placed in a collection tube for each DNA mixture. The DNA mixture was transferred to the MEGAquick-spin™ column assembled. To bind DNA, the sample was applied to the MEGAquick-spin™ column and centrifuged for 1 minute. The flow-through was discarded and the MEGAquick-spin™ column was placed back in the same 2 ml collection tube. 700µl of washing buffer was added to the column and centrifuged at 13,000 rpm for 1 minute. The flow-through was discarded and the MEGAquick-spin™ column was placed back in the same 2 ml collection tube. The assembled column was centrifuged for 1 minute at 13,000 rpm to dry the spin membrane. The MEGAquick-spin™ column was placed to a clean 1.5 ml microcentrifuge tube. 30µl of the Elution Buffer was applied directly to the center of the column without touching the membrane with the pipette tip. The column was incubated at room temperature for 1 minute and then centrifuged for 1 minute at 13,000 rpm. The MEGAquick-spin™ column was discarded and the microcentrifuge tube contained the eluted DNA was stored at -20°C.

6.2.5 DNA sequencing

6.2.5.1 *Strongyloides stercoralis*

Sequencing was carried out by First Base Laboratories Sdn. Bhd. For *Strongyloides stercoralis* targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene, sequencing was done in both directions using forward primer

(SS–FI: 5’- GTA ACA AGG TTT TCG TAG GTG A –3’) and reverse primer (SS–RI: 5’- ATT TAG TTT CTT TTC CTC CGC TT –3’).

6.2.5.2 Hookworm

To sequence hookworm species, sequencing was conducted by using forward primer NA (5’- ATG TGC ACG TTA TTC ACT -3’) for *N. americanus*, AD1 (5’- CGA CTT TAG AAC GTT TCG GC -3’) for *Ancylostoma* spp. and NC2 (5’- TTA GTT TCT TTT CCT CCG CT -3’) as a common reverse primer.

6.2.5.3 *Entamoeba* spp.

Sequencing of 16S-like ribosomal RNA gene of *Entamoeba* spp. was conducted 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*, ED1 (5’- TCT AAT TTC GAT TAG AAC TCT -3’) and ED2 (5’-TCC CTA CCTATT AGA CAT AGC -3’) to detect *E. dispar* and 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*.

6.2.5.4 *Giardia* spp.

For *Giardia* spp. targeting the TPI gene, sequencing was done in both directions using forward primer AL3544 (5′- CCC TTC ATC GGI GGT AAC TT -3′) and reverse primer AL3545 (5′- GTG GCC ACC ACI CCC GTG CC -3′).

6.2.6 Sequencing analysis

Sequences were edited via Applied Biosystems Sequence Scanner software v1.0 Sequence Trace Viewer and Editor (www. en.bio-soft.net/dna/ss). Edited sequences were aligned and consensus sequences were created for each isolates using the BioEdit (www.mbio.ncsu.edu) programme. Each consensus sequence was used for the identification of the parasite genotypes and sequences were searched using basic local alignment search tool (BLAST) (www.ncbi.nlm.nih.gov/blast) in order to get the 100% similarity with parasites genotypes sequences deposited in the GenBank.

6.2.7 RFLP (Restriction Fragment Length Polymorphism) for *Cryptosporidium* spp.

Restriction assays were conducted in a 20µL volume with 0.5 units of restriction enzymes and 10µL of PCR product per reaction. Mixes were incubated at 37°C for 8 hours. Digested products were subjected to electrophoresis through 1 % (w/v) agarose and visualized in a UV transilluminator after staining with RedSafe^{TM} Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc, Korea). The endonuclease enzymes used were *SspI* and *VspI* (Vivantis, Malaysia).

6.2.8 Summary of methodology

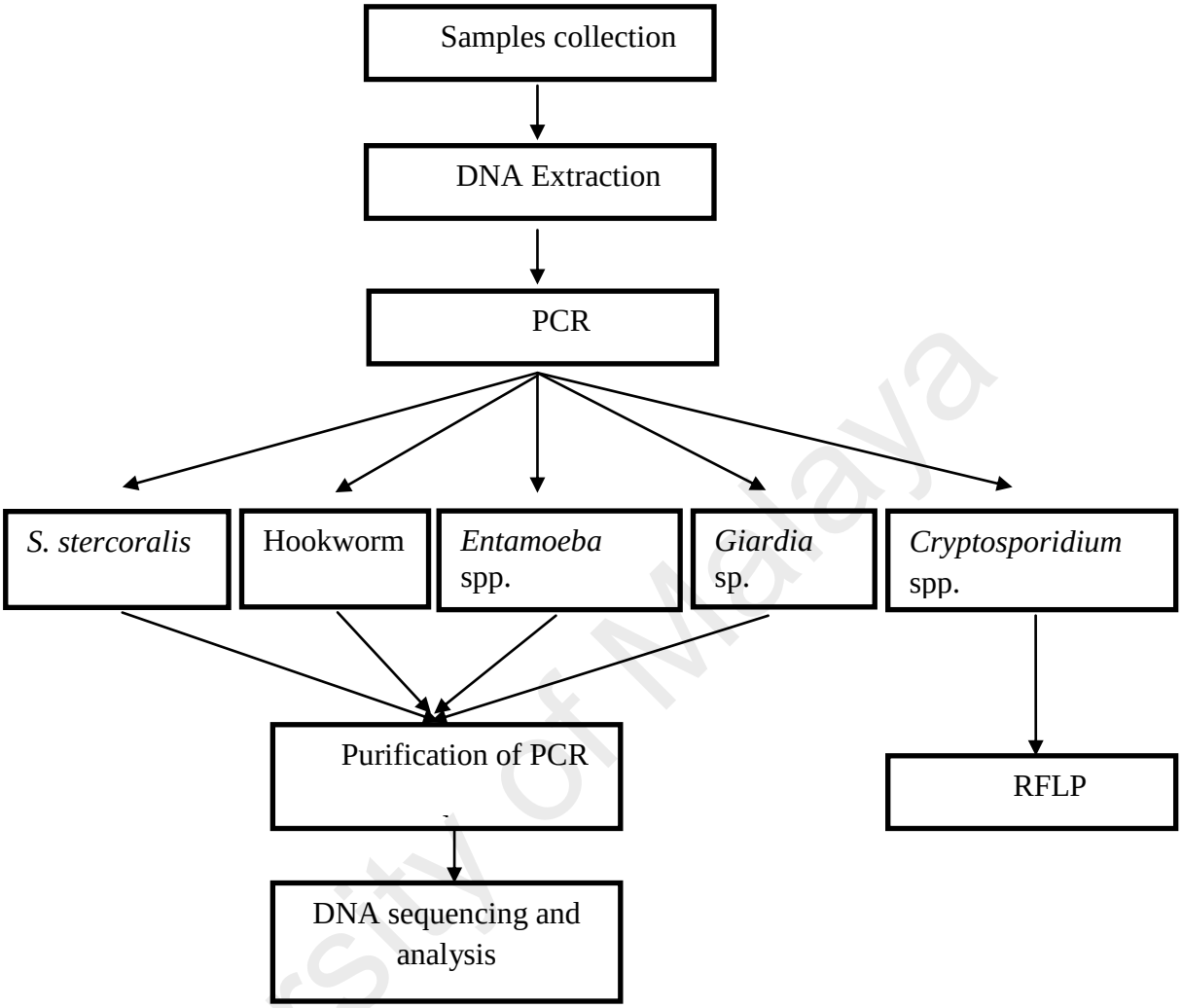

Figure 6.1: Summary of molecular characterization procedure of *S. stercoralis*, hookworm, *Entamoeba spp*., *Giardia* sp. and *Cryptosporidium* spp.

6.3 Results

6.3.1 *Strongyloides stercoralis*

Results for the microscopy screening of *S. stercoralis* were described in chapter 3. Microscopy did not detect the presence of any parasite despite 115 (37.6%) from 306 workers being seropositive of *S. stercoralis* using ELISA commercial kits (Chapter 5) (Table 6.1). Subsequent confirmation using a nested PCR against DNA from stool samples of the 115 ELISA positive individuals showed successful DNA amplification from three samples (2.6%) with a target amplicon of approximately 680bp.

All three positive amplifications were from males and living in this country for more a year, with two samples from India and one from Indonesia. Both the Indian workers were employed in food service sector and were also infected with *Ascaris lumbricoides* (Chapter 3). Meanwhile the worker from Indonesia was employed in the domestic sector. All of them were between 35 to 54 years old. Further molecular analysis also was conducted on negative samples both for ELISA and microscopic examination and they were confirmed negative.

Table 6.1: Detection of *Strongyloides stercoralis* by microscopy examination, ELISA and nested PCR. (N=306)

	Detection of *S. stercoralis*		
	Microscopy	**ELISA**	**Nested PCR**
Positive	0	115 (37.6%)	3 (0.98%)
Negative	306 (100%)	191 (62.4%)	303 (99.02%)
Total	306 (100%)	306 (100%)	306 (100%)

6.3.2 Hookworm

From a total of 388 stool samples collected from migrant workers, 51 samples (13.1%) were found positive by microscopy for hookworm infection (Chapter 3). The 51 positive hookworm samples were subjected to nested PCR analysis to detect *Necator americanus* and *Ancylostoma spp.* The PCR amplicons were successfully obtained from 42 (82.4%) of 51 samples with 81.0% (34 of 42) being *N. americanus* (approximately 250bp), 16.7% (7 of 42) *Ancylostoma* spp. (approximately 130bp) and the remaining one (2.4%) sample has mixed infection of both species (Table 6.2). When screened via PCR, all 337 samples found to be negative by microscopy showed no amplification. Sequence comparison using the Basic Local Alignment Search Tool (BLAST) confirmed that all eight *Ancylostoma* spp. were *Ancylostoma duodenale* (Figure 6.2).

Prevalence of *N. americanus* was markedly higher in male (82.4%, 28 of 34) compared to female (17.6%, 6 of 34). Workers aged below 25 years old showed highest infection rate with *N. americanus* (58.8%, 20 of 34). According to their nationality, Indonesia (44.1%, 15 of 34) reported the highest prevalence followed by Nepal (26.5%, 9 of 34), Myanmar (11.8%, 4 of 34), Bangladesh (8.8%, 3 of 34) and India (8.8%, 3 of 34). Meanwhile, according to their employment sector, food services (32.3%, 11 of 34) recorded the highest prevalence followed by domestic (20.6%, 7 of 34), plantation (20.6%, 7 of 34), construction (14.7%, 5 of 34) and manufacturing (11.8%, 4 of 34) (Table 6.2).

As for *A. duodenale*, higher prevalence also was reported in male (71.4%, 5 of 7) and in those workers aged below 25 years old (85.7%, 6 of 7). The species was reported only from workers in Indonesia (71.4%, 5 of 7) and Nepal (28.6%, 2 of 7) from

all working sectors except construction. One sample of mix infections was from Indonesian female from domestic sector (Table 6.2).

Sequences from all eight *A. duodenale* samples together with six reference sequences obtained from the GenBank database and one sequence from *Necator americanus* used as an outgroup, were analyzed in MEGA6 software and phylogenetic tree was constructed (Figure 6.2). All *A. duodenale* sequences were grouped together with *A. duodenale* reference sequence (EU344797.1). All sequences generated in this study were deposited in GenBank under accession numbers KX650194- KX650201.

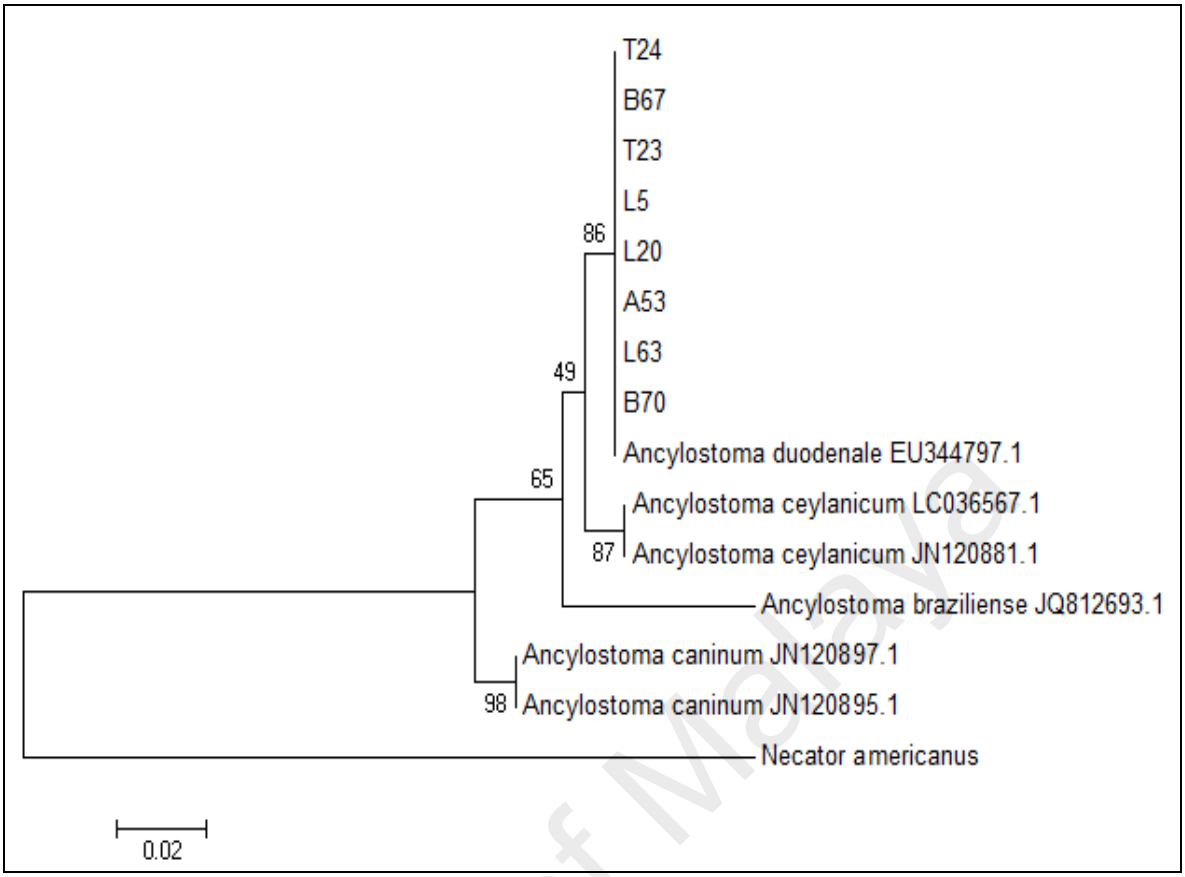

Figure 6.2: A phylogenetic tree based on partial ITS2 sequences of hookworm species constructed using MEGA6 program. Numbers above branches represent the percentage of 1,000 bootstrap replication trees in that branch. Accession numbers indicate sequences from the GenBank database.

Table 6.2: The prevalence of *N. americanus* and *A. duodenale* infections among migrant workers in Malaysia relative to factors such as sex, age, nationality, employment sector and years of residence in Malaysia.

Factors		PCR Positive	*N. americanus*		*A. duodenale*		*N. americanus and A.duodenale*	
			No.	%	No.	%	No.	%
Sex	**Male**	33	28	84.8	5	15.2	0	0
	Female	9	6	66.7	2	22.2	1	11.1
Age	**<25**	26	20	76.9	6	23.1	0	0
	25-34	8	7	87.5	1	12.5	0	0
	35-44	8	7	87.5	0	0	1	12.5
	45-54	0	0	0	0	0	0	0
	>55	0	0	0	0	0	0	0
Nationality	**Indonesia**	21	15	71.4	5	23.8	1	4.7
	Bangladesh	3	3	100.0	0	0	0	0
	Myanmar	4	4	100.0	0	0	0	0
	India	3	3	100.0	0	0	0	0
	Nepal	11	9	81.8	2	18.2	0	0
Employment Sector	**Construction**	5	5	100.0	0	0	0	0
	Manufacture	6	4	66.7	2	33.3	0	0
	Plantation	9	7	77.8	2	22.2	0	0
	Food Service	12	11	91.7	1	8.3	0	0
	Domestic	10	7	70.0	2	20.0	1	10.0
Years of residence in Malaysia	**< than 1 year**	20	14	70.0	6	30.0	0	0
	> than 1 year	22	20	90.9	1	4.5	1	4.5
Total		**42**	**34**	**81.0**	**7**	**16.7**	**1**	**2.4**

6.3.3 *Entamoeba* spp.

A total of 45 (11.6%) from 388 stool samples were found positive with *Entamoeba* spp. via microscopy screening. Of the 45 microscopy positive samples, 31 (68.9%) samples were successfully amplified using nested PCR. From the 31 PCR positive results, *E. dispar* (20/31; 64.5%) appeared to be the most predominant, followed by *E. histolytica* (8/31; 25.8%). Three samples were found having mixed infections of *E. dispar* and *E. histolytica* (3/31; 9.7%) (Table 6.3). No sample was found positive with *E. moshkovskii*.

Similar prevalence of infections was found between male and female for both *E. dispar* and *E. histolytica*. For *E. dispar*, higher infection rates were recorded from Indonesian (9/31; 29.0%), followed by Indian (5/31; 16.1%), Nepali (3/31; 9.7%), Myanmarian (2/31; 6.5%) and Bangladeshi (1/31; 3.2%). According to employment sector, higher prevalence was reported in food service sector (10/31; 32.3%), followed by domestic (8/31; 25.8%) and manufacturing (2/31; 64.5%) sectors.

As for *E. histolytica* infections, higher prevalence was recorded in workers from Indonesia (4/31; 12.9%), followed by workers from Nepal (2/31; 6.5%), India (1/31; 3.2%) and Myanmar (1/31; 3.2%). Meanwhile, according to employment sector, higher prevalence was found in domestic service (4/31; 12.9%), followed by food services (2/31; 6.5%) and manufacturing (2/31; 6.5%). Mix infection of both *E. dispar* and *E. histolytica* were reported in workers from Indonesian (2/31; 6.5%) and Nepali (1/31; 3.2%) with each of them working in domestic, food service and manufacturing sectors.

Table 6.3: The prevalence of *Entamoeba dispar* and *E. histolytica* among migrant workers in Malaysia relative to factors such as sex, age, nationality, employment sector and years of residence in Malaysia.

Factors		PCR Positive	*E. dispar*		*E. histolytica*		*E. dispar + E. histolytica*	
			No.	%	No.	%	No.	%
Sex	**Male**	20	13	65.0	5	25.0	2	10.0
	Female	11	7	63.6	3	27.3	1	9.1
Age	**<25**	9	5	55.6	3	33.3	1	11.1
	25-34	15	11	73.3	3	20.0	1	6.7
	35-44	4	3	75.0	0	0	1	25.0
	45-54	2	1	50.0	1	50.0	0	0
	>55	1	0	0	1	100.0	0	0
Nationality	**Indonesia**	15	9	60.0	4	26.7	2	13.3
	Bangladesh	1	1	100.0	0	0	0	0
	Myanmar	3	2	66.7	1	33.3	0	0
	India	6	5	83.3	1	16.7	0	0
	Nepal	6	3	50.0	2	33.3	1	16.7
Employment Sector	**Construction**	0	0	0	0	0	0	0
	Manufacture	5	2	40.0	2	40.0	1	20.0
	Plantation	1	0	0	0	0	1	100.0
	Food Service	12	10	83.3	2	16.7	0	0
	Domestic	13	8	61.5	4	30.8	1	7.7
Years of residence in Malaysia	**< than 1 year**	10	5	50.0	3	30.0	2	20.0
	> than 1 year	21	15	71.4	5	23.8	1	4.8
Total		**31**	**20**	**64.5**	**8**	**25.8**	**3**	**9.7**

6.3.4 *Giardia* spp.

From the total of 388 stool specimens from migrant workers, 42 (10.8%) specimens were found positive with *Giardia* spp. using microscopy technique. The 42 microscopy-positive specimens were analyzed by nested PCR to amplify the triosephosphate isomerase (TPI) gene of *Giardia duodenalis* and PCR amplicons were successfully obtained from 30 (30/42; 71.4%) samples (approximately 530bp). At the tpi gene, assemblages A and B were found in 13 (13/30; 43.3%) and 17 (17/30; 56.7%) samples, respectively. Based on the analysis targeting tpi gene, 13 isolates of the assemblages A were classified as A2 based on phylogenetic analysis. The neighbor-joining tree placed six representative sequences (A40, S2, S13, S35, T42 and T66) in one cluster with AII sequence references with high bootstrap support. Meanwhile, 8 sequences (B50, L101, L44, B1, A11, C31, T78 and A62) representing 17 isolates were identified as assemblage B (Figure 6.1).

Higher *G. duodenalis* infection was found in male (26/30; 86.7%) compared to female (4/30; 13.3%). Based on age factor, workers aged 34 and below (86.7%) recorded higher infection in both assemblages. Workers with assemblage A were Nepalese (7/13; 53.8%), Indonesian (4/13; 30.8%) and Indian (2/13; 15.4%) meanwhile assemblage B was recovered from Indonesian (6/17; 35.3%), Bangladeshi (6/17; 35.3%), Nepalese (4/17; 23.5%) and Indian (1/17; 5.9%). Based on employment sector, assemblage A was recorded only from workers in manufacturing (5/13; 38.5%), food service (5/13; 38.5%) and domestic sector (3/13; 23.1%), meanwhile assemblage B was reported in all 5 working sectors (domestic, 29.4%; food service, 23.5%; plantation, 17.6%; manufacturing, 17.6%; construction, 11.8%).

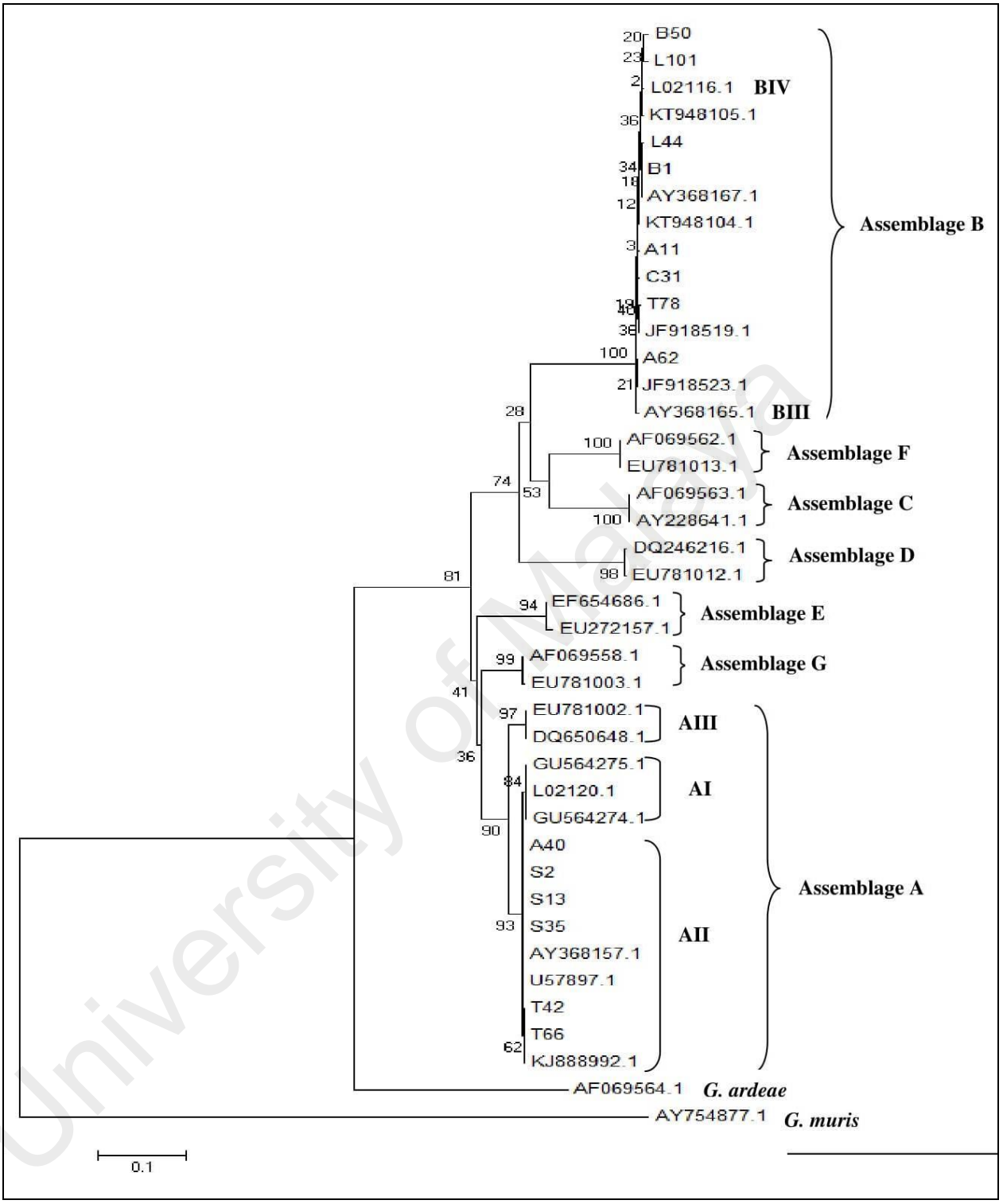

Figure 6.3: Phylogenetic relationship of *Giardia* spp. by neighbor-joining analysis of the triosephosphate isomerase (tpi) nucleotide sequences.

Table 6.4: The infections of *G. duodenalis* assemblages among migrant workers in Malaysia relative to factors such as sex, age, nationality, employment sector and years of residence in Malaysia.

Factors		PCR Positive	Assemblage A		Assemblage B	
			No.	%	No.	%
Sex	**Male**	26	10	38.5	16	61.5
	Female	4	3	75.0	1	25.0
Age	**<25**	10	6	60.0	4	40.0
	25-34	16	6	37.5	10	62.5
	35-44	3	1	33.3	2	66.7
	45-54	1	0	0	1	100.0
	>55	0	0	0	0	0
Nationality	**Indonesia**	10	4	40.0	6	60.0
	Bangladesh	6	0	0	6	100.0
	Myanmar	0	0	0	0	0
	India	3	2	66.7	1	33.3
	Nepal	11	7	63.6	4	36.4
Employment Sector	**Construction**	2	0	0	2	100.0
	Manufacture	8	5	62.5	3	37.5
	Plantation	3	0	0	3	100.0
	Food Service	9	5	55.6	4	44.4
	Domestic	8	3	37.5	5	62.5
Years of residence in Malaysia	**< than 1 year**	17	9	52.9	8	47.1
	> than 1 year	13	4	30.8	9	69.2
Total		**30**	**13**	**43.3**	**17**	**56.7**

6.3.5 *Cryptosporidium* spp.

From a total of 388 stool samples collected from migrant workers, 12 samples (3.1%) were found positive by microscopy for *Cryptosporidium* spp. infection (Chapter 3). All 12 samples were subjected to PCR-RFLP analysis to detect the species of *Cryptosporidium* isolates. The PCR amplicons were successfully obtained from 9 (75.0%) of 12 samples (approximately 833bp) (Figure 6.4). RFLP analysis of the nested-PCR secondary PCR product showed that all nine samples (100%) were *C. parvum* (Rafiei *et al*., 2014) (Figure 6.5).

Infection with *C. parvum* was mostly found in males (8/9; 88.9%) compared to 1 female (1/9; 11.1%) and can be found in every category of age. The infection can be found in workers from India (4/9; 44.4%), Indonesia (2/9; 22.2%), Bangladesh (1/9; 11.1%), Myanmar (1/9; 11.1%) and Nepal (1/9; 11.1%). Based on employment sector, most workers were from food service sector (7/9; 77.8%), meanwhile only one worker infected from manufacturing sector (1/9; 11.1%) and plantation sector (1/9; 11.1%). A total of 7 workers (7/9; 77.8%) resided in Malaysia for more than a year compared to only 2 workers (2/9; 22.2%) who resided less than a year. Most workers live in hostels provided by their employer (7/9; 77.8%) compared to only two workers (2/9; 22.2%) who are living in their own/rent house.

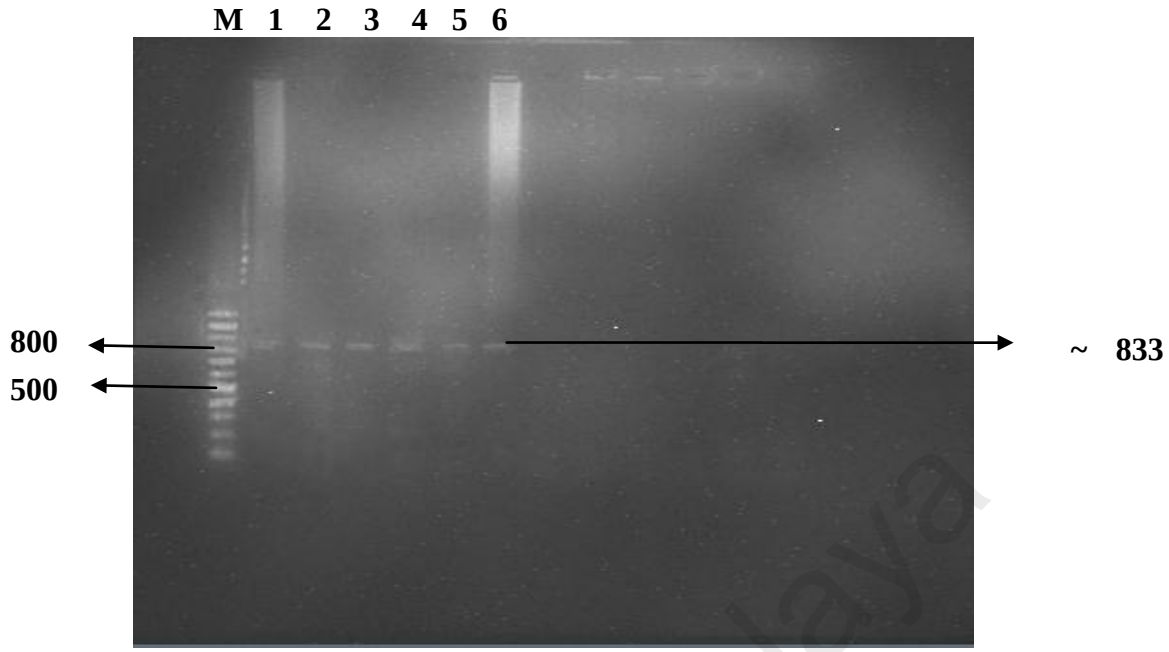

Figure 6.4: Nested PCR product of *Cryptosporodium* spp. SSU rRNA gene sequences from representative samples; M=100bp marker; Lane 1-6 = Positive isolates of *Cryptosporidium* spp.

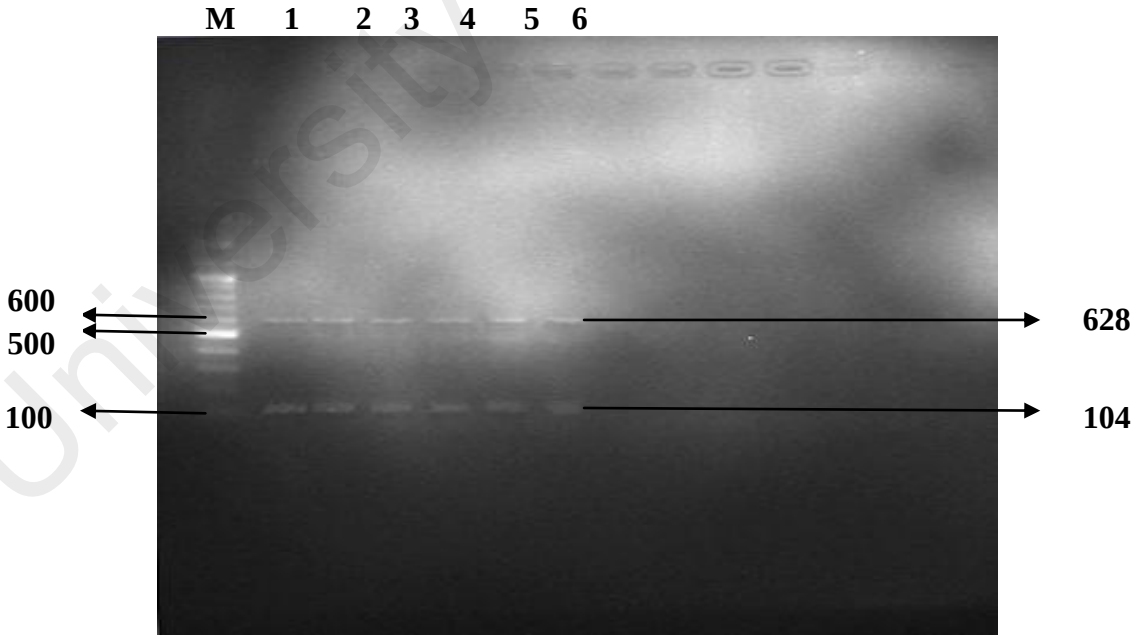

Figure 6.5: PCR-RFLP of *Cryptosporidium* SSUrRNA gene. Secondary PCR product was digested by *VSPI* restriction enzyme. Lane M= DNA size marker; Lanes 1-6 = *C. parvum* (628,104 bp).

6.4 Discussion

This study confirms the advantages of molecular characterization technique in differentiating organisms at species levels for the 5 human intestinal parasitic infections.

6.4.1 *Strongyloides stercoralis*

Nested PCR successfully characterized current active infections of *S. stercoralis* in three (2.6%) of 115 serological positive workers. Nested PCR was able to greatly improve sensitivity compared to faecal examination. Developments in the PCR have now permitted for reliable results to be obtained (Ahmad *et al.*, 2013).

All three positive samples were males and could be due to the higher involvement of males with outdoor activities compared to females especially in the plantation sector (Ahmad *et al*., 2013). However, in the present study, two males were employed in the food service sector and one in the domestic sector. The infections could be due to bad personal hygiene and improper way of handling sewage disposal (Lim *et al*., 2009; Ahmad *et al.*, 2013).

In the developed countries, *S. stercoralis* infections remain an issue among migrant workers as infections are still endemic in their country of origin. According to Schar *et al*. (2013), high infection rates up to 60% can be expected in the resource-poor countries with ecological and socioeconomic conditions which favour the spread of *S. stercoralis* while low prevalence in urban and developed countries where faecal contamination is rare with the exception of slum areas in the big cities (Schar *et al*., 2013).

Most studies in Malaysia used the low sensitivity conventional microscopy technique for the detection of *S. stercoralis* larvae in faecal samples (Rahmah *et al*., 1997; Ahmad *et al*., 2013). Although this study adopted this technique, it failed to detect any infection in any of the workers. In addition, most studies focused on STH only and excluded screening for *S. stercoralis* and studies that did however, used low sensitivity methods (Suresh *et al*., 2002). Similarly, most studies on refugees and migrants worldwide also used copro-diagnostic procedures with low sensitivity (71.8%) compared to copro-diagnostic procedures with moderate sensitivity (7.7%) and serological diagnostic procedures with high sensitivity (20.5%) (Schar *et al*., 2013). The frequent choice of diagnosis for *S. stercoralis* infections were the Koga Agar culture method and Baermann method, which are considerably time consuming and labour intensive. These methods involve examining stool samples collected over a few days and could mitigate the risk of missing low-intensity infections. Furthermore, the screening should include a minimum of three consecutive days' stool samples per person. Recently, the advancement in molecular studies has enabled high sensitivity diagnostic method of *S. stercoralis*. The current use of nested PCR has been successful applied for the screening of this parasite and should be applied in all future analysis and studies. In addition to better screening, workers should be exposed to health education campaigns and programs aimed at increasing community awareness of the importance of personal hygiene, sanitation, cleanliness and healthy behaviors in controlling *S. stercoralis* infections.

6.4.2 Hookworm

Slightly over 10% of the workers screened in this study were positive for hookworms which was consistent with previous studies in Malaysia conducted among

Orang Asli, rural communities, urban squatters and children with infection ranging between 3.0% to 12.8% (Nor Aini *et al*., 2007; Hakim *et al*., 2007; Al-Mekhlafi *et al*., 2008; Lim *et al*., 2009; Ngui *et al.*, 2011a; Sinniah *et al*., 2014). However, current diagnostic methods used were unable to identify the species of hookworm from the eggs and therefore employing molecular methods and sequencing to overcome this limitation is vital (Gruijter *et al*., 2005).

The PCR amplicons were successfully obtained with mostly *N. americanus* (81%) and the remaining were of *Ancylostoma* spp and only one sample was with mix infections. This finding was in agreement with previous studies highlighting *N. americanus* as the predominant human hookworm species (Gruijter *et al*., 2005; Ngui *et al*., 2012a). Sequence comparison using the Basic Local Alignment Search Tool (BLAST) confirmed that all 8 *Ancylostoma* spp. were *Ancylostoma duodenale* and is the first report of *Ancylostoma duodenale* in Malaysia.

Most Asian studies also reported high *N. americanus* infections compared to *A. duodenale*. In southern part of Thailand, high prevalence of *N. americanus* (99.9%) was reported among school children compared to *A. duodenale* (0.1%) (Anantaphruti *et al*., 2002). Another study in rural community in central Thailand recorded 92.0% *N. americanus* infection compared to *A. duodenale* (2.0%) (Jiraanankul *et al.*, 2011). While a study in northern Vietnam also recorded high infections of *N. americanus* (95%) (Verle *et al*., 2003). Conversely, a study in rural area of Laos found higher *Ancylostoma* spp. infection (9.4%) compared to *N. americanus* (5.9%), however the species was not elucidated (Sato *et al*., 2010). In West Bengal, India (Pal *et al*., 2007) and Hainan Province, China (Gandhi *et al*., 2001) also reported that hookworm

infections were more predominant to *N. americanus* with prevalence of 42.8% and 60.0%, respectively.

In the present study, *A. duodenale* infected workers were mainly from Indonesia and Nepal. Six workers were newly arrived workers to Malaysia, suggesting infections originated from their home country where infections were endemic due to poverty, poor hygiene practices and inadequate sanitation services. Infection in humans with *A. duodenale* can cause more severe pathological effects and produce symptoms with fewer worms compared to *N. americanus* (Bogitsh & Cheng, 1999). Furthermore, *A. duodenale* infection causes approximately five times greater blood loss resulting in a higher degree of iron deficiency than *N. americanus* infection (Pawlowski *et al*., 1991).

6.4.3 *Entamoeba* spp.

A relative small proportion of the workers were found positive for *Entamoeba* spp. with similar values with other studies conducted among rural communities in Malaysia ranging from 9.4% to 21.0% (Ngui *et al*., 2011a; 2012; Nor *et al*., 2003; Rajeswari *et al*., 1994; Hakim *et al*., 2007). However, these findings were based on microscopy screening which could not differentiate species (Ngui *et al*., 2012b). *E. histolytica* cysts and trophozoites were morphologically similar and difficult to differentiate between *E. dispar* and *E. moshkovskii*. Newer techniques have been developed for the detection of *E. histolytica* antigen using an enzyme-linked immunosorbent assay (ELISA) (Gonin & Louise, 2003; Redondo *et al.,* 2006; Zeehaida *et al.,* 2008) and PCR to amplify *Entamoeba* DNA (Fotedar *et al*., 2007; Katzwinkel-Wladarsch *et al*., 1994; Troll *et al.,* 1997; Ngui *et al*., 2012b).

Close to 70% of the positive samples were successfully amplified using nested PCR which resulted in identity of *E. dispar* (20/31; 64.5%) as the most predominant species, followed by *E. histolytica* (8/31; 25.8%) and mixed infections (3/31; 9.7%). The finding was in agreement with worldwide distribution of *Entamoeba spp*. (10%); pathogenic *E. histolytica* constitute 10% of the infections and non-pathogenic *E. dispar* for the remaining 90%. India also reported similar findings with 49.5% patients being infected with *E. dispar* meanwhile only 7.4% were infected with *E. histolytica* (Khairnar & Parija, 2007). Other worldwide studies including from Australia reported 70.8% patients infected with *E. dispar* compared to 4.5% with *E. histolytica* and 61.8% with *E. moshkovskii* (Fotedar *et al*., 2007). A study in northern Ghana also reported higher prevalence of *E. dispar* compared to *E. histolytica* with 92.3% and 1.3%, respectively (Verweij *et al*., 2003). 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 Netherland, 91.2% were identified as *E. dispar* and 6.7% were *E. histolytica* (Visser *et al*.,2006) meanwhile in Canada, 97.1% of the samples were *E. dispar* compared to 2.9% for *E. histolytica* (Gonin & Louise, 2003).

However, result from this study was in contrast with previous studies conducted in Malaysia. Ngui *et al.* (2012b) reported higher prevalence of *E. histolytica* (75.0%) compared to *E. dispar* (30.8%) from five rural villages in Peninsular Malaysia and among aborigine community in Pahang with *E. histolytica* (13.2%) being more prevalent compared to *E. dispar* (5.6%) (Noor Azian *et al*., 2006). Ngui *et al.*, (2012b) also reported the first detection of *E. moshkovskii* in Malaysia. A study in Bangladesh highlighted that infection with *E. moshkovskii* was common in children aged 2 to 5 years (Ali *et al*., 2003). Ngui *et al*. (2012b) also reported that 5.8% of infected *E.*

moshkovskii were children. However, *E. moshkovskii* was not present in the present study.

E. histolytica has the potential to cause dysentery and extraintestinal disease, meanwhile *E. dispar* is considered to be a harmless commensal (Petri, 1996; Walsh, 1986). Migrant workers in the present study were provided with suitable accommodation, clean water system, proper sewage toilets, and efficient waste disposal system. High prevalence of *Entamoeba* infection may be due to low standards of hygiene practice and lack of education about the diseases. The presence of *E. histolytica* in the study population must be considered as a public health problem, therefore parasite control strategies especially mass treatment and health education are recommended for all migrant workers as well as local population of Malaysia.

6.4.4 *Giardia* spp.

This study is the first in Malaysia to report on *Giardia* spp. infections among migrant workers (10.8%) via microscopy observation. The results were similar to other studies conducted in Malaysia among Orang Asli communities and children in rural areas with prevalence ranging between 0.2% - 29.2% (Noor Azian *et al*., 2007; Lim *et al*., 2008, Al-Mekhlafi *et al.*, 2005b; 2010; Anuar *et al*., 2012). PCR amplicons were successfully obtained from 30 isolates (30/42; 71.4%) and based on BLAST search of the GenBank database, all of the nucleotide sequences belonged to *G. duodenalis* which is in agreement with worldwide data that *G. duodenalis* is the only species found in human (Thompson *et al*., 2000). *G duodenalis* also has been found infecting other mammals including cattle, cats, dogs, horses, sheep and pigs (Thompson *et al*., 2000; Xiao, 1994; Olson *et al*., 1995).

Phylogenetic analysis highlighted that *G. duodenalis* was placed in two distinct lineage; assemblages A (13/30; 43.3%) and B (17/30; 56.7%). The isolates of assemblage A in this study belonged to sub-assemblage AII as also reported by other studies (Hussein *et al.,* 2009; Lebbad *et al*., 2011; Bonhomme *et al*., 2011; Huey *et al*., 2013). Assemblage B also commonly reported in human infected other mammals including beavers (Fayer *et al*., 2006), cattle (Coklin *et al*., 2007), dogs (Lalle *et al*., 2005; Read *et al*., 2004; Traub *et al*., 2004), horses (Traub *et al*., 2005), monkeys (Itagaki *et al*., 2005), muskrats (Sulaiman *et al*., 2003), rabbits (Sulaiman *et al*., 2003), and sheep (Castro-Hermida *et al*., 2007). This strongly suggests the potential zoonotic transmission between animal and human (Sulaiman *et al*., 2003). However, the isolates of assemblage B could not be assigned to any sub-assemblages due to high degree of nucleotide variation (Bonhomme *et al.,* 2011; Caccio *et al*., 2008; Lalle *et al*., 2009; Lebbad *et al.*, 2011, Levecke *et al*., 2009; Huey *et al*., 2013).

The presence of assemblage B (56.7%) was slightly higher compared to assemblage A (43.3%) in the current study. Global incidence of assemblage A and B differs from country to country (Feng & Xiao, 2011). In the present study, assemblage B was reported in Bangladeshi workers (35.3%) only, which was in agreement with a case control study in Bangladesh (Haque *et al*., 2005) where the presence of assemblage B (231/267; 86.5%) was significantly higher compared to assemblage A (20/267; 7.5%). In Nepal, higher prevalence of assemblage B (26/35; 74%) also was found in the isolates compared with assemblage A (7/35; 20%) (Singh *et al.,* 2009). However, present study recorded higher prevalence of assemblage A (63.6%) compared to assemblage B (36.4%) among the Nepalese. A study in India by Caccio *et al*. (2005) reported slightly higher prevalence of assemblage B (47.0%) compared to assemblage A

(32.0%) as also reported by Paintlia *et al*., (1998) (assemblage B=58%; assemblage A= 42.0%).

The presence of assemblage B and sub-assemblage AII in the samples of present study suggest that the mode of transmission of giardiasis among migrant workers in Malaysia may be human-to-human. However, further investigation should include multilocus genotyping of parasites from human and animals to understand the epidemiology, possibility of zoonotic transmission and public health importance of *G. duodenalis* among migrant workers in Malaysia.

6.4.5 *Cryptosporidium* spp.

The present molecular analysis of *Cryptosporidium* spp. among migrant workers in Malaysia successfully amplified SSU rRNA gene of *Cryptosporidium* spp. from 9 (9/388; 2.3%) stool samples. The prevalence reported was low compared to other previous studies conducted in Malaysia including among the Orang Asli with prevalence ranging from 5.5% to 20.1% (Kamel *et al*., 1994a; Lim *et al*., 1997), community cases of children below 7 years with 10.6% (Lai, 1992), HIV patients with prevalence ranging from 3.0% to 23.0% (Kamel *et al*., 1994b; Lim *et al*., 2005; Zaidah *et al*., 2008; Iqbal *et al*., 2012; Iqbal *et al*., 2015) and among the pediatric hospital cases ranging from 0.9% to 11.4% (Mendez *et al*., 1988; Mat Ludin *et al*., 1991; Lai, 1992; Ng & Shekhar, 1993; Menon *et al*., 1999; Menon *et al*., 2001).

Based on the SSU rRNA sequences, more than 20 *Cryptosporidium* species have been recognized (Plutzer & Karanis, 2009) and cryptosporidiosis in human was mainly

caused by *C. parvum* and *C. hominis* (Xiao *et al*., 1999; Kosek *et al*., 2001). PCR-RFLP analysis based on SSU rRNA gene on all 9 isolates of *Cryptosporidium* sp. inferred *C. parvum*. The dominance of *C. parvum* in this analysis was in agreement with recent study conducted among HIV/AIDS patients in Malaysia with 84.3% of 32 *Cryptosporidium* isolates was *C. parvum*, followed by 6.3% *C. hominis*, 6.3% *C. meleagridis* and 3.1% *C. felis* (Iqbal *et al*., 2015). Another study among HIV/AIDS patients in Malaysia also reported the dominant of *C. parvum* with 64% from 25 samples, followed by 24.0% *C. hominis*, 8.0% *C. meleagridis* and 4% *C. felis* (Lim *et al*., 2011). Zaidah *et al*., (2008) also reported that *C. parvum* was the only species found among 9 HIV patients in Kota Bharu. Previously, the distribution and genotyping of *Cryptosporidium* species base on gp60 gene among the 18 isolates of HIV-infected patients in Malaysia also reported that 72.2% was *C. parvum* and 27.7% was *C. hominis* (Iqbal *et al*., 2012).

However, results from previous studies failed to show significance between *Cryptosporidium* infection and gender. The result in this study reported more male workers (8/9; 88.9%) were infected with *C. parvum* compared to female (1/9; 11.1%) with infections were found in all age levels. However this study was only limited to adult workers and was not unable to compare infections with ages younger than 21 years old.

Based on nationality factor, most infected workers were from India (4/9; 44.4%), followed by Indonesia (2/9; 22.2%), Bangladesh (1/9; 11.1%), Nepal (1/9; 11.1%) and Myanmar (1/9; 11.1%). Recent study in Andhra Pradesh, India reported high prevalence of *Cryptosporidium* infection (25%) from 306 cases, with 35% to 36%

adults, 17% children and 20% infants (Manocha *et al*., 2014). In North India also reported high prevalence of infection with 73.0% *C. hominis* and 24% *C. parvum* (Sharma *et al*., 2013). Similarly, Das *et al*. (2006) also reported *C. hominis* (87.5%), *C. parvum* (10%) and *C. felis* (2.5%) from children in Kolkata, India. The high infections in the population were possibly due to the close contact with animals in rural areas and farms and the highly endemic occurrence of cryptosporidiosis among livestock could be the sources of zoonotic transmission to human (Kali, 2014). In Indonesia, a community based study in Surabaya reported 8.2% of *C. parvum* oocysts were detected in diarrhea samples and indicated that close contact with cats, rain, flood and crowded living condition as significant risk factors (Katsumata *et al*., 1998).

In the present study, most of the infected workers resided in Malaysia for more than a year. The transmission among the workers could be due to improper personal hygiene and in the crowded living condition as most of them are living in the hostel provided by the employer. The outcome of this study is important in order to enable proper control strategy and hygiene programme by the responsible agency to prevent *Cryptosporidium* sp. infections among this migrant population (Lim *et al*., 2008).

6.5 Conclusion

Molecular characterization has been successful applied for the identification up to species level of all IPIs.

The use of nested PCR targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene was successfully applied in the detection of *S. stercoralis* infection in three migrant workers.

Using internal transcribed spacer 2 and 28S ribosomal RNA region of *N. americanus* and *Ancylostoma* spp., PCR amplicons resulted in the detection of both *N. americanus* and for the first time in Malaysia, *A. duodenale* in this targeted group.

While, nested PCR targeting 16S-like ribosomal RNA gene was used to genetically characterize *E. histolytica* and *E. dispar* in which *E. dispar* was the most predominant infection in migrant workers. The presence of *E. histolytica* albeit low in the study population still plays a role in public health.

Molecular characterization of *G. duodenalis* isolated from migrant workers in Malaysia was conducted and successfully amplificated the triosephosphate isomerase (TPI) gene. Targeting the tpi gene, the presence of assemblage B and sub-assemblage AII suggest that the mode of transmission of giardiasis amongst these workers were possibly human-to-human. However, further investigation is required to include multilocus genotyping of parasites from human and animals to have a better understanding of the epidemiology of this infection.

Based on the SSU rRNA gene, the *C. parvum* amplicons were successfully detected in all 9 human isolates. The possible associated risk factor could be due to crowded living conditions among the workers. Further studies should be conducted to determine the significance of *Cryptosporidium* sp. transmission among the migrant workers in Malaysia.

CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

7.1 General discussion

The health status of marginalized population in Malaysia is well documented particularly on parasitic infections and toxoplasmosis. A review of one hundred and one published articles on intestinal parasitic infections in Malaysia (1970 to 2013) highlighted that intestinal parasitic infections continue to be a public health concern especially among the poverty-stricken communities including the Orang Asli, plantation and rural communities, squatter, fishing communities, new villages and the flat dwellers (Sinniah *et al*., 2014). Some studies have shown that prevalence of infections fluctuated among slum dwellers (Chia *et al*., 1978; Sinniah *et al*., 2014), flat dwellers (Kan, 1983; Sinniah *et al*., 2014) and rural communities (Bisseru & Aziz, 1970; Sinniah *et al*., 2014). In addition, human toxoplasmosis also persists among the Malaysian population particularly in the clinically suspected cases and HIV-AIDS patients (Nissapatorn & Anuar, 2004) and noted several factors linked to the acquisition of infection. Another target study group is among migrants with a few studies among the clinical suspected patients and workers of specific sectors (Table 7.1).

Large numbers of workers originating from more than 12 countries in Asia (Indonesia, Bangladesh, Thailand, Philippines, Pakistan, Myanmar, Nepal, India, Cambodia, China, Vietnam, Laos and Sri Lanka) enter Malaysia annually to fill the low-skilled labor market primarily in sectors such as construction, domestic, food services, manufacturing and plantation. Therefore, there is great interest to determine the health status of these workers as they come from countries endemic to many diseases. Despite the mandatory health screening prior to entering the country for

communicable diseases such as AIDS, malaria and tuberculosis, little is known on the non-communicable disease status. These diseases have public health implications as it can potentially be transmitted to the general public through bad hygiene and sanitation. The present study successfully recruited 610 migrant workers from five working sectors to determine their parasitic health status based on a questionnaire survey, microscopy and molecular characterisation. However, only 388 stool samples in addition to 484 blood samples were available for screening (Table 7.2). Many refused to donate either blood or/and stool samples as recruitment was on voluntary basis and the screening was non-mandatory under FOMEMA (agency involved in the implementation, management and supervision of a nationwide mandatory health screening programme for all legal migrant workers in Malaysia), Ministry of Health and Immigration Department of Ministry of Home Affairs Malaysia upon entry or residing in Malaysia. Other reasons for not participating include disgusted with faeces handling and/or preoccupied with matters related to work.

Most workers recruited were from domestic sector (n=148), followed by construction (n=139), food service (n=128), plantation (n=102) and manufacturing (n=93) sector. The participants came from varied demographic background (age, level of education, nationality, religion and marital status, types of accommodation), however most were housed with clean water, proper sewage toilets and waste disposal system. Most workers did not engage in any risk behavior, such as smoking, consumption of alcohol and illegal drugs. More than 80% were fully covered for medical treatment with accessibility to private or government hospital/clinic. The workers also were briefed on occupational health and safety background and were provided with personal protective equipment (PPE) at work.

The results from the parasitic screening were based on three diagnostic methods adopted to detect the presence of helminthes and protozoan infections among the workers namely; microscopy, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Seven helminthes (*A. lumbricoides, T. trichiura, N. americanus, A. duodenale, S. stercoralis, E. vermicularis* and *H. nana*) and 4 protozoan (*E. dispar, E. histolytica, G. duodenalis* and *C. parvum*.) infections were detected with high prevalence of *A. lumbricoides* with 43.3% (n=168), followed by *T. trichiura* (n=37, 9.5%), *N. americanus* (n=35, 9.0%), *G. duodenalis* (n=30, 7.7%), *E. dispar* (n=23, 5.9%), *E. histolytica* (n=11, 2.8%), *C. parvum* (n=9, 2.3%), *A. duodenale* (n=8, 2.1%), *H. nana* (n=7, 1.8%), *S. stercoralis* (n=3, 0.98) and *E. vermicularis* (n=2, 0.5%) among the workers. Details of the parasite species recovered with their diagnostic method are described in Table 7.3 and Table 7.4.

ELISA positive result for anti-Toxoplasma IgG indicates past infection and IgM of recent infection, and positive anti *Strongyloides* antibody indicates patient may be seropositive for *S. stercoralis* infection. Results recorded high seroprevalence of *S. stercoralis* was detected in the targeted population. However, this result was not unequivocally able to prove current active infection but a persisting level of antibody from a past infection (Yori *et al*., 2006; Ahmad *et al*., 2013). Based on microscopic examination, all of the 306 faecal samples provided were negative for larvae. Siddiqui and Berk (2001) reported that detection of *S. stercoralis* larvae by microscopy using only single stool sample lacked sensitivity and failed in 70 % of cases. While nested PCR targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene in the detection of *S. stercoralis* was the most sensitive diagnostic method in the detection of current infection, which successfully detected three positive workers and should be used in all future analysis and studies of *S. stercoralis*.

Among the significant explanatory factors association with the high prevalence of parasitic infections in this country were sex, age, nationality, employment sector and years of residence in Malaysia. *A. lumbricoides* infection among the workers was significantly related to sex, nationality, employment sector and years of residence factor. Similarly, significantly higher infections of *A. lumbricoides* and *T. trichiura* were linked mainly to males compared to females. This could be due to more involvement of males with more outdoor activities compared to females.

The demographic profiles of respondents comprised predominantly of volunteers from rural areas in their respective countries where IPIs are still very much prevalent and a major concern among the poor and deprived communities, particularly workers from India and Nepal. This finding was in agreement with studies in India which suggested that inadequate sanitary and poor drainage was likely to have contributed to disease prevalence (Fernandez *et al*., 2002; Dhanabal *et al*., 2014). Similarly, parasitic infections in Nepal were also reported linked to rapid, unplanned urbanization, open defaecation and other unhygienic habits, as well as a lack of health awareness (Uga *et al*., 2004; Rabindranath *et al*., 2006; Singh *et al*., 2013). It was also shown that workers with employment history of less than a year or newly arrive workers were most likely to be infected as they were also most likely to have no history of taking any anthelmintic drugs in the last 12 months. This is not surprising as the mandatory medical screening procedure upon entry to this country excludes examination for IPIs and not required to be administrated with any anthelmintic drugs (FOMEMA, 2015).

Serological analysis of *T. gondii* found four demographic factors associated with the infections namely; age, nationality, employment sector and years of residence in Malaysia. Highest prevalence of infection was found amongst workers from Nepal. Variations in prevalence of infection among workers from different countries were most likely due to dietary habits, risk behavior, environmental conditions, socioeconomic status and hygiene (Chan *et al.,* 2008). The high infections among Nepalese could be due to the habitual ingestion of minced raw meat or cooking meat insufficiently common among certain ethnic groups (Rai *et al*., 1994; Rai *et al*., 1999). In the present study, all workers (n=484) originated from rural areas in their respective countries where parasitic infections are still very much prevalent and a major concern among the poor and deprived communities. Correlations were observed between seropositivity of toxoplasmosis and consumption of unboiled water. Toxoplasmosis was also reported in many studies, particularly among disadvantaged and indigenous communities living in rural and remote areas (de Moura *et al*., 2006; Sroka *et al.*, 2006; Sroka *et al*., 2010a) Despite showing high infections among those in manufacturing, the current results may be biased as most (91.1%) working sectors were dominated by a particular nationality i.e., Nepalese.

The high prevalence of parasitic infections among the migrant community in this study provided an insight into the conditions under which the subjects previously and currently live, and reflects the availability of environmental sanitation as well as the socioeconomic status. Transmission of parasitic infections within the community is predominantly dependent on human behaviour, particularly during food preparation and intake, defaecation, personal hygiene, and cleanliness. Furthermore, most parasites found in this study can be easily treated with the appropriate anthelmintic (Table 7.3 and Table 7.4).

Table 7.1: Historical timeline of parasitic infections studies among migrants in Malaysia.

References	Samples	Migrant population	Parasitic analysis	No. of samples	No. positive (%)
Zainul *et al*, 1992	Blood	Women with still births	Toxoplasmosis	144	51 (35.7)
Suresh *et al*., 2002	Stool	Clinical samples/ Workers	STH and Protozoa	173	62 (36)
Rajah *et al*., 2002	Stool	Clinical samples/ workers	Blastocystis	173	10 (5.8)
Kamarulzaman & Khairul Anuar, 2002	Blood	Clinical suspected case (worker)	Leishmaniasis	A case report	1
Khairul Anuar *et al*., 2002	Blood	Clinical samples/ workers	Blood parasites	241	2 (0.83)
Zurainee, 2002	Blood	Workers	Serological detection: Amoebiasis, Echinococcosis, Filariasis (*Brugia malayi* and *Wuchereria bancrofti*), Leishmaniasis, Malaria, Schistosomiasis, Trypanosomiasis	698	266 (38.1)
Nissapatorn *et al*, 2002	Blood	HIV-AIDS/HBD	Toxoplasmosis	303	152 (50.0)
Nissapatorn *et al*, 2003a	Blood	HIV/AIDS, HKL	Toxoplasmosis	301	75 (25.0)
Nissapatorn *et al*, 2003b	Blood	AIDS, HKL	Toxoplasmosis	406	6 (1.4)
Chan *et al*., 2008a	Blood	Plantation workers/ Detention camp	Toxoplasmosis	501	171 (34.1)
Chan *et al*., 2008b	Blood	Plantation workers/ Detention camp	Toxoplasmosis	501	171 (34.1)
Amal *et al*., 2008	Blood	Plantation workers/ Detention camp	Toxoplasmosis	501	171 (34.1)
Chan *et al*., 2009	Blood	Plantation workers/ Detention camp	Toxoplasmosis	336	138 (42)

Table 7.2: Participants employment according to employment sectors in Malaysia.

Sector	Total participants (Questionnaire and consent to participate)	Stool sample returned	Blood sample collected	Total stool and blood samples collected
	N (%)	N (%)	N (%)	N (%)
Construction	139 (22.8)	47 (12.1)	68 (14.0)	10 (3.3)
Manufacture	93 (15.2)	61 (15.7)	93 (19.2)	61 (19.9)
Plantation	102 (16.7)	71 (18.3)	102 (21.0)	71 (23.2)
Service	128 (21.0)	104 (26.8)	115 (23.8)	94 (30.7)
Domestic	148 (24.3)	105 (27.1)	106 (21.9)	70 (22.9)
Total	**610 (100)**	**388 (63.6)**	**484 (79.3)**	**306 (50.2)**

Table 7.3: Prevalence of helminth infection among migrant workers in Malaysia in relation to factors; sex, age, nationality, employment sector and years of residence.

		Helminth						
		A. lumbricoides	*T. trichiura*	*N. americanus*	*A. duodenale*	*S. stercoralis*	*E. vermicularis*	*H. nana*
Type of samples	**Stool**	√	√	√	√	√	√	√
	Blood	-	-	-	-	√	-	-
Total samples examined		388	388	388	388	306[b]	388	388
Method of detection	**Microscopy**	√	√	√[a]	√[a]	√	√	√
	Serology	-	-	-	-	√	-	-
	PCR	-	-	√	√	√	-	-
No. of positive samples	**Microscopy**	168 (43.3)	37 (9.5)	51 (13.1)[a]	51 (13.1)[a]	0	2 (0.5)	7 (1.8)
	Serology	-	-	-	-	115 (37.6)	-	-
	PCR	-	-	35 (68.6)	8 (15.7)	3 (0.98)	-	-
Sex								
	Male (n=304)	145 (47.7)	35 (11.5)	28 (9.2)	5 (1.6)	3/243 (1.2)[b]	2 (0.7%)	7 (2.3)
	Female (n=84)	23 (27.4)	2 (2.4)	7 (8.3)	3 (3.6)	0/63[b]	0	0
	P-value	0.001[c]	0.001[c]	0.804	0.271	0.375	0.456	0.160
Age								
	<25 (n=114)	59 (51.8)	20 (17.5)	20 (17.5)	6 (5.3)	0/93[b]	0	2 (1.8)
	25-34 (n=145)	63 (43.4)	12 (8.3)	7 (4.8)	1 (0.7)	0/110[b]	0	3 (2.1)
	35-44 (n=90)	32 (35.6)	3 (3.3)	8 (8.9)	1 (1.1)	2/70 (2.9)[b]	1 (1.1)	1 (1.1)
	45-54 (n=29)	9 (31.0)	2 (6.9)	0	0	1/25 (4.0)[b]	0	1 (3.4)
	>55 (n=10)	5 (50.0)	0	0	0	0/8[b]	1 (10.0)	0
	P-value	0.113	0.010[c]	0.002[c]	0.079	0.137	0.001	0.920
Nationality								
	Indonesia (n=167)	44 (26.3)	16 (9.6)	16 (9.6)	6 (3.6)	1/124 (0.8)[b]	1 (0.6)	0

Bangladesh (n=70)	29 (41.4)	6 (8.6)	3 (4.3)	0	0/53[b]	1 (1.4)	2 (2.9)
Myanmar (n=23)	4 (17.4)	6 (26.1)	4 (17.4)	0	0/12[b]	0	0
India (n=47)	32 (68.1)	1 (2.1)	3 (6.4)	0	2/41 (4.9)[b]	0	1 (2.1)
Nepal (n=81)	59 (72.8)	8 (9.9)	9 (11.1)	2 (2.5)	0/76[b]	0	4 (4.9)
P-value	<0.001[c]	0.010[c]	0.312	0.292	0.097	0.076	0.076
Employment Sector							
Construction (n=47)	17 (36.2)	10 (21.3)	5 (10.6)	0	0/10[b]	1 (2.1)	0
Manufacture (n=61)	44 (72.1)	3 (4.9)	4 (6.6)	2 (3.3)	0/61[b]	0	3 (4.9)
Plantation (n=71)	18 (25.4)	11 (15.5)	7 (9.9)	2 (2.8)	0/71[b]	1 (1.4)	2 (2.8)
Food Service (n=104)	61 (58.7)	8 (7.7)	11 (10.6)	1 (1.0)	2/94 (2.1)[b]	0	2 (1.9)
Domestic (n=105)	28 (26.7)	5 (4.8)	8 (7.6)	3 (2.9)	1/70 (1.4)[b]	0	0
P- value	<0.001[c]	0.001[c]	0.875	0.629	0.587	0.299	0.162
Years of residence							
< than 1 year (n=134)	78 (58.2)	17 (12.7)	14 (10.4)	6 (4.5)	0/122[b]	0	2 (1.5)
> than 1 year (n=254)	90 (35.4)	20 (7.9)	21 (8.3)	2 (0.8)	3/184 (1.6)[b]	2 (0.8)	5 (2.0)
P- value	<0.001[c]	0.004[c]	0.476	0.015[c]	0.156	0.303	0.738
Treatment	Albendazole Mebendazole	Albendazole Mebendazole	Albendazole Mebendazole	Albendazole Mebendazole	Ivermectin Albendazole	Albendazole Mebendazole Pyrantel pamoate	Praziquantel Niclosamide Nitazoxanide

[a] Detected as hookworms
[b] Number infected / variables from each factor (%)
[c] Significant at 0.05

Table 7.4: Prevalence of protozoan infection among migrant workers in Malaysia relation to factors; sex, age, nationality, employment sector and years of residence.

		Protozoa					
		E. dispar	*E. histolytica*	*Giardia duodenalis*		*Cryptosporidium parvum*	*T. gondii*
				A	B		
Type of samples	**Stool**	√	√	√	√	√	-
	Blood	-	-	-	-	-	√
Total samples examined		388	388	388	388	388	484
Method of Detection	**Microscopy**	√	√	√	√	√	-
	Serology	-	-	-	-	-	√
	PCR	√	√	√	√	√	-
No. of positive samples	**Microscopy**	45 (11.6)[a]	45 (11.6)[a]	42 (10.8)[b]	42 (10.8)[b]	12 (3.1)[c]	-
	Serology	-	-	-	-	-	278/484 (57.4)
	PCR	23 (5.9)	11 (2.8)	13 (3.4)	17 (4.4)	9 (2.3)	-
Sex							
	Male (n=304)	15 (4.9)	7 (2.3)	10 (3.3)	16 (5.3)	8 (2.6)	213/375 (56.8)[d]
	Female (n=84)	8 (9.5)	4 (4.8)	3 (3.6)	1 (1.2)	1 (1.2)	65/109 (59.6)[d]
	P-value	0.115	0.229	0.899	0.106	0.403	0.598
Age							
	<25 (n=114)	6 (5.3)	4 (3.5)	6 (5.3)	4 (3.5)	2 (1.8)	84/142 (59.2)[d]
	25-34 (n=145)	12 (8.3)	4 (2.8)	6 (4.1)	10 (6.9)	3 (2.1)	97/183 (53.0)[d]
	35-44 (n=90)	4 (4.4)	1 (1.1)	1 (1.1)	2 (2.2)	3 (3.3)	61/111 (55.0)[d]
	45-54 (n=29)	1 (3.4)	1 (3.4)	0	1 (3.4)	1 (3.4)	26/35 (74.3)[d]
	>55 (n=10)	0	1 (10.0)	0	0	0	10/13 (76.9)[d]
	P-value	0.587	0.547	0.366	0.417	0.880	0.078
Nationality							
	Indonesia (n=167)	11 (6.6)	6 (3.6)	4 (2.4)	6 (3.6)	2 (1.2)	144/247 (58.3)[d]
	Bangladesh (n=70)	1 (1.4)	0	0	6 (8.6)	1 (1.4)	33/72 (45.8)[d]

Myanmar (n=23)	2 (8.7)	1 (4.3)	0	0	1 (4.3)	4/14 (28.6)[d]
India (n=47)	5 (10.6)	1 (2.1)	2 (4.3)	1 (2.1)	4 (8.5)	20/52 (38.5)[d]
Nepal (n=81)	4 (4.9)	3 (3.7)	7 (8.6)	4 (4.9)	1 (1.2)	77/99 (77.8)[d]
P-value	0.287	0.577	0.029[e]	0.301	0.135	<0.001[e]
Employment Sector						
Construction (n=47)	0	0	0	2 (4.3)	0	42/68 (61.8)[d]
Manufacture (n=61)	3 (4.9)	3 (4.9)	5 (8.2)	3 (4.9)	1 (1.6)	71/93 (76.3)[d]
Plantation (n=71)	1 (1.4)	1 (1.4)	0	3 (4.2)	1 (1.4)	46/102 (45.1)[d]
Food Service (n=104)	10 (9.6)	2 (1.9)	5 (4.8)	4 (3.8)	7 (6.7)	58/115 (50.4)[d]
Domestic (n=105)	9 (8.6)	5 (4.8)	3 (2.9)	5 (4.8)	0	61/106 (57.5)[d]
P- value	0.049[e]	0.332	0.055	0.997	0.009[e]	<0.001[e]
Years of residence						
< than 1 year (n=134)	7 (5.2)	5 (3.7)	9 (6.7)	8 (6.0)	2 (1.5)	119/180 (66.1)[d]
> than 1 year (n=254)	16 (6.3)	6 (2.4)	4 (1.6)	9 (3.5)	7 (2.8)	159/304 (52.3)[d]
P- value	0.670	0.440	0.007[e]	0.267	0.415	<0.001[e]
Treatment	Non-pathogenic Harmless	Paromomycin Iodoquinol Metronidazole Tinidazole	Metronidazole Tinidazole Nitazoxanide		Nitazoxanide	Pyrimethamine Sulfadiazine Spiramycin Clindamycin

[a] Detected as *Entamoeba* spp.
[b] Detected as *Giardia* sp.
[c] Detected as *Cryptosporodium* spp.
[d] Number infected/ variables from each factor (%)
[e] Significant at 0.05

7.2 Conclusion

The parasitic health status of migrant workers in Malaysia was successfully determined according to the objectives described below;

- Migrant workers in Malaysia were provided with suitable living accommodations and were supplied with complete basic amenities including clean water system, proper sewage toilets, and efficient waste disposal system. The workers also were fully covered for medical treatment with the accessibility to private or government hospitals/clinics and all were provided with personal protective equipment (PPE) and adhered to wearing the gear at work at all times.

- The screening of stool samples using formalin ethyl-acetate concentration technique and modified ziehl-neelsen staining method recovered a total of 8 species of parasites with four nematode species (*Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis* and hookworms), one cestode (*Hymenolepis nana*) and three protozoan species (*Entamoeba histolytica/dispar, Giardia* spp. and *Cryptosporidium* spp.) infecting the workers with infections significantly influenced by socio-demographic (nationality), and environmental characteristics (years of residence in this country, employment sector and educational level).

- Slightly more than half of the workers were seropositive for *Toxoplasma gondii* (57.4%) with 52.9% being seropositive for anti-*Toxoplasma* IgG only, 0.8% seropositive for anti-*Toxoplasma* IgM only and 3.7% seropositive with both IgG and IgM antibodies. Samples positive for both IgG and IgM antibodies were further tested for IgG avidity and all showed high avidity, suggesting chronic infection. Four significant factors affected seropositivity namely, age, nationality, employment sector and years of residence in Malaysia.

- Over a third of the workers were seropositive (37.6%) to *S. stercoralis* infection with no association to any for the factors tested.

- Nested PCR targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene of *S. stercoralis* was highly sensitive in the detection of current infections and should be applied in all future analysis and studies. This study successfully detected three workers with current *S. stercoralis* infections.

- PCR methods successfully amplified internal transcribed spacer 2 and 28S ribosomal RNA region of *N. americanus* and *Ancylostoma* spp. The PCR amplicons successfully obtained *N. americanus* and *A. duodenale.* This is the first time *A. duodenale* was reported in Malaysia. This finding may have important implications for public health and for the control of hookworm diseases in Malaysia, especially *A. duodenale* which has higher degree of iron deficiency and anemia compared to *N. americanus*.

- Nested PCR targeting 16S-like ribosomal RNA gene successfully recovered *E. dispar* as the most dominant infection among workers compared to *E. histolytica.* The presence of *E. histolytica* in the study population was small however is carries a public health risk, therefore parasite control strategies especially mass treatment and health education are recommended for all migrant workers as well as local population of Malaysia.

- Amplification of the triosephosphate isomerase (TPI) gene from *G. duodenalis* isolates successfully obtained the presence of assemblage B and sub-assemblage AII. This suggested that the mode of transmission of giardiasis among migrant workers in Malaysia may be human-to-human. However, further investigation should be conducted involving multilocus genotyping of parasites from human and animals to understand the epidemiology of *G. duodenalis* infection based on molecular genotyping approach.

- Based on the SSU rRNA gene, the *C. parvum* amplicons were successfully detected in 9 human isolates. The possible associated risk factor could be due to crowded living conditions among the workers in the hostel provided by the employer. Further analysis should be done to determine the significance of *Cryptosporidium* sp. transmission among the migrant workers in Malaysia.

Limitations of the study

Of the 610 volunteers recruited, only 388 stool samples and 484 blood samples were available for screening. Many refused to donate either blood or/and stool samples for reasons including disgusted with faeces handling and/or preoccupied with matters related to work. Higher participation was expected if this study had the full support from the Ministry of Health Malaysia and other related agencies such as FOMEMA including the employer of the migrant workers.

Recommendations

These findings highlight the urgent need especially to refine current health polices for Malaysia to include STH and other parasites in the mandatory health screening list of those applying for entry, work permits and residence in Malaysia. Recommendations also include the implementation of mass drug administration for all newly arrive workers as stated by World Health Organization (2015). Moreover, this should be accompanied by health education campaigns and programs aimed at increasing community awareness of the importance of personal hygiene, sanitation, cleanliness, healthy behaviors in controlling parasitic infections and the potential in transmission of diseases.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

PUBLICATIONS

1. **Sahimin, N.,** Yvonne A.L. Lim, Ariffin, F., Behnke, J.M., Lewis, J.W. & Mohd Zain, S.N. (2016). Migrant Workers in Malaysia: Current Implications of Sociodemographic and Environmental Characteristics in the Transmission of Intestinal Parasitic Infections. PLoS Neglected Tropical Diseases, 10(11): e0005110. doi:10.1371/journal.pntd.0005110. (ISI-Cited Publication)

2. **Sahimin, N.,** Yvonne A.L. Lim, Ariffin, F., Behnke, J. M., Basáñez, M. G., Walker, M., Lewis, J.W., Nordin, R., Abdullah, K. A. & Mohd Zain, S.N. (2017). Socio-demographic determinants of *Toxoplasma gondii* seroprevalence in migrant workers of Peninsular Malaysia. Parasites & Vectors. Accepted with minor revision.

3. **Sahimin, N**., Yvonne A.L. Lim, Douadi, B., Mohd Khalid, M. K. N., Wilson, J. J. & Mohd Zain, S.N. (2017). Hookworm infections among migrant workers in Malaysia: molecular identification of *Necator americanus* and *Ancylostoma duodenale.* Submitted to Acta Tropica.

PRESENTATIONS

1. **Sahimin, N.,** Mohd Zain, S. N. & Yvonne A.L. Lim. (2015). Preliminary study of intestinal parasitic infections amongst migrant workers in Malaysia. 51st Annual Scientific Conference of The Malaysian Society of Parasitology and Tropical Medicine.

2. **Sahimin, N.,** Mohd Zain, S. N. & Yvonne A.L. Lim. (2015). Intestinal parasitic infections amongst migrant workers in Malaysia. 2nd International Conference on Tropical Medicine & Infectious Diseases.

3. Sahimin, N., **Mohd Zain, S. N.,** Yvonne A.L. Lim. Behnke, J.M., Lewis, J.W. & Ariffin, F. (2015). Intestinal parasitic infections amongst migrant workers in Malaysia. BSP Spring Meeting 2015. British Society for Parasitology.

4. Sahimin, N., **Mohd Zain, S. N.** & Yvonne A.L. Lim. (2016).The relationship between seroprevalence of toxoplasmosis with sociodemographic characteristics and period of residence of migrant workers in Malaysia. BSP Spring Meeting 2016. British Society for Parasitology.

APPENDIX D

Published Paper I:

Sahimin, N., Yvonne A.L. Lim, Ariffin, F., Behnke, J.M., Lewis, J.W. & Mohd Zain, S.N. (2016). Migrant Workers in Malaysia: Current Implications of Sociodemographic and Environmental Characteristics in the Transmission of Intestinal Parasitic Infections. PLoS Neglected Tropical Diseases, 10(11): e0005110. doi:10.1371/journal.pntd.0005110. (ISI-Cited Publication)