SEROPREVALENCE, RISK FACTORS OF LEPTOSPIROSIS SEROPOSITIVITY AND EFFECTIVENESS OF LEPTOSPIROSIS HEALTH INTERVENTION MODULE IN IMPROVING KNOWLEDGE, ATTITUDE, BELIEF AND PRACTICE ON LEPTOSPIROSIS AMONG WET MARKET WORKERS IN KELANTAN

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LIST OF SYMBOL AND ABBREVIATIONS

ANOVA	Analysis of Variance
CI	Confidence Interval
CSF	Cerebrospinal fluid
DGM	Dark Ground Microscopy
DNA	Deoxyribonucleic acid
df	Degree of freedom
ELISA	Enzyme-linked Immunosorbent Assay
EMJH	Ellinghausen-McCullough-Johnson-Harris
HUSM	Hospital Universiti Sains Malaysia
IgM	Immunoglobulin M
IgG	Immunoglobulin G
IMR	Institute for Medical Research
IQR	Interquartile range
KABP	Knowledge, Attitude, Belief and Practice
LHIM	Leptospirosis Health Intervention Module
LHIP	Leptospirosis Health Intervention Program
MAT	Microscopic Agglutination Test
MOH	Ministry of Health
OR	Odds ratio
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
RCT	Randomized Controlled Trial
RM ANOVA	Repeated Measures Analysis of Variance

ROC	Receiver Operating Characteristics
SD	Standard Deviation
SE	Standard Error
SEL	Sensitized Erythrocyte Lysis
SPSS	Statistical Package for Social Science
USM	Universiti Sains Malaysia
WHO	World Health Organisation

ABSTRAK

Seroprevalen, Faktor-faktor Risiko Leptospirosis dan Keberkesanan Modul Intervensi Kesihatan Leptospirosis dalam Meningkatkan Pengetahuan, Sikap, Kepercayaan dan Amalan tentang Leptospirosis di Kalangan Pekerja Pasar Basah di Kelantan

Leptospirosis merupakan penyakit bawaan haiwan yang memberi kesan kepada manusia dan haiwan di seluruh dunia. Penyakit ini diketahui mempunyai hubungkait dengan pekerjaan yang terdedah kepada persekitaran yang tercemar. Kajian ini bertujuan untuk menentukan seroprevalen, faktor-faktor berkaitan leptospirosis dan keberkesanan Modul Intervensi Kesihatan Leptospirosis di kalangan pekerja pasar basah di Kelantan. Dalam fasa pertama, satu kajian keratan rentas telah dijalankan melibatkan 232 pekerja pasar basah yang memenuhi kriteria kajian dan dipilih secara rawak dari dua pasar basah utama di Kelantan. Maklumat berkenaan sosiodemografi, ciri-ciri berkenaan pekerjaan dan aktiviti rekreasi dikumpulkan menggunakan borang kaji selidik KABP berkenaan leptospirosis yang telah divalidasi. Sampel darah diambil dan dianalisa menggunakan ujian aglutinasi mikroskopik (MAT). Dalam fasa kedua, satu kajian intervensi telah dijalankan. Peserta dari fasa pertama dibahagikan kepada kumpulan kawalan dan kumpulan intervensi berdasarkan tempat kerja mereka. Kumpulan intervensi menerima program intervensi berdasarkan Modul Intervensi Kesihatan Leptospirosis manakala kumpulan kawalan tidak menerima program intervensi. Pengetahuan, sikap, kepercayaan dan amalan peserta dinilai menggunakan borang kaji selidik KABP yang telah divalidasi sebelum dan enam minggu selepas intervensi. Purata umur peserta adalah 42.6 (14.68) tahun dan majoriti adalah wanita (63.4%). Seroprevalen keseluruhan leptospirosis di kalangan pekerja

pasar basah adalah 33.6% dan serovars yang paling dominan adalah Autumnalis dengan 18.2%. Faktor-faktor yang berkaitan dengan seropositif leptospirosis adalah umur (AOR 1.02; 95% CI: 1.004, 1.043) dan tidak menggunakan sarung tangan semasa bekerja (AOR 2.45; 95% CI: 1.02, 5.87). Terdapat peningkatan yang signifikan dalam markah pengetahuan, sikap, kepercayaan dan amalan di kalangan kumpulan intervensi berbanding kumpulan kawalan. Bagi bahagian pengetahuan, kumpulan intervensi menunjukkan markah yang lebih tinggi ($p<0.001$) berbanding kumpulan kawalan dengan purata perbezaan yang dilaraskan adalah 12.93 (95% CI: 8.47, 17.39). Bagi bahagian sikap, kumpulan intervensi menunjukkan markah yang lebih tinggi ($p=0.001$) berbanding kumpulan kawalan dengan purata perbezaan yang dilaraskan adalah 5.55 (95% CI: 2.28, 8.81). Bagi bahagian kepercayaan, kumpulan intervensi menunjukkan markah yang lebih tinggi ($p<0.001$) berbanding kumpulan kawalan dengan purata perbezaan yang dilaraskan adalah 7.21 (95% CI: 3.43, 10.99). Bagi bahagian amalan, kumpulan intervensi menunjukkan markah yang lebih tinggi ($p<0.001$) berbanding kumpulan kawalan dengan purata perbezaan yang dilaraskan adalah 7.35 (95% CI: 3.64, 11.05). Penemuan dalam kajian ini menunjukkan seroprevalen leptospirosis di kalangan pekerja pasar basah di Kelantan adalah tinggi dan berkaitan dengan umur dan tidak menggunakan pakaian pelindung di tempat kerja. Modul Intervensi Kesihatan Leptospirosis adalah berkesan dalam meningkatkan pengetahuan, sikap, kepercayaan dan amalan berkaitan leptospirosis di kalangan pekerja tersebut. Oleh itu, adalah penting untuk meningkatkan kesedaran berkaitan penyakit ini di kalangan pekerja dan Modul Intervensi Kesihatan Leptospirosis boleh digunakan sebagai alat Pendidikan kesihatan di kalangan kumpulan berisiko ini.

Kata kunci: *leptospirosis, pekerja pasar basah, seroprevalen, intervensi*

ABSTRACT

Seroprevalence, Risk Factors of Leptospirosis and Effectiveness of Leptospirosis Health Intervention Module in Improving Knowledge, Attitude, Belief and Practice on Leptospirosis Among Wet Market Workers in Kelantan

Leptospirosis is a zoonotic disease which affect human and animal globally. The disease is known to be related to occupations which are exposed to contaminated environment. This study aims to determine the seroprevalence, factors associated with leptospirosis and effectiveness of Leptospirosis Health Intervention Module among wet market workers in Kelantan. In Phase One, a cross sectional study was conducted among 232 wet market workers who fulfilled the study criteria and were randomly selected from two main wet markets in Kelantan. Information regarding sociodemographic, work-related characteristics and recreational activities were collected using validated KABP questionnaire on leptospirosis. Blood samples were collected and analysed using microscopic agglutination test (MAT). In Phase Two, an intervention study was conducted. Respondents from phase one were divided into control and intervention groups based on their workplace. Intervention group received the intervention program based on Leptospirosis Health Intervention Module and control group received no intervention. Knowledge, attitude, belief and practice of respondents were assessed using validated KABP questionnaire before and after six weeks of the intervention. The mean age of respondents was 42.6 (14.68) years old and majority of them were female (63.4%). The overall seroprevalence of leptospirosis among wet market workers was 33.6% and the predominant serovars was Autumnalis with 18.2%. The factors associated with leptospirosis seropositivity

was age (AOR 1.02; 95% CI: 1.004, 1.043) and not using glove during work (AOR 2.45; 95% CI: 1.02, 5.87). There were significant increase of knowledge, attitude, belief and practice scores among intervention group compared to control group. For knowledge section, intervention group showed significantly higher score ($p<0.001$) compare to control group with the adjusted mean difference between groups was 12.93 (95% CI: 8.47, 17.39). For attitude section, intervention group showed significantly higher score ($p=0.001$) compare to control group with the adjusted mean difference between groups was 5.55 (95% CI: 2.28, 8.81). For belief section, intervention group showed significantly higher score ($p<0.001$) compare to control group with the adjusted mean difference between groups was 7.21 (95% CI: 3.43, 10.99). For practice section, intervention group showed significantly higher score ($p<0.001$) compare to control group with the adjusted mean difference between groups was 7.35 (95% CI: 3.64, 11.05). The findings in this study showed that the seroprevalence of leptospirosis among wet market workers in Kelantan was high and associated with age and not using protective clothing at work. The Leptospirosis Health Intervention Module was effective in improving the knowledge, attitude, belief and practice regarding leptospirosis among the workers. Thus, it is important to increase awareness regarding this disease among the workers and Leptospirosis Health Intervention Module can be used as a tool for health education for this risk group.

Keywords: *leptospirosis, wet market workers, seroprevalence, intervention*

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Leptospirosis is a zoonotic disease caused by bacteria of the genus *Leptospira*. Before the advent of modern medicine, leptospirosis was known by various names such as "rice field jaundice", "autumn fever", "seven-day fever", "cane-cutter's disease", "swine-herd's disease", and "mud fever". The disease was described by Adolph Weil in 1886 when he encountered patients with jaundice, rash, splenomegaly, renal impairment and conjunctivitis with history of outdoor activities. Later it was known as Weil's disease (Adler, 2015; Levett, 2001). The leptospiral were later identified in the early 1900s as the causative agent of the potentially fatal Weil's disease in human. Since then leptospiral have been isolated from human and almost all mammalian species all over the world (Adler, 2015; Levett, 2001). The leptospiral can be divided into two species, *L. interrogans* which triggers disease in human *and L. biflexa*, which do not caused harm to human. At present, there are more than 200 serovars recognized within the *L. interrogans* species (Adler and de la Pena Moctezuma, 2010; Levett, 2001). In Malaysia, 37 serovars of leptospiral have been identified from human and animal samples (El Jalii and Bahaman, 2004).

Leptospiral can infect human through direct or indirect contact with urine of infected animals. Wide range of wild and domestic animals such as horses, rodents, cows, goats, pigs and dogs can be a carrier for the bacteria. These animals can carry leptospiral in their renal without having any sign or symptom. The animals then excrete the leptospiral in their urine to the surroundings during their lifetime and contaminate

the environment. The survival of the leptospiral in the environment depend on several factors. Human get infected when in contact with contaminated environment through cuts in skin and mucous membrane (Victoriano *et al.*, 2009).

The incubation period of leptospirosis is between two to 20 days. The presentation of the disease is variable and sometime not specific. Many patients present with symptoms and signs similar to those seen in many other febrile illnesses such as dengue, malaria and typhoid (WHO, 2003). This make diagnosis of leptospirosis a challenge for medical practitioner. The presentation can be divided into four broad clinical categories; mild influenza like illness, Weil's syndrome, meningitis, meningoencephalitis, and pulmonary haemorrhage with pulmonary failure. The severity of the disease depends on the virulence of the infecting serovars and health status of the patient (Haake and Levett, 2015). High index of suspicion is the key for early detection and diagnosis of the disease. Leptospirosis can be treated with antibiotics. However, early treatment is essential to avoid complication and for better disease outcome (Ministry of Health, 2011).

The annual incidence of leptospirosis is estimated at 0.1 to 1 in every 100000 people worldwide. In tropical climates, the number can increased up to 10 or more per 100000 people (WHO, 2015). Leptospirosis causes significant morbidity and mortality with estimation of more than one million cases and 58,900 deaths yearly all over the world. Majority of the cases occurred in tropical and world's poorest regions such as Africa and Southeast Asia (Costa *et al.*, 2015). Figure on leptospirosis can be higher as the true extent of cases remain unknown due to the challenge in diagnosing the condition, under reporting and lack of surveillance (Bernadette *et al.*, 2010; Hartskeerl, 2006).

In Malaysia, leptospirosis is endemic and becoming an emerging public health concern (Benacer *et al.*, 2016a; Tan *et al.*, 2016). The number of reported cases has been steadily increasing over the years (Benacer *et al.*, 2016a; Yaakob *et al.*, 2015) with Malaysian Ministry of Health (MOH) reported the highest number of cases and deaths due to leptospirosis in 2014 (Thayaparan *et al.*, 2015). The Malaysian incidence rate of leptospirosis in 2014 was 25.94 per 100000 population with mortality rate of 0.31 per 100000 population (Ministry of Health, 2015).

The emergence of zoonotic disease in the human population is a complex phenomenon with multifactorial causes (Taylor *et al.*, 2001). A number of factors responsible for the occurrence of the zoonotic disease have been studied over the years. This include climatic condition, availability of animal carriers and human factors. Tropical climate and high annual rainfall contribute to survival and transmission of leptospirosis in this country. The bacteria can survive for weeks in warm and humid conditions. Annual flooding in some parts of Malaysia also play a role in transmission of the disease (Adler and de la Pena Moctezuma, 2010; Garba *et al.*, 2017a). The country climate provides suitable habitat for a wide range of animals that can become carriers for the leptospiral. These are the challenges for leptospirosis control and preventive measures in Malaysia (Benacer *et al.*, 2013a; Benacer *et al.*, 2013b; Mohamed-Hassan *et al.*, 2012).

Although many animals can be a carrier for leptospires, rodents were the main sources for infection in human (Haake and Levett, 2015). This is the reason human leptospirosis is seen in urban and institutional areas as rodents can adapt to different types of environment. A study on rodents at National Service Training Centres in Terengganu and Kelantan noted that 17.9% of rats caught were positive for

leptospirosis. The rats were positive for serovars *Icterohaemorrhagiae, Canicola, Ballum, Pyrogenes* and *Hebdomadis (Mohamed-Hassan et al., 2010)*. In a study on water and soil samples from selected urban sites in Malaysia, Benacer *et al.* (2013a) found that 23.2% samples contained leptospiral isolates. The water samples were collected from lakes, swamps and effluent drain waters, while soil samples were collected from roadsides near housing areas, wet and night markets. The author suggested, presence of leptospiral in drain effluent waters from night and wet markets could be related to improper waste disposal. This becomes a food source for rodents, cats and dogs which may be the carrier of leptospiral. Benacer *et al.* (2013b) found that, 20 out of 300 rodents samples collected from urban sites including wet market areas were positive for leptospirosis. This information indicates that present of leptospiral at human surrounding were mainly related to present of rodents.

Human activities are important contributors in leptospirosis transmission. Occupations such as agricultural workers, veterinarians, sewer workers, abattoir workers and military personnel were noted to be at risk for infection. These occupations require contact with water and soil that may have been contaminated by infected animal urine (Victoriano *et al.,* 2009; WHO, 2012). Shafei *et al.* (2012) reported seroprevalence of leptospirosis among town service workers in Kelantan were 24.7%. This study also found that garbage collectors and town cleaners had the highest prevalence at 27.4% and 26.0% compared to landscaper and lorry driver at 23.8% and 17.9%. Similarly, Samsudin *et al.* (2015) also found high seroprevalence level among municipal service workers (34.8%) in Selangor. Another study on oil palm plantation workers in Malaysia noted seroprevalence of 28.6% with the highest prevalence among fruit collectors (59.2%), harvesters (24.5%) and pesticide applicators (24.5%) (Ridzuan *et*

al., 2016b). Work-related factors that were associated with infection include present of rodent or carrier animals at workplace, lack of hand washing practice, not using proper personal protective equipment (PPE) and type of jobs. (Ridzuan *et al.*, 2016c; Samsudin *et al.*, 2015; Shafei *et al.*, 2012).

Similarly, recreational activities such as water sport, swimming and canoeing in river also predisposed human to leptospirosis infection (Pappas *et al.*, 2008). Several leptospirosis outbreaks were reported following recreational water sport activities in recent years (Morgan *et al.*, 2002; Reisberg *et al.*, 1997; Sejvar *et al.*, 2003). Morgan *et al.* (2002) reported a leptospirosis outbreak following a triathlon event in 1998. A total of 52 athletes and 14 residents were diagnosed with leptospirosis during the outbreak which saw 21 hospital admission (Morgan *et al.*, 2002). During Borneo Island Echo-Challenge in year 2000, 304 international athletes from 26 countries involved in leptospirosis outbreak which recorded 29 hospital admissions. (Garba *et al.*, 2017b; Sapian *et al.*, 2012; Sejvar *et al.*, 2003). This goes to emphasize the importance of leptospirosis transmission related with human activities (Garba *et al.*, 2017a).

World Health Organization (WHO) had outline general measures for prevention and control of leptospirosis which targeted the infection source, route of transmission to human and treatment of human infection (WHO, 2003). The prevention and control of leptospirosis is complex due to variety of infection sources existed and varies transmission conditions. There are more than 200 serovars existed with variety of animal species that can act as carrier for the pathogen making control of leptospirosis infection a challenge. Each measure taken at reduction of infection source needs to be

tailored based on local condition (Hartskeerl *et al.*, 2011). It is important to establish knowledge regarding animal species that become the reservoir of leptospiral and type of circulating serovars at local setting. Control measures can then be targeted to the specific animals. These measures include the reduction of animal reservoir populations, separation of animal reservoirs from human habitations, animal immunization and keeping environment clean to avoid rodents' infestation (John, 2005; WHO, 2003; Zavitsanou and Babatsikou, 2008).

Leptospirosis infection can also be prevented by interrupting route of transmission of the disease. This can be achieved if the person at risk is aware of the risk factors for human infection and, if possible, the infection source. Risk of infection can be minimized by avoiding contaminated environment, using protective equipment or clothing and covering wound with waterproof dressing when exposure is likely such as during occupational and recreational exposure. It is important for people who involved in high risk occupations, travel or hobbies to gain knowledge regarding the disease especially on preventive measures. Awareness and education to these high risk groups and community in general can help prevent leptospirosis infection in humans (Rao *et al.*, 2003). Knowledge on ecological, epidemiological, risk factors and cultural characteristics of local community is essential in developing effective and acceptable intervention strategy (WHO, 2003).

1.2 Rationale of Study

Leptospirosis is an important zoonotic disease and has become a public health concern in Malaysia. The infection is known to be associated with occupations that predisposed to contaminated water and present of carrier animals especially rodents. Human activities at wet markets provide suitable environment for survival of leptospiral and provide source of food favouring presence of rodents. These were supported by information on soil, water and rodents' samples at wet market sites that showed present of pathogenic leptospiral in the surrounding. However, there is a paucity of information regarding leptospirosis infection among wet market workers in term of seroprevalence and the distribution of leptospiral serovars in the environment. These study findings will provide baseline epidemiological data on leptospirosis at wet market areas which is important for prevention and control planning of the disease.

The occurrence of leptospirosis is closely related to the transmission of infection from animals' carriers to human host. The link of transmission is associated with many factors that increase the probability of being infected. Many studies had been carried out to investigate the determinants for leptospirosis in other occupational risk groups, however the information on associated factors specific to wet market workers is still scarce. Understanding of these factors within the context of wet market workers will provide a guide for health authority to plan measures of prevention and control of the disease.

Figure on leptospirosis cases in Malaysia are increasing over the years and many measures to prevent and control leptospirosis have been taken. In 2016, the Project 3 LRGS Leptospirosis had developed a new health promotion module on leptospirosis

which targeted the public especially high-risk groups. This module was developed with the objective to educate public especially risk groups regarding leptospirosis so that they can increase their knowledge and take appropriate measures to prevent and control leptospirosis infection. Therefore, we hope that this study will provide baseline epidemiological data regarding risk and determinants of leptospirosis among wet market workers and provide information on effectiveness of the newly developed Leptospirosis Health Intervention Module.

1.3 Research Questions

1. What is the seroprevalence of leptospirosis among wet market workers in Kelantan?
2. What are the factors associated with seropositivity of leptospirosis among wet market workers in Kelantan?
3. Is the 'Leptospirosis Health Intervention Module' effective in improving knowledge, attitude, belief and practice on leptospirosis among wet market workers in Kelantan?

1.4 Research Objectives

1.4.1 General Objective

To study the seroprevalence, factors associated with leptospirosis seropositivity and to evaluate the effect of Leptospirosis Health Intervention Module on knowledge, attitude, belief and practice among wet market workers in Kelantan.

1.4.2 Specific Objectives

1. To determine the seroprevalence of leptospirosis among wet market workers in Kelantan.
2. To determine the factors associated with seropositivity of leptospirosis among wet market workers in Kelantan.
3. To study the effect of Leptospirosis Health Intervention Module by comparing the mean score changes of knowledge, attitude, belief and practice on leptospirosis at baseline and six weeks post intervention between intervention and control groups.

1.5 Hypothesis Statements

1. There are associations between socio-demographic, work-related and recreational activities factors with seropositivity of leptospirosis among wet market workers in Kelantan.
2. There are significant mean difference of knowledge, attitude, belief and practice scores changes between control and intervention group at baseline and six weeks post intervention among wet market workers in Kelantan.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Leptospirosis

Leptospirosis is a widespread zoonotic disease caused by spiral-shaped bacteria of genus *Leptospira*. Before the advent of modern medicine, leptospirosis was described by various names such as “rice field jaundice” in China, “autumn fever” or “seven-day fever” in Japan. In Europe and other places, the febrile illness was related with occupations, giving rise to terms such as “cane-cutter’s disease”, “swine-herd’s disease”, and “mud fever” (Adler, 2015). In 1886, Adolph Weil described the condition which came with jaundice, rash, splenomegaly, renal dysfunction and conjunctivitis. Later it was known as Weil’s disease. At that time, the source of infection was unknown, but the disease was noted to be associated with outdoor activities in which the person had contact with water (Adler, 2015; Levett, 2001).

The leptospiral was first isolated in Japan in early 1900s. Inada *et al.* (1916) injected blood of Weil’s disease patients into guinea-pigs and successfully reproducing the disease in the animals. At that time, Weil’s disease was commonly described in coal miners in Japan. The Japanese researchers also described the pathological changes in diseased animals, tissue distribution, characteristics of the pathogens, mode of transmission, urinary excretion and immune response to the disease. At almost the same time, two groups of researchers in Europe succeeded in transmitting the infection to guinea-pigs and demonstrated the leptospiral in guinea-pig tissues named the organism Spirochaeta nodosa and Spirochaeta icterogenes respectively. Since then

leptospiral have been isolated from human and almost all mammalian species all over the world (Adler, 2015; Levett, 2001; WHO, 2003).

2.2 Aetiology of Leptospirosis:

Leptospiral has been identified as the aetiological agent for leptospirosis since early 1900s. It is a gram-negative bacteria with thin, helically coiled and slow growing aerobes. It measures from 5 to 25 micrometres in length and 0.1 to 0.3 micrometres in diameters (Adler and de la Pena Moctezuma, 2010; Rao *et al.*, 2003). The bacteria from genus *Leptospira* can be divided into two species; *L. interrogans* which triggers disease in human *and L. biflexa*, which do not cause harm to human. At present, there were more than 200 serovars recognized within the *L. interrogans* species (Adler and de la Pena Moctezuma, 2010; Levett, 2001). In Malaysia, 37 serovars of leptospiral have been identified from human and animal samples (El Jalii and Bahaman, 2004).

Leptospirosis is a zoonotic disease which means the bacteria are carried by animals before it is transmitted to human. The bacteria are widespread in the environment which reflect its many reservoir hosts. The main reservoirs for leptospiral in animals are *Icterohaemorrhagiae* infection in the brown rat, *Hardjo* in cattle and sheep and *Canicola* in pigs and dogs. Other carrier animals have limited geographical spread due to host distribution limitation and other unrecognized factors (Ellis, 2015; Victoriano *et al.*, 2009). However, isolation of leptospiral have been documented in many wild and domestic mammals since its discovery a decade ago. The maintenance of infection in animals' population depend on factors such as population density and environmental conditions. A study of brown rat in New Zealand showed maintenance of *Ballum*

infection in high density population found in rubbish dumps (Ellis, 2015; Hathaway and Blackmore, 1981).

The life cycle of leptospiral include shedding in the urine, survival in the environment, acquiring new host and dissemination to kidney of animal host. Once the bacteria in the renal system, it can be excreted through urine for long period of time without causing disease in the animal. In the environment, survival of leptospiral depends on several factors. Conditions favourable for the survival of the bacteria include humid environment such as rivers, flood, ponds and stagnant water where it can survive for weeks (Adler and de la Pena Moctezuma, 2010; Victoriano *et al.*, 2009). Leptospirosis is mainly a zoonotic disease and humans served as accidental hosts when in contact with infected animal urine. Majority of human infections occur in warm and humid climates. Poor sanitation, rodent infestation and poor domestic animal management systems lead to environmental contamination (Haake and Levett, 2015).

2.3 Transmission of Leptospirosis

Leptospires can enter human body through skin cuts and abrasions or mucous membrane found in conjunctival and oral cavity. The infection can occur when there is direct or indirect contact with infected animals' urine. Certain professions such as veterinarians, agricultural workers, animal shelter workers, army personnel and laboratory technologists have increased risk of exposure to infected animals and contaminated environments. Some outdoors recreational activities such as fishing, swimming, kayaking, rafting and canoeing are shown to have association with leptospirosis. Leptospirosis incidents related to these activities are increasing due to popularity of the sports over the years (Lau *et al.*, 2010). The risk of infection also

depends on local distribution of leptospires in environment and the degree of exposure (Monahan *et al.*, 2009). These infections can be avoided by using suitable personal protective equipment that meet the needs of the activities such as protective clothing, masks and boots while covering cuts and abrasion. They should also be educate regarding seeking medical attention if indicated (Lau *et al.*, 2010; Steneroden *et al.*, 2011).

2.4 Clinical Features and Treatment

The period between exposure and appearance of symptoms in leptospirosis is between two to 20 days. The presentation of the disease in human is variable and can mimic other febrile illnesses. Patient can present with symptoms and signs similar to other infectious diseases such as influenza, dengue, malaria and typhoid (WHO, 2003). Typical symptoms include sudden onset of fever with chills and headache. Generally, the headache is severe accompanied by photophobia and retro-orbital pain. Patient commonly complaint of myalgia which involved calves and lower back. Conjunctival suffusion is frequent in leptospirosis but uncommon with other febrile illness. Other unspecific symptoms and signs include cough, nausea, vomiting, abdominal pain, jaundice and rash can also present with the disease (Haake and Levett, 2015). Overlapping of presentation with other infectious diseases make diagnosis of leptospirosis a challenge for medical practitioner.

Several laboratory investigations abnormality can be associated with leptospirosis infection. Patients may have leucocytosis and thrombocytopenia. They can also have reduction in white blood cells, red blood cells and platelets counts which suggest bone marrow suppression. Severe infection can also cause multiple organs dysfunctions

which include the brain, lungs, liver and kidneys. One of the most recognizable forms of leptospirosis is Weil's disease which consists of combination of jaundice and renal dysfunction. Patients are typically noted to have elevations of liver transaminases and direct bilirubin. Kidneys involvement are characterized by elevations of serum urea nitrogen and creatinine levels. Meanwhile, urinalysis showed present of pyuria, haematuria, and elevated urine protein levels. In cases where brain and lung are involve, abnormality of cerebrospinal fluid examination and chest radiograph may be found (Haake and Levett, 2015).

To assist management of the disease, four broad clinical categories have been described; mild influenza like illness, Weil's syndrome, meningitis, meningoencephalitis, and pulmonary haemorrhage with pulmonary failure. The severity of human leptospirosis ranges from mild, self-limited disease to life threatening condition depending on involvement of multiple organ systems. Health status of the patient and virulence of the infecting serovars also contribute to severity of the disease (Haake and Levett, 2015; WHO, 2003). High index of suspicion is key for early detection of leptospirosis which is essential to avoid complication and for better disease outcome.

2.5 Common Laboratory Diagnosis of Leptospirosis

Laboratory investigations are important to confirm leptospirosis infection as the presentation of the disease can overlapped with many other febrile illnesses. Laboratory investigations are also important to determine the type of serovars, source of infection and animals' reservoir to help in control strategies. Diagnosis of leptospirosis can be accomplished by detection of the pathogens or its components in

body samples, isolation of the organism in cultures or detection of antibodies (Hartskeerl *et al.*, 2011; Schreier *et al.*, 2013). During initial phase of the infection, leptospires appear in blood and later invade other tissues and organs.

The body immune system reacts by producing specific antibodies against the leptospires. This occurs about five to seven days after onset of the infection. Initially IgM antibodies appear earlier than IgG antibodies which then persist for months to years in the body. The antibodies produce by the body are directed at common antigens which are shared by different leptospires serovars or serovars-specific and serogroup-specific antigens. The common antigens antibodies can react to different leptospires serovars which produce cross-reaction phenomenon, usually seen at initial stage of the infection. Cross-reaction phenomenon can also be seen with other microorganism groups which vary between types of serological methods (WHO, 2003).

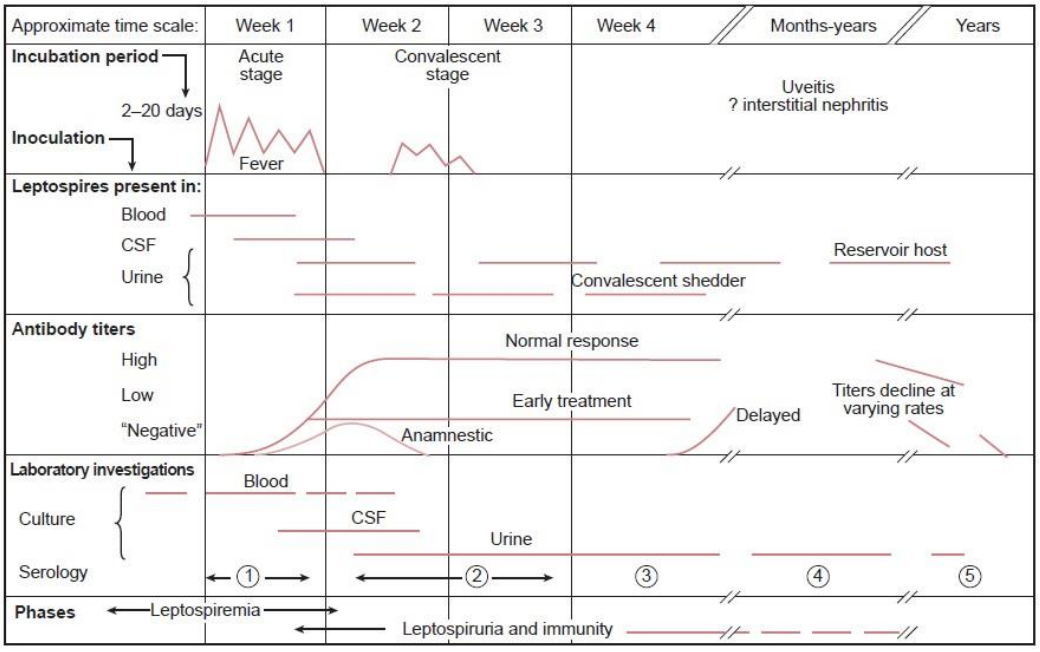


Figure 2.1 Natural history and laboratory investigations of leptospirosis (adapted from Levett, Clinical Microbiology Review, vol.14, no.2, 2001, p.303)

2.5.1 Culture

Leptospires can be isolated and cultured from patient samples during acute phase and immune phase of the infection. Leptospires can be cultured from blood, CSF, peritoneal dialysate and urine samples. The bacteria present early in blood, CSF and peritoneal dialysate. Samples should be taken before the administration of antibiotics as it can affect the recovery of the bacteria. Leptospires grows slowly, doubling time at six to eight hours in culture medium at optimal temperature of 28C to 30C. The growth can be detected after a week up to 4 months. Low sensitivity to leptospiral with the culture technique was reported in studies (Fornazari *et al.*, 2012; Wagenaar *et al.*, 2000). Therefore, culture does not aid in management of early phase infection and yield lower positive results compare to other methods. However positive culture results is definite proof of infection (Cameron, 2015; Haake and Levett, 2015; WHO, 2003).

2.5.2 Serological Methods

Serological methods are laboratory tests which detect the presence of specific anti-leptospiral antibodies in the serum. Since antibodies can remain for months to years after infection subside, positive serological test alone does not confirm a current infection state. Seroconversion or rise in titre in repeated serum samples are required for diagnosis of recent or current infection (WHO, 2003). There are different serological tests available that can be divided into two types; genus-specific and serogroup specific tests. Most leptospirosis cases are diagnose by serological test due to limitation of capacity for culture and molecular methods (Adler and de la Pena Moctezuma, 2010; Haake and Levett, 2015).

2.5.2.1 Serogroup Specific Tests

Microscopic Agglutination Test (MAT)

MAT is the "gold standard" for serodiagnosis of leptospirosis due to its high specificity compare to other tests. MAT is a serological test which used live cultures of leptospires serovars as antigens to react with patient's serum. The live cultures must be representative of entire serogroups and local serovars. The reaction between antibodies in samples and antigens are examined under dark field microscopy to determine agglutination and titres. Skilled personnel are required to perform and interpret MAT. Cross-reaction between serogroups can occur, especially in early phase samples. Positive MAT results are established by observation of 50 % agglutination of live leptospires compare to control suspension. Rising titres in repeated samples are required to confirm diagnosis of acute infection. As for sero-surveys in epidemiological study, a titre of ≥100 is sufficient to conclude as past exposure (WHO, 2003).

Rapid Screening test

Rapid screening tests are developed to assist management of leptospirosis in acute phase. The rapid screening tests detect IgM class antibodies that appear in the first week of infection, thus allowing treatment to be initiated early. Unlike MAT and culture, rapid screening tests are not confirmatory test. However, these tests are helpful in diagnosing leptospirosis in early phase while awaiting result of confirmatory test. Rapid screening tests are also useful due to cheaper cost, less complicated procedures and consumed less time to be performed.

2.5.2.2 Genus-specific tests

Enzyme-linked immunosorbent assay (ELISA)

Another common serological test for leptospirosis is enzyme-linked immunosorbent assay (ELISA). ELISA is a genus-specific tests and not suitable to identify serogroup or serovars of the leptospires. It can detect leptospirosis earlier compare to the MAT because it targeted the IgM class antibodies. It is also sensitive and specific for leptospirosis detection and can be used to determine seroprevalence for epidemiological study. However the test may be negative for certain serogroups such as serogroup Grippotyphosa and serogroup Australis (WHO, 2003).

2.5.3 Molecular Diagnosis

In the early phase of the disease, serological tests have less sensitivity due to delay development of antibodies against the pathogens. The molecular methods which can directly detect leptospires at molecular levels are more useful to diagnose leptospirosis in early phase (Bharti *et al.*, 2003). A common molecular method used for confirming leptospirosis is polymerase chain reaction (PCR). PCR is a method of diagnosing leptospirosis by detecting and identifying segments of the bacteria DNA. The specific DNA are amplified from clinical samples such as blood and urine to detectable levels. However, there are disadvantages of PCR such as it requires sophisticated expensive equipment, specific laboratory space, and skilled personnel. Thus limiting it used in management of leptospirosis patient (Haake and Levett, 2015; WHO, 2003).

2.6 Epidemiology of Leptospirosis

Leptospirosis is the most widespread zoonotic disease worldwide. Its infection depends on interaction of three major factors which are epidemiology, host and

pathogen. Sanitation, rainfall, flood and housing are epidemiological factors that contribute to leptospirosis occurrence. Human occupations, recreational activities and travels are epidemiological factors related to the hosts. Meanwhile, specific host aspects are age, immune status, comorbidities, and skin integrity. Leptospires differ in term of virulence, animal carriers and survival in the environment which influence the degree of exposure, ability to cause disease and severity of disease in human. The interaction between these factors determine the distribution and burden of leptospirosis in humans population (Haake and Levett, 2015).

Globally, leptospirosis occurs in diverse geographical settings due to large spectrum of animals' host that can carry the pathogen in their renal tubules. However, the incidence of leptospirosis is higher in humid and warm countries where survival of leptospires in environment are favourable. Resource poor region face similar challenge where sanitation and overcrowding are a problem. It is estimated that the number of leptospirosis exceed one million cases every year around the globe. The number of death due to leptospirosis is estimated at 58900 cases each year. The estimated incidence of leptospirosis was 14.77 cases per 100000 population and the mortality incidence due to leptospirosis was estimated at 0.84 deaths per 100000 population worldwide. Geographically the incidence of leptospirosis is high in Oceania, South-East Asia, Caribbean, and East Sub-Saharan Africa (Costa *et al.*, 2015; Lozano *et al.*, 2012).

The figures on leptospirosis cases is underestimated as leptospirosis was not listed as notifiable disease in many countries in previous years (Pappas *et al.*, 2008). However, as the disease became more significant in term of numbers and clinical consequences,

the awareness to monitor the disease trend and burden increased. Many countries have listed the disease as a notifiable disease, including Malaysia (Ministry of Health, 2011). WHO had reported the leptospirosis disease burden by region through the Leptospirosis Burden Epidemiology Reference Group (LERG) meeting report. Based on geographical region, the highest annual median incidence was recorded in Africa region (95.5 cases per 100 000 population). This is followed by Western Pacific region and Americas region with 66.4 cases per 100 000 population and 12.5 cases per 100 000 population respectively. South-East Asia and Europe recorded median annual incidence of 4.8 cases per 100 000 population and 0.5 cases per 100 000 population respectively (WHO, 2013).

Victoriano *et al.* (2009) categorised countries in Asia-Pacific region into three groups based on the incidence of leptospirosis; low incidence (<1 cases per 100 000 population), moderate incidence (1 to 10 cases per 100 100 population) and high incidence countries (>10 cases per 100 000 population). The low incidence countries include Australia, Hong Kong, Japan, South Korea and Taiwan. Malaysia was recorded as moderate incidence countries together with American Samoa, China, India, Indonesia, New Zealand, Palau, Philippines and Mongolia. Bangladesh, Cambodia, Fiji, India, Laos, Nepal, Sri Lanka and Vietnam were classified as high incidence countries (Victoriano *et al.*, 2009). Many of the high and moderate incidence countries are tropical countries with agriculture as a major economic sector.

Leptospirosis is endemic in many tropical countries including Malaysia. With the rising number of incidences and outbreaks of the disease, leptospirosis gain growing attention from health authority (Benacer *et al.*, 2016b). The current information on

leptospirosis in Malaysia showed that the incidence trend of the disease is increasing by the year. From 2004 to 2012, there were 12,325 cases of leptospirosis reported in Malaysia. The cases showed increasing trend over the years with the highest figure was recorded in 2012. The annual incidence of leptospirosis in 2004 was 0.97 cases per 100 000 population and increased to 12.47 cases per 100 000 population in 2012. Based on geographical distribution, the states with highest incidences of leptospirosis were Selangor, Pahang, Kelantan and Perak (Benacer *et al.*, 2016b).

Cases of leptospirosis in Malaysia were contributed by the humid and warm climates which has become suitable habitats for carrier animals and survival of the bacteria in the environment. The variety of animal reservoirs for leptospiral which include wild life, domestic animals, pets and rodents play an important role in continued present of the bacteria in the human surrounding. Other major factors which contribute to the transmission of the disease in the population include occupational activities, natural disaster and recreational exposure. Occupational exposures had been studied which demonstrated high risk groups in Malaysia such as town service workers, agricultural workers and market workers (Ridzuan *et al.*, 2016b; Samsudin *et al.*, 2015; Suhailah *et al.*, 2018). The nature of work in these groups expose the workers to leptospiral in the environment. Natural disaster such as flood lead to increase of leptospirosis cases in Malaysia during the monsoon season. This is due to population displacement, poor sanitation, disruption of clean water supply and migration of wild life and rodents' population during the period.

2.7 Risk Groups

There are groups of humans that have more probability to get infected by leptospiral as a result of their occupation or recreational exposures. As leptospirosis exposure differs between areas depending on local distribution of animal carriers, transmission and human behaviours, human risk groups are different between locations. Many studies had been conducted among these risk groups to determine the risk and determinants of leptospirosis in their setting. Even though the population disease incidence is low, the burden of disease can be higher in certain risk groups. As leptospirosis infection attribute by many factors, changes in animals' distribution and human activities may result in new risk groups. Continuous surveillance is important to monitor the disease and planning of control and prevention program (WHO, 2003).

Among the occupational risk groups are cattle farmers, agricultural workers, veterinarians, abattoir workers, sewer workers and town service workers. These occupations involved tasks which expose workers to possible animals' carrier or contaminated environment. Rafizah *et al.* (2013) conducted a study on febrile cases of 10 hospitals in North-eastern Malaysia. From 999 subjects, 84 were found to be leptospirosis positive by MAT (titre cut off point 1≥400) which gave the seroprevalence of 8.4%. Majority of the positive results were high risk occupational group with agricultural workers recorded the highest number of cases. Eight leptospiral serovars were identified in the study and the predominant serovars was Sejroe (Rafizah *et al.*, 2013).

A study among high risk population was conducted in Andaman Islands, India. Participants were from high risk occupational groups including sewage workers, forest

department workers, agricultural workers, butchers, animal handlers and white-collar workers as control group. Total of 611 blood samples from participants were analysed by MAT using titre of 1≥50 as positive result. The researchers found that overall leptospirosis seroprevalence level of 52.7% among high risk group compared to 14.7% among control population. Based on type of occupations, agricultural workers had the highest seroprevalence (62.5%) followed by sewage workers (39.4%), animal handlers (37.5%), butchers (30.0%) and forestry workers (27.3%). The commonest serovars identified in the study were Grippotyphosa and Australis (Sharma *et al.*, 2006).

Dreyfus *et al.* (2014) had conducted a study on seroprevalence of leptospirosis among abattoir workers in New Zealand. The study was carried out in 2009 involving 567 workers from 8 abattoirs. The samples were tested using MAT and cut-off point titre of 1≥48 was considered positive. The findings of the study noted leptospirosis seroprevalence level were different between workers of sheep abattoirs, deer abattoirs and beef abattoirs. The highest seroprevalence was noted among sheep abattoir workers (10.0%-31.0%), followed by deer abattoir workers (17.0%-19.0%) and beef abattoir workers (5.0%). Based on the interview with participants, the researchers postulated that the high seroprevalence at sheep abattoirs were due to more animal processing at the plants and sheep were observed to urinate spontaneously once stunned which were rarely seen in beef (Dreyfus *et al.*, 2014).

In a study conducted by (Shafei *et al.*, 2012) among town service workers in Kelantan in 2008 demonstrated the seroprevalence of 24.7%. The study was conducted among 296 workers of Kota Bharu Municipal Workers comprising of garbage collectors, town cleaners, landscapers and lorry drivers. MAT titre of 1≥100 was used for seropositive

results. Based on jobs categories, garbage collectors and town cleaners had the highest proportion for positive leptospiral antibodies with 27.4% and 26.0% respectively. The respondents were positives to 12 different serovars with predominant Patoc and Bataviae serovars (Shafei *et al.*, 2012).

Samsudin *et al.* (2015) reported a seroprevalence of 34.8% of antibodies against leptospiral among municipal workers in Selangor. The study was conducted among 89 workers from Ampang Jaya Municipal Council (MPAJ) in 2012 using MAT titre cut-off point of 1≥50 for positive results. Different job categories were found to have different seroprevalence level; garbage collectors (41.5%), town cleaners (33.3%), public workers and public health workers (0%) which indicated different degree of exposure to contaminated environment. The study also found that the respondents were positive to 10 different leptospiral serovars with Sarawak and Bataviae were the predominant serovars (Samsudin *et al.*, 2015).

In 2014, Ridzuan *et al.* (2016b) conducted a seroprevalence investigation among oil palm plantation workers in 10 plantations in Melaka and Johor. The workers were categorized based on their task which include fruit collectors, harvesters, pruners, pesticide applicators, fertilizer applicators, drivers and nursery workers. The researchers found that seroprevalence level of 28.6% among the workers. The highest seroprevalence based on job categories were fruit collectors (59.2%), harvesters (24.5%) and pesticide applicators (24.5%). The samples analysis showed positive results with nine different leptospiral serovars. The predominant serovars found in the study were serovars Sarawak and Patoc (Ridzuan *et al.*, 2016b).

A seroprevalence study on cattle farmers in Kelantan was conducted by Hafiz *et al.* (2017). The study was conducted in 2015 involving six districts. MAT analysis with titre of 1≥100 was considered as positive results. A total of 120 cattle farmers involved in the study which yield overall leptospirosis seroprevalence of 72.5%. The samples were positive to 15 different leptospiral serovars with Sarawak and Patoc were the predominant serovars. Cattle farmers were high risk groups due to daily exposure to polluted environment and direct contact with animals which can be a carrier for leptospiral bacteria (Hafiz *et al.*, 2017).

To date, only one recent local study regarding leptospirosis among market workers was published. In 2016, Suhailah *et al.* (2018) conducted a cross sectional study on four wet markets and 20 food premises in Selangor. The respondents in the study were consisted of 111 food handlers and 120 wet market workers. The researchers found that the overall leptospirosis seroprevalence among the workers was 46.3%. The seroprevalence among wet market workers were 43.3% while food handlers had 49.5% seroprevalence level. The samples in the study were reactive to six different serovars with predominant Sarawak and Patoc serovars. The researchers suggested that work place rats' infestation as a possible explanation for high seroprevalence results among the workers in the study (Suhailah *et al.*, 2018).

2.8 Factor Associated with Leptospirosis

Risk factors are any attributes, characteristics or exposures that increases the possibility of developing a disease or acquiring an infection or injury in an individual. Figure 2.2 shows the factors contributing to leptospirosis infection. Risk factors of leptospirosis are closely related to three major determinants which are epidemiologic

factors, host factors and the pathogen factors and their interactions. Example of epidemiologic factors are sanitation condition, housing, climate and natural disaster. Leptospirosis infection is also related to income, occupation, recreational activities and behaviours which is related to the human host. The intrinsic factors linked to human are age, genetic factors, skin integrity and immune system. These factors interact with leptospiral which determine the type of exposure and route of transmission of the disease. The characteristic of the leptospiral infection will determine the severity of the disease in human which reflects their virulence, pathogenicity, motility and level of exposure. The reservoir host will determine the present and distribution of the pathogen in epidemiological setting (Haake and Levett, 2015).

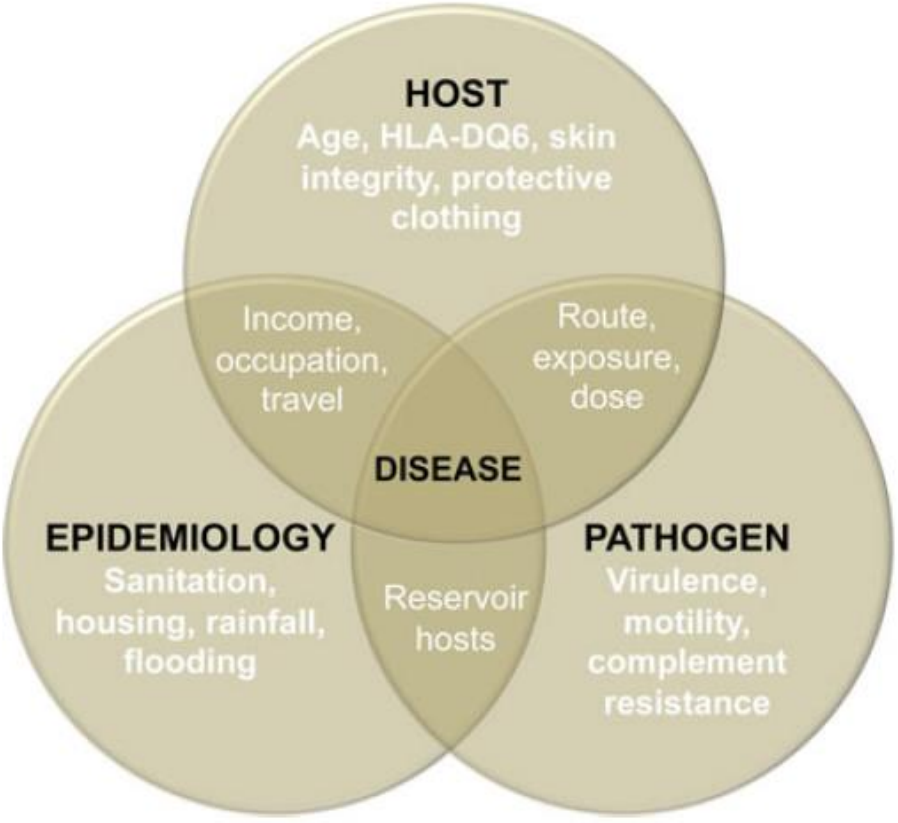


Figure 2.2 Factors contributing to leptospirosis

(adapted from Leptospira and Leptospirosis, Current Topics in Microbiology and Immunology, p.67)

2.8.1 Sociodemographic Factors

Increasing in age was found to be associated with leptospirosis. Dreyfus *et al.* (2014) found that age was related to leptospirosis among abattoir workers in New Zealand. Aging by one year increased the odds of being seropositive by 9% of the baseline prevalence among the workers. The researchers postulated that the effect of aging might be explained by reduction in body immune function which predisposed human to infection.

Another study on seroprevalence and associated factors for leptospirosis in Colombia noted similar effect of age on leptospirosis seropositivity. The study was conducted in 2013 where 353 participants were randomly selected from urban community of Cali district. The study found that an increased in one year of age increased the odds to be leptospirosis seropositive by 1.03. Other sociodemographic factors associated with leptospirosis were female, single status and low-income group. Among the postulated reason for these findings were present of domestic exposure, single person pone to involve in risk activities and low economic group reflect the living conditions such as open sewers, accumulation of garbage and unpaved roads. These factors lead to increase population of rodents and stray animals in the surrounding (Escandon-Vargas *et al.*, 2017).

This finding was in contrast with the result of study by Azfar *et al.* (2014). The study among town service workers in Kelantan documented a protective factor with aging against leptospirosis infection. The researchers suggested that with increasing age, a person awareness and protective practices regarding leptospirosis increase as he or she learned about the disease from other sources. Younger age was related with risk

behaviours such as involvement with outdoor sport activities thus increasing their risk (Azfar *et al.*, 2014).

Beside age, gender is a significant sociodemographic factor associated with leptospirosis seropositivity. Many previous studied on high risk occupational groups focused on outdoor, manual labour jobs which are dominated by male gender. Men are also prone to engage in risky outdoor activities. These predispose them to get infected by leptospirosis compare to women. This pattern explains the finding of male gender associated with leptospirosis in previous studies (Costa *et al.*, 2015; Felzemburgh *et al.*, 2014; Garba *et al.*, 2017b; Lau *et al.*, 2016).

Leptospirosis cases were also related to living condition which reflect the socioeconomic status of a person. The infectious disease was associated with poverty where the living condition is unhygienic. The cases of leptospirosis are reported high in slum area due to lack of basic sanitation, improper garbage disposal system and present of carrier animals (Costa *et al.*, 2017; Maciel *et al.*, 2008). These determinants were indirectly indicated by monthly income of the family. Many studies had reported the relationship between socioeconomic status and leptospirosis (Escandon-Vargas *et al.*, 2017; Haake and Levett, 2015). Escandon-Vargas *et al.* (2017) documented that low socioeconomic class had significant association with leptospirosis seropositivity in a study conducted in Cali districts, Colombia. The researchers noted that low socioeconomic status was related to poor living condition with attracted stray animals. These predisposed the population to contaminated environment. Another sociodemographic factor associated with leptospirosis is educational level. Higher educational level is related to better awareness regarding disease and health literacy.

Furthermore, they are better at hygiene practice and preventive measure that put them at lower risk for leptospirosis infection (Dias *et al.*, 2007; Johnson *et al.*, 2004).

2.8.2 Work Related Factors

Leptospirosis has been associated with occupations which are related to animals and exposure to outdoor environment such as agricultural workers, animal handlers, army personnel, town service workers and veterinarians (Haake and Levett, 2015; WHO, 2003). There are many determinants for leptospirosis exposure at workplace which are unique depending on type of work, condition at workplace and present of animal carriers.

Work position and job task were found to be risk factors for leptospirosis. These have to do with the degree of exposure to contaminated environment and risk to get injury during the work procedure. Dreyfus *et al.* (2014) reported work position as a risk factors for leptospirosis among abattoir workers especially in sheep and deer abattoirs. Workers at the start of the slaughter board had the highest prevalence for leptospirosis and the risk reduce along the slaughter line. This was thought due to splashing of urine when the animal was stunned. The workers were also exposed when working with the carcasses and organs of the genital-urinary tract of the animals which were contaminated with leptospiral (Dreyfus *et al.*, 2014). The researchers also noted a different in seroprevalence between sheep abattoir, deer abattoir and beef abattoir workers. Sheep and deer abattoirs processed more animals than beef abattoirs which might explained the difference. This finding was supported by a local study by Shafei *et al.* (2012). The seroprevalence study on town service workers in Kelantan revealed that job category with the highest seroprevalence was garbage collectors, followed by

town cleaners, lorry drivers and landscapers. Garbage collectors had prolonged exposure and contact with environment that might be contaminated with urine of infected animal.

Ridzuan *et al.* (2016a) conducted a study to examine the work-related factors associated with leptospirosis among plantation workers. The findings of the study documented that not using rubber glove, working with present of hand wound and did not practice hand washing with soap after work were associated with leptospirosis seropositivity. The fact that plantation workers used hand to conduct their labour work explained the increase in risk when not using glove. The workers had direct contact with polluted environment which exposed them to leptospiral (Ridzuan *et al.*, 2016a). Similar finding was noted in a leptospirosis outbreak in Australia following a flood event where nine person was reported to contract the disease. Smith *et al.* (2013) found that all confirmed cases had direct exposure to flood. The cases also reported of non-compliant to glove and enclosed footwear during wading through flood water. Furthermore, the cases had cuts and scratches at the time of exposure. Although concrete evidence was not available, the researchers presumed that infection occurred during wading the flood water and cleaning up after the disaster (Smith *et al.*, 2013). The association between present of wounds and cuts with leptospirosis were also reported in study among farm workers and kennel workers. Desai *et al.* (2009) documented risk of leptospirosis increased when strawberry farm workers worked in the presence of hand wounds. Meanwhile, a case control study conducted among kennel workers in Nigeria reported a significant association between presence of wounds and contracting leptospirosis (Awosanya *et al.*, 2013).

A cross sectional study conducted in Thailand among pond cleaning workers following an outbreak in 1999. During the outbreak, 80 cases of leptospirosis were reported after pond cleaning activities which include clearing up the water, pulling up foliage, and removing debris. Total of 43 samples were tested positive for IgM antibodies against leptospiral during the outbreak where 500 people participated in the event. It was reported that wearing trousers was a protective factor against leptospirosis infection. However, having more than two wounds on the body were associated with the infection (Phraisuwan *et al.*, 2002). While using protective clothing can help prevent leptospirosis infection at workplace, certain unhealthy work habit lead to increase of risk to contract the infection. Campagnolo *et al.* (2000) investigated an outbreak of leptospirosis in Missouri in 1998 after a slaughter facility worker was diagnosed with leptospirosis. During the investigation, nine out of 163 people tested were positive to leptospirosis. Study on risk factors noted washing hands during work was found to have protective effect against infection. However, smoking cigarette and drinking beverage while working with infected pigs were found to be risk factors for leptospirosis. These evidences suggest towards oral cavity as route of entry for the bacteria (Campagnolo *et al.*, 2000). This finding was supported by a study in Kenya among slaughterhouse workers. The researchers examined the determinant for leptospirosis infection among the workers. They documented that eating at slaughterhouse and smoking increased risk for leptospirosis seropositivity. The researchers suggested that the behaviours increases the possibility of transmitting leptospiral from contaminated hands to the oral cavity (Cook *et al.*, 2016).

2.8.3 Recreational Factors

Currently, there is a rising trend of leptospirosis cases related to outdoor recreational activities. The ability of leptospiral to survive in moist environment make recreational activities involving water body a risk (Wynwood *et al.*, 2014). As the incubation period for leptospirosis can be as long as 3 weeks (Ministry of Health, 2011), the relationship between water exposure and the disease can be unclear and lead to delay diagnosis. Thus, it is important for medical practitioner to have detail history of exposure as leptospirosis can be mistaken for other febrile illness.

A systematic review regarding determinants of leptospirosis documented that water activities such as swimming and fishing were related to cases of leptospirosis. The study revealed that 17 investigations regarding swimming activities showed an increased risk for leptospirosis infection with odds ratios ranging from 1.5 to 87.0. However, the researchers noted two studies with high odds ratio from the review had lack of external validity. One study was a case control study of an outbreak investigation (OR 87.0) and the other study (OR 27.0) used a small sample (Mwachui *et al.*, 2015).

Morgan *et al.* (2002) reported a leptospirosis outbreak investigation among athletes and residents of Springfield, Illinois following a triathlon sport event in 1998. The event involved competitive running, cycling and swimming activities. A total of 876 athletes participated in the sport activity. Out of 474 athletes and 248 symptomatic residents tested, 52 athletes and 14 residents were tested positive for leptospirosis. Hospital admission was reported for 21 cases. Majority of ill cases reported of fever,

headache and muscle pain. Swallowing lake water during the event was found to be associated with the leptospirosis infection in the outbreak (Morgan *et al.*, 2002).

A leptospirosis outbreak was reported following Sabah Eco-Challenge in 2000. The sport event involved 304 athletes from 26 countries which included water and jungle activities such as jungle trekking, swimming, kayaking, spelunking, climbing and biking. Infected athlete developed high grade fever, chills, headache, muscle pain and diarrhoea. Out of 80 athletes who met the clinical definition, 29 admissions to hospital were reported. No severe complication or death was documented following the outbreak. Epidemiological investigation suggested the cases were related to swimming activity during the event. The researchers also suggested that cuts and abrasions from the vigorous activities and prior heavy rainfall contributed to high attack rate of the outbreak (Sejvar *et al.*, 2003).

Several case reports of leptospirosis documented the risk of water recreational activities. (Teichmann *et al.*, 2001). A 38 years old diver presented with fever associated with chills, muscle pain, joint pain and headache and admitted on the fourth day of the disease. The patient had previously participated in an iron man event in Philippines which consisted of 40 kilometres run through rainforest and 7 kilometres canoeing. MAT result was reactive to serovars Autumnalis, Bataviae, Hardjoe and Australis. The patient developed renal impairment during hospitalization which require hemofiltration. His condition improved after antibiotic treatment and was discharged after 12 days of admission (Teichmann *et al.*, 2001). Abb (2002) documented a leptospirosis case in a 38 years old man following history of repeated swimming in a river. The patient was preparing for a triathlon event when he developed

fever, headache and muscle pain. Blood investigations demonstrated renal and hepatic impairment. Serial blood investigations confirmed the diagnosis of leptospirosis and he was well after administration of antibiotic (Abb, 2002).

Although leptospirosis cases are observed more due to occupational exposure, outbreak cases after recreational activities are on the rise due to popularity of the sport activities and growing of international travel. Advise on preventive behaviours and awareness regarding the symptoms of the disease for the athletes and public in general can assist in preventing leptospirosis and its complications. Prophylaxis antibiotic can be considered for those involved in risk activities to reduce the risk of infection. Health care provider must consider the probability of leptospirosis infection in symptomatic person with history of exposure to outdoor sport activities especially involving water activities (Monahan *et al.*, 2009; Pavli and Maltezou, 2008).

2.5 Control and Prevention of Leptospirosis

WHO has outlined strategies for control and prevention of leptospirosis. These measures for reducing cases of leptospirosis were complicated due to present of large number of leptospiral serovars and wide range of animals' carriers. Leptospiral has been isolated from almost all mammals from all over the world. It can infects wild and domestic animals including pets which make total prevention of exposure to human impossible (Ellis, 2015). Furthermore, the bacteria can survive in environment for weeks in warm and humid condition. However, risk of leptospirosis infection to human can be reduce using strategies focusing on source of infection, route of transmission and at the level of human host. These strategies are effective if planned and

implemented based on the knowledge of local epidemiological setting due to different environment characteristics from place to place (WHO, 2003; Wynwood *et al.*, 2014).

As leptospiral are carried by wide range of animals, measures to control the infection source need to be tailored according to local condition. The main animal reservoirs for human infection are rodents. Thus, reduction of leptospirosis cases can be achieved by reducing rodents' habitat at human surrounding. Rodents control can be accomplished by educating the public regarding the importance of reducing the animal carriers' population to control the disease. Hygienic practice at home and workplace, eliminating food source, good sanitation and tackling the determinants for rodents activity need to be strengthen to achieve the goal of source reduction (CDC, 2015; Ellis, 2015).

Animal vaccination is also an effective method in controlling leptospirosis in domestic animals and pets. Vaccination protects animals from infection by strengthening the immune system against leptospiral. The use of animal vaccination is limited by expense, quality, availability and type of antigens relevance to local epidemiological setting. Animal vaccination has been used mainly in dogs, cattle and pigs. Currently vaccines may contain one or more antigen serovars against leptospiral and local epidemiological information regarding circulating serovars are important for effective vaccination program (Ellis, 2015; WHO, 2003).

In certain conditions where vaccination is not available, other measures can be used to reduce human contact to animal carriers. Animal infected with leptospiral can be isolated and treated with antibiotic. This method can prevent further infection to other

animals and human. Beside treating the animals, fences and screens can be used to separate the animals from human living areas. Animal's excreta should be disposed properly to reduce environmental contamination which can introduce the bacteria into the surrounding. Effective measures depend on local setting as there is no general control practice suitable to all condition (WHO, 2003).

Beside focusing on source of infection, route of transmission need to be identified for planning of control measures. Thorough investigation on exposure history and risk activities may reveal the mechanism of infection and help tailoring suitable preventive measures. Using personal protective equipment during risk activities which exposed to polluted environment can reduce transmission of leptospiral into human body (Haake and Levett, 2015). For example Ridzuan *et al.* (2016a) reported oil palm plantation workers who did not use glove during work had an increased risk for leptospirosis infection compared to workers who used glove. Personal protective equipment such as boots, gloves and masks protect the eye, mucous membrane and wound from contaminated environment (Ridzuan *et al.*, 2016a).

Another important aspect in prevention of leptospiral transmission is wound care. Public should be educated to avoid risk activities and risk areas if one had cut or wound on the skin as it can become portal of entry for the bacteria. The skin wound should be covered with dressings if the activity is unavoidable. After the activity, the wound should be wash and clean. Other measures to reduce the transmission of disease include increasing awareness regarding potential risks and measures to minimize exposure, strict hygienic measures when handling animals and its products and adhere to standard safety procedures at workplace (Haake and Levett, 2015; WHO, 2003). In

country like Japan, modernization of agricultural sector by using machinery to substitute human workforce reduce the incidence of leptospirosis dramatically (Yanagihara *et al.*, 2007).

Apart from reducing infection source and interrupting transmission of disease, WHO recommended prevention measures focusing on human host. These include raising public awareness, using antibiotic prophylaxis and human immunization. Raising awareness of the disease in public and risk groups are important aspect of prevention and control of leptospirosis. Public not only need to know the disease and methods to reduce risk of infection, but also sought early medical attention if leptospirosis infection is suspected. This can be achieved through health education to the general population especially risk groups (WHO, 2003).

In 2014, Azfar *et al.* (2018) conducted an intervention study among town service workers in Kelantan. The study examined the effect of health educational module regarding leptospirosis on knowledge, attitude and practice of the workers. The module consisted of varies activities such as interview, mind mapping, role play, animation presentation, demonstration, hands on and games. It covered topics on cause, risk factors, signs and symptoms, complications, treatment, prevention and control of leptospirosis which were delivered in a two days program. The study found that the health educational intervention significantly improved the knowledge, attitude and practice of the workers in intervention group compared to control group.

The finding was supported by Bipin *et al.* (2010). The researchers conducted an intervention study involving illiterate community in Navsari district, India. The

intervention consisted of street play and poster exhibition on aetiology, transmission of disease, symptoms, control and preventive measures regarding leptospirosis. The interventions were delivered to the community in one month duration in local dialect. Post assessment of the program showed significant improvement in knowledge regarding leptospirosis among the villagers (Bipin *et al.*, 2010). These evidences showed that health education is effective in improving knowledge and promoting preventive practice among community and risk groups.

Another method of prevention for leptospirosis infection among risk groups is using prophylaxis antibiotic. Antibiotic prophylaxis can be considered by travellers to endemic areas, army personnel and rescue teams during disaster situation. For people planning on activities that expose them to contaminated environment, pre-exposure prophylaxis antibiotic which consist of weekly dose of Doxycycline 200mg can be considered. Post-exposure prophylaxis of daily Doxycycline for five to seven days can be recommended in case of leptospirosis outbreak. Azithromycin can be used in case of allergy to Doxycycline or in pregnant lady (Ministry of Health, 2011). Takafuji *et al.* (1984) conducted a randomized controlled trial among US army personnel who go through a three weeks jungle operation in Panama. The study found that the leptospirosis attack rate was significantly lower in intervention group compared to control group. In another study by Galloway *et al.* (2009) found that prophylaxis antibiotic reduce severe cases of disease and was cost effective in managing leptospirosis cases.

2.6 Conceptual Framework

Figure 2.3 illustrates the conceptual framework of this study. From previous literatures, wet market workers were considered risk group for leptospirosis infection due to exposure to rodents and contaminated environment. The conditions of their workplace are suitable for rodents to breed and populate. Warm and humid surrounding at market areas prolong the survival of leptospiral outside the animal carriers (Adler and de la Pena Moctezuma, 2010; Suhailah *et al.*, 2018).

Environment are contaminated with leptospiral when the rodents excreted the bacteria through their urine. Transmission of leptospiral to wet market workers can occur when there is direct or indirect contact with the urine of infected animals. The bacteria can enter the human body through mucous membrane and cuts on the skin. The risk of infection in wet market workers are considered high as they are expose to the bacteria during their working hours. Workplace determinants for leptospirosis infection include duration of employment, present of open garbage disposal and sighting of rats at workplace. The risk is higher if the workers do not practice protective behaviours such as using protective clothing, eat or drink during working and do not practice hand washing after work. Beside work-related factors, sociodemographic and recreational activities were documented to contribute to risk of infection (Haake and Levett, 2015; WHO, 2003). Thus, the focus of the present study includes risk and determinants of leptospirosis among wet market workers and the effect of intervention program to modifiable risk factors.

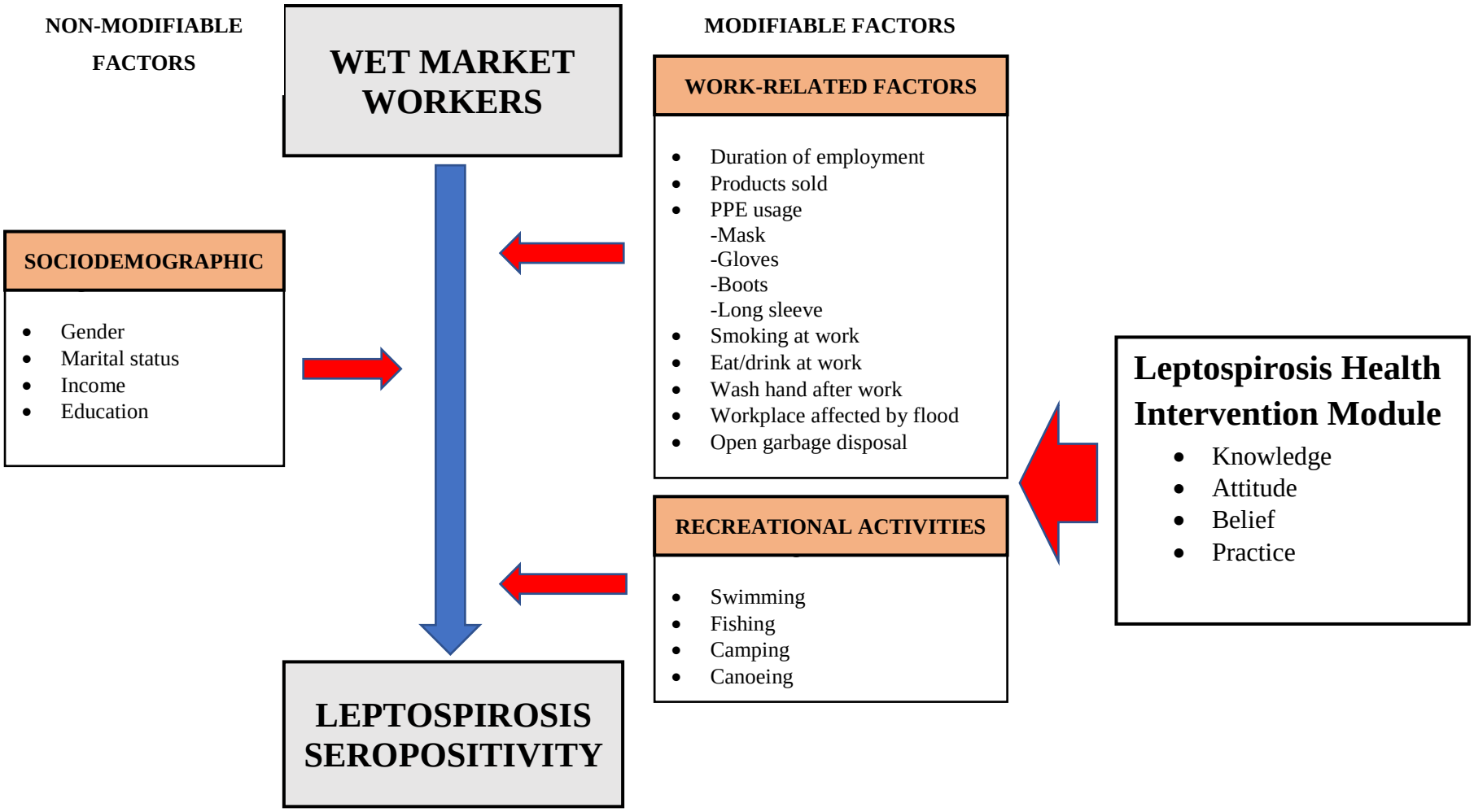


Figure 2.3 Conceptual framework of the study

CHAPTER 3

METHODOLOGY

This study composed of two phases; phase one and phase two.

A. Phase One

3.1 Study Design

This study was a cross sectional study.

3.2 Study Location

This study was conducted in two main wet markets located in Kota Bharu and Pasir Mas districts in the state of Kelantan, which is located in the north-eastern part of Peninsular Malaysia. The selected wet markets were Siti Khadijah Market in Kota Bharu district and Pasir Mas Market in Pasir Mas district. These two districts were chosen in this study because these areas had the highest number of leptospirosis in Kelantan in 2015.

3.3 Reference Population

The reference population was all wet markets workers in Kota Bharu and Pasir Mas districts in Kelantan.

3.4 Source Population

The source population was wet market workers in Siti Khadijah Market and Pasir Mas Market.

3.5 Sampling Frame

The sampling frame was the list of wet market workers at Siti Khadijah Market in Kota Bharu district and Pasir Mas Market in Pasir Mas district who fulfil the study criteria.

3.6 Study Criteria

3.6.1 Inclusion Criteria

1. Wet market workers who were 18 years old and above.

2. Wet markets workers who had work more than three months at Siti Khadijah Market and Pasir Mas Market.

3.6.2 Exclusion Criteria

1. Wet market workers who were not Malaysian citizen.

2. Wet market workers who were not available during the study period.

3.7 Sampling Method

Siti Khadijah Market and Pasir Mas Market in Kota Bharu and Pasir Mas districts were purposely selected in this study because these markets were the main wet markets in both districts. The list of wet market workers in Siti Khadijah Market and Pasir Mas Market were obtained from Kota Bharu and Pasir Mas Municipal Council respectively. Systematic random sampling was applied to select participants from the lists. Participants were selected at fix periodic interval started at number one. The sampling interval was calculated by dividing the number of workers with the calculated sample size. If the selected worker refuses to participate or unavailable, the worker of adjacent shop lot was selected.

3.8 Sample Size Estimation

Sample size calculation was done for all objectives. Objective 2 (usage of PPE during work, sample size =232) had the biggest sample size and therefore was used as sample size for this study.

a) Objective 1: To study the seroprevalence of leptospirosis among wet market workers in Kelantan.

For objective 1, sample size was calculated using one proportion formula.
z = 1.96 Δ = 10% p = 0.35 (Samsudin *et al.*, 2015)

$$n = \left(\frac{z}{\Delta}\right)^2 p(1-p)$$

$$n = \left(\frac{1.96}{0.10}\right)^2 0.35(0.65)$$

= 87 subjects
Considering 20% drop out, n = 104

b) Objective 2: To study the factors associated with seropositivity of leptospirosis among wet market workers in Kelantan.

Sample size was calculated using PS Software Version 3.0 (Dupont and Plummer Jr, 2009).

P0= proportion of exposure in non-disease subject – seronegative (from study)

P1= proportion of exposure in diseased subject – seropositive (expert opinion)

OR = minimum OR of disease/event between cases and controls

m= number of controls per cases

Table 3.1 Sample size calculation for objective 2 (numerical variables)

Variables	SD	alpha	power	Detectable difference	m	Sample size*
Age	10.57	0.05	0.8	5	1	170
Income	375	0.05	0.8	250	1	86
Duration of employment (years)	8.24	0.05	0.8	5	1	105

*Total sample size + 20% dropout

Table 3.2 Sample size calculation for objective 2 (categorical variables)

Variables	P0	P1	alpha	power	m	Sample size*
Gender(male)	0.97	0.8	0.05	0.8	1	129
Marital status (single)	0.13	0.3	0.05	0.8	1	218
Education level (lower)	0.5	0.7	0.05	0.8	1	223
Average work per week	0.36	0.56	0.05	0.8	1	230
PPE used during work						
Boot	0.61	0.41	0.05	0.8	1	232
Glove	0.32	0.12	0.05	0.8	1	158
Long sleeve sheet	0.81	0.61	0.05	0.8	1	192
Wash hand with soap after work	0.90	0.70	0.05	0.8	1	148
Eat or drink while working	0.43	0.63	0.05	0.8	1	232
Smoking while working	0.25	0.45	0.05	0.8	1	211
Sighting rats/rodents at work site	0.58	0.78	0.05	0.8	1	201
Camping	0.04	0.24	0.05	0.8	1	110
Horse riding	0.03	0.23	0.05	0.8	1	103
Gardening	0.51	0.71	0.05	0.8	1	220
Swimming	0.08	0.28	0.05	0.8	1	136
Fishing	0.29	0.50	0.05	0.8	1	213

Reference : (Zainuddin *et al*., 2014)

*Total sample size + 20% dropout

3.9 Study Period

The study period was from December 2016 until April 2018.

3.10 Operational Definitions

a) Wet market workers

A wet market is an open food market. Wet market workers are those who worked at the market and involved with activities of selling fresh meat, fish, fruits, vegetables, dried processed foods and others.

b) Leptospirosis seropositive:

Leptospirosis seropositive was defined as positive sera by microscopic agglutination test (MAT) for anti-leptospiral antibodies. MAT titre of ≥1 in 100 titres was considered positive for evidence of past exposure to leptospires.

c) Leptospirosis seronegative:

Leptospirosis seronegative was defined as negative sera by microscopic agglutination test (MAT) for anti-leptospiral antibodies. MAT titre of <1 in 100 titres was considered positive for evidence of past exposure to leptospires.

d) Monthly income categories

Monthly income categories are based on poverty line index 2014 by Economic Planning Unit (2018). Monthly household income are categorized into hardcore poverty (household monthly income less than RM 580), poverty (household monthly income between RM 580 to RM 940) and above poverty (household monthly income more than RM 940).

3.11 Research Tools and Materials

3.11.1 Knowledge, Attitude, Belief and Practice Questionnaire

A newly developed and validated knowledge, attitude, belief and practice (KABP) questionnaire was used to collect information from wet market workers in this study. The questionnaire was developed in Bahasa Malaysia by a panel of experts (epidemiologist, occupational health specialist, microbiologist, health educationist and medical statistician) following a thorough literature reviews and eight focus group discussions (FGDs) of urban and rural communities in Kelantan and Selangor. It was later validated by two parameter logistic item response theory for knowledge section; Exploratory factor analysis and confirmatory factor analysis (CFA) for attitude, knowledge, belief and practice sections (Zahiruddin *et al.*, 2018). Results for validation study were as follow:

a) Knowledge section

For difficulty parameter, all knowledge items were within acceptable range of -3 to +3. For discrimination, most items were within the acceptable range, with K5(ii), K5(iii) and K(iv) exceed the cut-off by small margin. All items were kept due to acceptable difficulty and discrimination values. Internal consistency reliability by Cronbach's alpha was 0.867. Table 3.3 showed the result for item response theory analysis for knowledge section.

b) Attitude section

Items were grouped into 2 factors; Affect factors and Behaviour-cognitive factor. Thirteen items were kept with standardized factor loading ranging from 0.47 to 0.95.

Both factors had acceptable internal consistency reliability (Affect factor=0.67 and Behaviour cognitive factor = 0.85). The model had good model fit (x2 (df=62) = 262.51, p<0.001; CFI_{robust} = 0.92; TLI_{robust} = 0.90; $RMSEA_{robust}$ = 0.08; SRMR = 0.06).

c) Belief section

Final analysis resulted in three-factor model with 6 items which had good model fit (x2 (df=6) = 31.49, p<0.001; CFI_{robust} = 0.97; TLI_{robust} = 0.93; $RMSEA_{robust}$ = 0.10; SRMR = 0.04). The composite reliability of Benefit factor was below cut-off value of 0.7 (Raykov's rho = 0.59), and for Barriers and Self efficacy factors, the reliability was 0.80 and 0.87 respectively.

d) Practice section

The finding from validation study showed no interpretable correlation between item. Thus, the items were described per item instead of total score.

Table 3.3 Item Response Theory analysis results for knowledge section in validation study

Concept measured	Items	Difficulty, b (SE)	Discrimination, a (SE)	Standardized loadings (λ)	χ^2 (df=8)	*p*-value
Cause	K1, K2, K3	-0.67-0.88 (0.09-0.16)	0.71-1.22 (0.10-0.13)	0.58-0.77	26.76-35.76	0.001
Exposure routes	K5(i)-K5(vii)	-0.63-0.85 (0.04-0.16)	0.69-6.45 (0.10-0.74)	0.57-0.99	18.90-46.87	<0.001
Symptoms and signs	K6(i)-K6(iii)	-1.07-0.52	1.17-2.19 (0.13-0.22)	0.76-0.91	33.82-38.43	<0.001
Detection methods	K4	-0.37 (0.08)	1.17 (0.12)	0.76	16.23	0.039
Complications	K7(i)-K7(iv)	-1.50-0.38 (0.10-0.14)	0.76-1.22 (0.10-0.14)	0.60-0.77	24.83-34.10	<0.001
Prevention aspects	K8(i)-K8(vi)	-2.35-1.20 (0.20-0.32)	0.62-1.05 (0.09-0.13)	0.53-0.72	14.97-129.43	0.001-0.060

Zahiruddin *et al.* (2018)

Table 3.4 Confirmatory Factor Analysis results for attitude and belief section in validation study

Section	Factors	Items	Factor loading, λ	Reliability
Attitude	Affect	A7, A8, A10, A13	0.39-0.78	0.67
	Behaviour - Cognitive	A1, A2, A3, A4, A5, A6, A9, A11, A12	0.46-0.87	0.85
Belief	Benefit	B14, B16	0.58-0.72	0.59
	Barrier	B3, B7	0.63-0.95	0.80
	Self-efficacy	B1, B13	0.84-0.92	0.87

Zahiruddin *et al.* (2018)

The questionnaire consisted of six sections; sociodemographic, environment, knowledge, attitude, belief, and practice sections.

A. Sociodemographic variable

Age, gender, race, marital status, number of children, monthly income, educational level, place of work, type of product sold, duration of employment, number of working day per week, personal protective equipment used during work, history of leptospirosis, family history of leptospirosis, sighting of rodents, smoking status, eating and drinking during work, hand washing practice after work and recreational activities.

B. Environment variable

Distance from house to the waterfall, pond, river and paddy field, animal ownership, house or workplace affected by flood, garbage disposal nearby to the house or workplace.

C. Knowledge section

There were 8 items in knowledge section with three options of "true", "false" and "unsure". This section covered the knowledge on cause, exposure routes, symptoms and signs, detection methods, treatment, complications, and prevention aspects of leptospirosis. A correct answer scored as "1", whereas an incorrect or unsure answer scored as "0". The scores for each item of knowledge section were summed to get an overall score. The overall score was divided by 24 to get percentage of overall score.

D. Attitude section

There were 10 items in attitude section with five Likert-scale options from "strongly agree", "agree", "unsure", "disagree" to "strongly disagree". The score of attitude was recorded from 1 to 5. However, the scores were reversed for items (A5, A6, A8 and A10) with negatively arranged responses. The scores for each item of attitude section were summed to get an overall score. The overall score was divided by 50 to get percentage of overall score.

E. Belief section

There were 5 items in belief section with five Likert-scale options from "strongly agree", "agree", "unsure", "disagree" to "strongly disagree". The score was recorded from 1 to 5. The scores were reversed for items (B2 and B5) with negatively arranged responses. The scores for each item of belief section were summed to get an overall score and divided by 25 to get percentage of overall score.

F. Practice section

Practice section include preventive and risk reduction practice on leptospirosis. There were 17 items in practice section with five Likert-scale options from "always", "most of the time", "seldom", "never" to "not related". P15 and P16 were not assessed in this study as the items were regarding practices during flood. The score was recorded from 0 to 4. However, the scores were reversed for items (P2, P4, P5 and P17) with negatively arranged responses. The scores for each item of practice section were summed to get an overall score. The overall score was divided by 68 to get percentage of overall score.

3.11.2 Microscopic Agglutination Test (MAT)

MAT is the 'gold standard' test for human leptospirosis infection. MAT test for present of antibody against leptospirosis in serum which can indicate past exposure. A titre of $\geq$ 1:100 was used as cut off point for leptospirosis seropositive subjects in this study (Levett, 2001; WHO, 2003). Samples were analysed in Microbiology Laboratory in Universiti Putra Malaysia. The samples were tested against a panel battery of live reference serovars as recommended by World Health Organisation (WHO) which were:

1. *L.biflexa* serovar Patoc
2. *L.interorrogans* serovar Autumnalis
3. *L.interrogans* serovar Bataviae
4. *L. interrogans* serovar Canicola
5. *L. interrogans* serovar Celledoni
6. *L.interrogans* serovar Hardjobovis (IMR LEP 27)
7. *L.interorrogans* serovar Ichterohaemorrhagiae
8. *L. borgpetersenii* serovar Javanica
9. *L.interorrogans* serovar Pomona
10. *L.interorrogans* serovar Pyrogenes
11. *L.interorrogans* serovar Hardjoprajitno
12. *L.interorrogans* serovar Melaka (IMR LEP 1)
13. *L.interorrogans* serovar Terengganu (IMR LEP 115)
14. *L.interorrogans* serovar Sarawak (IMR LEP 175)
15. *L.interorrogans* serovar Copenhageni (IMR LEP 803/11)
16. *L.interorrogans* serovar Australis
17. *L.interorrogans* serovar Lai (IMR LEP 22)
18. *L. borgpetersenii* serovar Tarassovi
19. L. *interrogans* serovar Djasiman
20. *L. interrogans* serovar Grippotyphosa

3.11.3 Equipments Used for Blood Taking

1. Syringe – a 5 ml syringe (Terumo / Luer-Lok Tip)
2. Needle – a 21 gauge size needle
3. Non-sterile disposable latex gloves
4. Alcohol swabs
5. Serum separator (blood collection) tube
6. Tourniquet
7. Cotton wool ball
8. Sharp bin
9. Labels
10. Rack – to place the serum specimen tubes (holder)*
11. Cooler box*
12. Ice packs*
13. Scaling film*

*Equipment used for transportation of blood samples to the Microbiology Laboratory in Universiti Sains Malaysia for initial processing and then to Microbiology Laboratory in Universiti Putra Malaysia for MAT analysis.

3.12 Data Collection

3.12.1 Data Collection Using KABP Questionnaire

Participants from both markets were identified. A total of 232 workers were selected to participate in this study as per calculated sample size, 116 from each market. Prior to data collection, co-researchers were trained regarding the KABP questionnaire to reduce interrater bias. The researcher and co-researchers used face to face interview guided method to obtain information from the participants. The study protocol was explained, and written consent was obtained from all participants. The validated KABP questionnaire on leptospirosis was used by researchers to collect data from the participants.

3.12.2 Venous Blood Sampling

Venous blood sampling procedure for MAT was done according to recommended procedure (WHO, 2010):

1. A tourniquet was placed above the venepuncture site.
2. The venepuncture site was then disinfected meticulously with 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venepuncture site outwards.
3. 5ml of blood was collected for analysis from each participant.
4. The tourniquet was removed, and pressure was applied with cotton wool ball to venepuncture site until bleeding stops.
5. The specimens were transferred to the serum separator tubes and caps were tightly secured.
6. The tubes were labelled with respondent's identification (ID) number.
7. The tubes were inverted and put on the tube rack. These serum separator tubes were stored in the cooler box and transported to the Microbiology Laboratory in USM Health Campus Kelantan.

3.12.3 Separation of Blood Serum

Separation of serum from blood samples were carried out at Microbiology Laboratory, Universiti Sains Malaysia Health Campus, as recommended by manufacturer;

1. The tube was left in an upright position for at least 30 before centrifugation to allow the blood to clot.
2. It was then centrifuged for at least 10 minutes at 1300 to 2000 RPM within one hour of collection.
3. The serum was transferred to a plastic screw-cap vial.
4. The separated serum was kept and stored at -20^0C until MAT was performed.

3.12.4 Microscopic Agglutination Test

The microscopic agglutination test (MAT) was performed with a panel of live leptospires. The serovars used in the MAT for the present study were Patoc, Autumnalis, Bataviae, Canicola, Celledoni, Hardjobovis (IMR LEP 27), Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Hardjoprajitno, Melaka (IMR LEP 1), Terengganu (IMR LEP 115), Sarawak (IMR LEP 175), Copenhageni (IMR LEP 803/11), Australis, Lai (IMR LEP 22), Tarassovi, Djasiman and Grippotyphosa. Live leptospiral cell suspensions representing 20 serovars were added to serially diluted serum specimen in a microtiter well plates and incubated at 30°C for two hours. Agglutination was examined by dark field microscopy at a magnification of 100x. Agglutination was checked by observing free leptospires in each well and compared it with the ones in the control wells. Positive agglutination was considered when the approximate numbers of free leptospires are <50% compared to the control wells. The titre result for each sample against each serovars was labelled at the microtiter plate. The titre dilution done was 1 in 50, 100, 200, 400 and 800. The titre result will be the last dilution that show <50% of the free leptospires compared to the control well. A titre of ≥ 1:100 was used as cut off titre for leptospirosis seropositive subjects in this present study.

3.13 Statistical Analysis

3.13.1 Descriptive Characteristics of Respondents

All data were entered into IBM Statistical Program for Social Sciences (SPSS) Version 24.0 software for Windows. Data were checked and cleaned. Preliminary data screening was done for missing values. The data set was then evaluated for normality

and outliers. Normality was checked using histogram and normality tets. Outliers were checked for the possibility of data recording error, data entry errors or were true outliers. Sociodemographic characteristics, occupational characteristics and environmental characteristics of all the respondents were tabulated for descriptive statistics. The continuous variables were described using the mean and standard deviation (SD) for data with normal distribution and median and interquartile range (IQR) for skewed data. For categorical variables, they were described in frequency and percentage (%).

The continuous variables were age and duration of employment. The categorical variables were gender, marital status, education level, place of work, type of product sold, usage of personal protective equipment (PPE) during working, history of leptospirosis infection, family history of leptospirosis infection, sighting of rats at home and workplace, smoking while working, eating or drinking while working, wash hand after work, recreational activities, distance of house to river/waterfall, distance of house to paddy field, distance of house to main drain, household animal ownership, house area affected by flood, workplace area affected by flood, accumulation of garbage near to the house, accumulation of garbage near to the workplace and garbage disposal.

3.13.2 Seroprevalence Of Leptospirosis Among Wet Market Workers in Kota Bharu and Pasir Mas Districts

Seroprevalence of leptospirosis among wet market workers in Kelantan was determined by microscopic agglutination test (MAT). MAT titre of ≥1 in 100 was considered positive, indicating evidence of past exposure. Seroprevalence of

leptospirosis was calculated and presented as proportion and 95% confidence interval (CI).

3.13.3 Factors Associated with Leptospirosis Seropositivity Among Wet Market Workers in Kota Bharu and Pasir Mas Districts

Univariable and multivariable analysis were performed to determine the associated factors for leptospirosis seropositivity among wet market workers. The factors were evaluated based on sociodemographic factors, work-related factors and recreational activity factors. Summary of independent variables were shown in table 3.5. The outcome variable was the result of the MAT analysis for leptospirosis which was coded as "1" for seropositive result and "0" for seronegative result.

Variables with *p*-value of less than 0.25 from simple logistic regression (SLogR) and clinically important were selected for multiple logistic regression (MLogR) analysis. MLogR was used to evaluate factors associated with seropositivity among wet market workers after controlling for other variables. Preliminary main effect model was obtained after comparing model using backward likelihood ratio and forward likelihood ratio methods. Multicollinearity was checked using correlation matrix. All possible two-way interactions were checked. Fitness of the model was tested by Hosmer-Lemeshow goodness of fit test. Other than that, the classification table and area under receiver operation characteristics (ROC) curve were also used to determine the fitness of the model. The final model was presented with adjusted odds ratio (AOR) and 95% confidence interval (CI), Wald statistics and *p*-value. The level of significance was set at *p*-value of less than 0.05.

Table 3.5 Independent variables for logistic regression analysis

Factors		Independent Variables
Sociodemographic	1.	Age
	2.	Gender (Male*, Female)
	3.	Marital status (Single*, Married, Widower)
	4.	Monthly income, RM (0-580, 581-940, >940*)
	5.	Educational level (No formal education, Primary school, Secondary school, Form 6/higher education*)
Work-related	1.	Place of work (Siti Khadijah Market*, Pasir Mas Market)
	2.	Duration of employment (≤5 years*, >5 years)
	3.	Number of days working per week (≤5 days*, 6 days, 7 days)
	4.	Type of product sold (Others*, Processed food, Fruits and vegetables, Fresh meat)
	5.	Usage of mask at work (Yes*, No)
	6.	Usage of glove at work (Yes*, No)
	7.	Usage of boot at work (Yes*, No)
	8.	Usage of long sleeve at work (Yes*, No)
	9.	Rodents sighting at work (No*, Yes)
	10.	Smoke at work (No*, Yes)
	11.	Eat and drink at work (No*, Yes)
	12.	Washing hand after work (Yes*, No)
	13.	Workplace affected by flood (No*, Yes)
	14.	Open garbage disposal at workplace (No*, Yes)
Recreational activity	1.	Overall risk activities (No*, Yes)
	2.	Gardening (No*, Yes)
	3.	Swimming (No*, Yes)
	4.	Fishing (No*, Yes)

*Reference group

B. Phase Two

3.14 Study Design

Phase two of this study was a quasi-experimental study design.

3.15 Study Area

Study areas were similar to phase one of the study which were Siti Khadijah Market in Kota Bharu district and Pasir Mas Market in Pasir Mas district.

3.16 Reference Population

The reference population was all wet markets workers in Kota Bharu and Pasir Mas districts in Kelantan.

3.17 Source Population

The source population was wet market workers in Siti Khadijah Market and Pasir Mas Market.

3.18 Sampling Frame

The sampling frame was the list of wet market workers at Siti Khadijah Market in Kota Bharu district and Pasir Mas Market in Pasir Mas district who fulfil the study criteria.

3.19 Sampling Method

All participants in phase one were selected to participate in phase two. Participants in Siti Khadijah Market were assigned to intervention group and participants in Pasir Mas Market were assigned to control group. The ratio of intervention to control group was 1:1.

3.20 Study Period

The study period was from December 2016 until April 2018.

3.21 Sample Size Estimation

For phase two of this study sample size was estimated using PS Software Version 3.0 (Dupont and Plummer Jr, 2009), based on a study in Malaysia Zainuddin et al. (2014). To compare the mean score of knowledge, attitude, belief and practice on leptospirosis between control and intervention group at baseline and six weeks post intervention among wet market workers in Kelantan.

Table 3.6 Sample size calculation for objective 3

Variables	SD	Detectable mean difference	Calculated sample size	Sample size
Total knowledge score	8.48	6	32 for each group	40 for each group Total = 80
Total attitude score	10.96	6	53 for each group	67 for each group Total = 134
Total practice score	13.31	6	78 for each group	98 for each group Total = 196

Reference: (Zainuddin *et al.*, 2014)

3.22 Research Tools and Materials

3.22.1 Knowledge, Attitude, Belief and Practice Questionnaire

The same questionnaire used in phase one was used in phase two.

3.22.2 Leptospirosis Health Intervention Module

The Leptospirosis Health Intervention Module (LHIM) is a module prepared for the purpose of educating public especially people in high risk groups regarding leptospirosis. The module was prepared by a panel of experts including epidemiologists, occupational health specialists, microbiologists, health educationist and medical statisticians. The LHIM was developed following extensive literature reviews and serial discussions among the experts to ensure good content validity and relevancy of information regarding leptospirosis. The module consisted of four scopes on leptospirosis and varies activities which were shown in table 3.7.

Table 3.7 Contents of Leptospirosis Health Intervention Module

Scope	Contents	Activities
Scope 1	Introduction to leptospirosis	• Lecture
Scope 2	Diagnosis and treatment	• Video presentation • Lecture • Match and win • Role play
Scope 3	Risk for infection	• Lecture • Small group discussion
Scope 4	Prevention and control	• Lecture • Poison box • Hand on: i. Hand washing techniques ii. Personal protective equipment • Where am I?

a) **Scope one: Introduction to leptospirosis**

i. Lecture which covers introduction on leptospirosis, cause of leptospirosis, mode of transmission, incubation period, current situation of leptospirosis in Malaysia, symptoms and signs of leptospirosis and clinical staging of disease.

b) **Scope two: Diagnosis and treatment**

i. Video presentation and lecture were regarding sample types for leptospirosis laboratory analysis (blood, urine, cerebrospinal fluid and tissue samples), type of leptospirosis laboratory analysis (rapid test, MAT, PCR, culture, immunostaining test) and treatment for leptospirosis.

ii. Match and win (Padan dan menang) activity was conducted afterward to evaluate understanding of participants regarding the scope. Participants need to select type of samples and match it to the anatomy location of human body.

iii. Role play was conducted by dividing participants into groups of six. Groups were given specific scenarios and their group will act according to the scenarios given. Example of scenario was ‘a person developed fever and jaundice after flood incident’. The other participants then give their opinions regarding the role play. Facilitators were to guide and be the time keeper to ensures the role-playing activities run smoothly.

c) Scope three: Risk for infection

i. Lecture which covers definition of high risk groups and high-risk areas for leptospirosis, example of high risk groups and high-risk areas and factors for rodents infestation.

ii. Small groups discussion was done by dividing participants into groups of 4 to 5 people. Then the group discussed regarding high risk groups and areas and listed on a paper. Then, the group presented their findings to other groups. Other participants and facilitators will give opinion regarding their presentation.

d) **Scope four**: **Prevention and control of leptospirosis**

i. Lecture which covers prevention and control measures on leptospirosis for public and high-risk groups; prevention and control measures when visiting high risk areas.

ii. Poison box activity where participants were selected by random need to pick an envelope which contain a question. The participants will then answer the question. Other participants and facilitators will give opinion regarding their answer.

iii. Hand washing technique was introduced in this program was to ensure the cleanliness and good hand hygiene technique that could prevent diseases including leptospirosis. First, the participants were given hand-outs regarding the seven steps of hand washing technique. They will read it and go through the theory with the attending facilitator. Then, the facilitator will start the demonstration and repeat it few times until all the participants able to do the seven steps hand washing technique by themselves. Next, the facilitator will assess each participant regarding the hand washing technique. The participants passed the test when they were able to demonstrate the seven steps of hand washing technique correctly without referring to the hand-outs or other participants.

iv. Hands-On Personal Protective Equipment (PPE) Session where facilitators with the aid of PPE (rubber gloves, boots and face mask) explained to the participants regarding the correct way to use the PPE, the importance of compliance to PPE usage and the maintenance of the respective PPE. Then, each participant demonstrated how to wear the rubber gloves, boots and face mask while the facilitators gave their comments. This was to further increase the awareness of the participants regarding the importance of PPE usage and compliance to it.

v. 'Where am I?' activity was conducted by using pictures which showed good and bad practice at a food stall. Participants need to find and list both type of practice and present the finding to facilitators. Other participants and facilitators will give comments on their presentation.

The module was tested among 10 health staffs to assess on relevancy, clarity and comprehension of the content of the module. For relevancy, 70.0% of respondents answered, "the item is very relevant to the domain" while 30.0% answered "the item is relevant to the domain". For clarity, 60.0% answered "the sentence is clear" and 40.0% answered "the sentence is very clear". As for comprehensible, 70.0% answered "the sentence is comprehensible" and 30.0% answered "the sentence is very comprehensible". The module was also tested regarding informative, readability, arrangement, attractiveness and user friendliness of the module. The assessment was summarized in table 3.8 and table 3.9.

Table 3.8 Relevancy, clarity and comprehension result of Leptospirosis Health Intervention Module

Variable	1 n (%)	2 n (%)	3 n (%)	4 n (%)
Relevancy	0 (0.0)	0 (0.0)	3 (30.0)	7 (70.0)
Clarity	0 (0.0)	0 (0.0)	6 (60.0)	4 (40.0)
Comprehension	0 (0.0)	0 (0.0)	7 (70.0)	3 (30.0)

For relevancy of the module content, the option answers were

1 = the item is not relevant to the domain

2 = the item is somewhat relevant to the domain

3 = the item is relevant to the domain

4 = the item is very relevant to the domain

For clarity, the option answers were,

1 = the sentence is not clear

2 = the sentence is somewhat clear

3 = the sentence is clear

4 = the sentence is very clear

For comprehension, the option answers were

1 = the sentence is not comprehensible

2 = the sentence is somewhat comprehensible

3 = the sentence is comprehensible

4 = the sentence is very comprehensible

Table 3.9 Face validity result of Leptospirosis Health Intervention Module

Variable	Agree n (%)	Unsure n (%)	Not agree n (%)
Informative	10 (100.0)	0 (0.0)	0 (0.0)
Readability	9 (90.0)	1 (10.0)	0 (0.0)
Arrangement	10 (100.0)	0 (0.0)	0 (0.0)
Attractiveness	4 (40.0)	5 (50.0)	1 (10.0)
User friendliness	8 (80.0)	2 (20.0)	0 (0.0)

3.22.3 Leptospirosis Health Intervention Program

The intervention in this study was named as Leptospirosis Health Intervention Program (LHIP). The program was based on Leptospirosis Health Intervention Module. The lectures and activities during the program were carried out by experts and trained staffs whom also involved in developing of the module. It was carried out in January 2017 after preintervention data collection completed. The event took place at Royal Guest House Kota Bharu which was about 100 meters from Siti Khadijah Market. Royal Guest House Kota Bharu was chosen as the event location due to its suitable facilities and short distance from study location. The participants were informed regarding the program two weeks prior the actual event. They were also given invitation card and short message service (sms) to remind them regarding the program. Those who were unable to attend on the agreed date were given option to join on the later date. The program's activities were shown in table 3.10.

Table 3.10 Tentative of Leptospirosis Health Intervention Program

Program tentative	
8.00 am	Registration
8.30 am	Scope one: Lecture on 'Introduction to Leptospirosis'
9.00 am	Scope two: Video Presentation and Lecture on 'Diagnosis and Treatment'
10.00 am	Tea break
10.30 am	Scope two: 'Match and win' activity Role play activity
11.30 pm	Scope three: Lecture on 'Risk for Infection'
12.15 pm	Scope three: Small group discussion on 'Risk for Infection'
1.00 pm	Lunch break
2.15 pm	Scope four: Lecture on 'Prevention and Control of Leptospirosis'
2.45 pm	Scope four: Activity on hand washing technique Activity on personal protection equipment (PPE)
4.00 pm	Scope four: 'Poison box' activity
4.30 pm	Scope four: 'Where am I?' activity
5.00 pm	Tea break and dismiss

3.23 Data Collection

Data collection for phase two of this study was conducted using the same KABP questionnaire as phase one. Prior to data collection co-researchers were trained regarding the KABP questionnaire to reduce interrater bias. The researcher and co-researchers used face to face interview guided method to obtain information from the participants. The preintervention KABP data collection was conducted concurrent with data collection in phase one. The leptospirosis health intervention program was conducted after completion of preintervention data collection. The postintervention data collection was carried out six weeks after the intervention program.

3.24 Statistical Analysis

All data was entered into Statistical Program for Social Sciences (SPSS) Version 24.0 software for Windows. Baseline sociodemographic characteristics of participants were compared between intervention and control groups. Significant different of baseline characteristics between groups were controlled using multivariable analysis (gender and monthly income). To determine the effect of Leptospirosis Health Intervention Module (LHIM), multi-way ANOVA was performed to compare the preintervention and postintervention changes in knowledge, attitude, belief and practice score outcome between intervention and control groups.

First, data exploration was performed to obtain descriptive statistics for all variables. Data cleaning was done to check for any missing value and error in data entry before analysis and evaluated for normality and outliers. Missing value were excluded from analysis. Normality was checked by using histogram. Numerical data were expressed as mean and standard deviation (SD) whereas categorical data was expressed as frequency and percentage (%). The one-way ANOVA was used for univariable analysis to check for significant different of the preintervention and postintervention changes in knowledge, attitude, belief and practice score outcome score between intervention and control groups.

The effect of gender and monthly income on KABP score changes were also examined at univariable analysis. Mean changes of knowledge, attitude, belief and practice scores were then compared between intervention and control groups by adjusting for effect of gender and monthly income to get the preliminary main effect model. Two-way interactions were checked between groups vs gender and group vs monthly

income. Assumption for multi-way ANOVA were check using histogram for normality assumption, scatter's plot and Levene's test for equal variances and overall model fitness.

3.25 Ethical Consideration

Ethical clearance was obtained from Research and Ethic Committee (Human), School of Medical Sciences, Health Campus, Universiti Sains Malaysia, on 25th of July 2016 (Appendix A). Approval from each Municipal and District Councils were obtained in October 2016 (Appendix B and appendix C).

Prior to the data collection, the wet market workers were approached, and details of the study methods and procedures were explained. Participants were informed that their participation was entirely voluntary, and they may reserve their rights to withdraw from the study, refuse to answer any question or leave whenever they want, all without any penalty.

The data collection, blood sampling and intervention program were conducted after participants gave their consent. The blood samples were only used for the objectives of this study. The blood samples were disposed according to the standard operating procedure of University. The confidentiality of the data and blood samples were strictly maintained, whereby only the author, supervisors and co researchers could access the data. The research only reported group data and not individual data.

3.26 Study Flow Chart

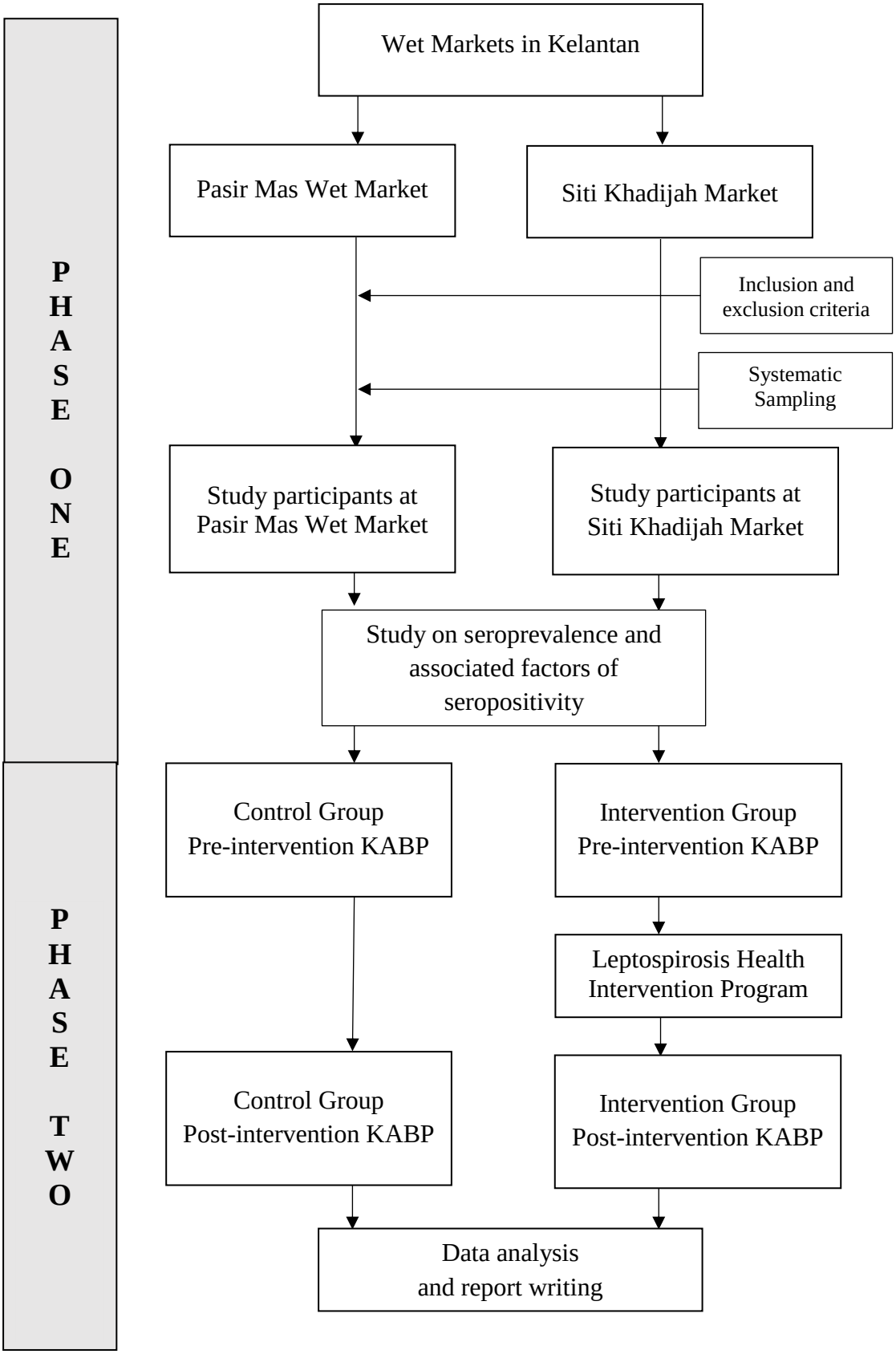


Figure 3.1 Flow chart of study

CHAPTER 4

RESULTS

A. Phase One

4.1 Descriptive Characteristics of Respondents in Phase One

4.1.1 Sociodemographic Characteristics of Respondents

Total of 232 wet market workers from Siti Khadijah Market and Pasir Mas Market participated in phase one of this study. Table 4.1 describes the sociodemographic characteristics of respondents. All study respondents were Malay. The mean (SD) age of the respondents was 42.6 (14.68) years old ranging from 18 to 79. Majority of respondents were female (63.4%) and the median (IQR) of monthly income was RM 800 (500). As for level of education, 59.1% had at least secondary school education.

Table 4.1 Sociodemographic characteristics of respondents (n=232)

Variables	Mean (SD)	Frequency (%)
Age (years)	42.6 (14.68)	
Gender		
Male		85 (36.6)
Female		147 (63.4)
Marital status		
Single		47 (20.3)
Married		175 (75.4)
Widower		10 (4.3)
Monthly income (RM)*		
0-580		68 (29.3)
581-940		75 (32.3)
>940		89 (38.4)
Educational level		
No formal education		19 (8.2)
Primary school		30 (12.9)
Secondary school		137 (59.1)
Form 6/ Higher education		46 (19.8)

*Economic Planning Unit (2018)

4.1.2 Work-related Characteristics of Respondents

Table 4.2 shows the work-related characteristics of respondents. 232 wet market workers from Siti Khadijah and Pasir Mas Markets participated in this study. The median (IQR) duration of employment and number of days working per week were 83.5 (168) months and 7 (1.0) days respectively.

Table 4.2 Work-related characteristics of respondents (n=232)

Variables	Frequency	(%)
Place of work		
Siti Khadijah Market	116	(50.0)
Pasir Mas Market	116	(50.0)
Duration of employment (month)		
≤5 years	98	(42.2)
>5 years	134	(57.8)
No. of days working per week		
≤5 days	23	(9.9)
6 days	71	(30.6)
7 days	137	(59.1)
Type of product sold		
Fresh meat (Chicken/meat/fish)	21	(9.1)
Fruits and vegetables	38	(16.4)
Processed food	74	(31.9)
Others	99	(42.7)
PPE use at work		
Mask	13	(5.6)
Gloves	37	(15.9)
Boots	35	(15.1)
Long sleeve shirt	159	(68.5)
Rats or rodents sighting at work	184	(79.3)
Smoke at work	34	(14.7)
Eat or drink at work	127	(54.7)
Wash hands with soaps after work	188	(81.0)
Workplace area affected by flood	158	(68.1)
Open garbage disposal at workplace	108	(46.6)

4.1.3 Household Characteristics of Respondents

More than half of respondents reported rats or rodents sighting at home. Less than 25.0% of respondents lived within 200 meters from waterfall, river, pond or paddy field. Most respondents had domestic animals around housing area. Table 4.3 shows the details of household characteristics of respondents.

Table 4.3 Household characteristics of respondents (n=232)

Variables	Frequency (%)
Rats or rodents sighting at home	155 (66.8)
Distance from house to waterfall/river/pond	
<100 meters	30 (12.9)
100 to 200 meters	24 (10.3)
>200 meters	53 (22.8)
NA	125 (53.9)
Distance from house to paddy field	
<100 meters	27 (11.6)
100 to 200 meters	26 (11.2)
>200 meters	47 (20.3)
NA	132 (56.9)
Distance from house to main drain	
<100 meters	36 (15.5)
100 to 200 meters	20 (8.6)
>200 meters	45 (19.4)
NA	131 (56.5)
Present of domestic animals around housing area	214 (92.2)
Housing area affected by flood	140 (60.3)
Open garbage disposal at housing area	88 (62.1)

4.1.4 Recreational Activities Among Respondents

Table 4.4 reported the recreational activities engaged by the respondents. The main recreational activities done by the wet market workers are gardening (28.4%), followed by swimming (9.1%) and fishing (6.0%).

Table 4.4 Recreational activities engaged by respondents (n=232)

Variables	Frequency (%)
Overall risk activities	78 (33.6)
Gardening	66 (28.4)
Swimming	21 (9.1)
Fishing	14 (6.0)
Camping	3 (1.3)
Canoeing	3 (1.3)

Respondents may answer more than one category

4.2 Seroprevalence of Leptospirosis Among Wet Market Workers

The seroprevalence result for both markets were similar with 39 respondents from each market were found positive. The overall seroprevalence for leptospirosis among respondents was 33.6% (95% CI: 27.5, 39.7). Table 4.5 shows the seroprevalence of leptospirosis in Siti Khadijah Market and Pasir Mas Market.

Respondents' blood samples can be positive to more than one leptospiral serovars. A total of 137 positive results were obtained from the MAT analysis. Table 4.6 showed the serovars distribution among 137 positive MAT results on all serovars in this study. Serovars Autumnalis was the predominant serovars found positive with 18.2% of overall positive results. No sample was reactive against serovars Lai (IMR LEP 22) and Celledoni.

Table 4.5 Seroprevalence of leptospirosis among wet markets workers according to workplace (n=232)

Variables	n	MAT titre 1≥100	
		Frequency (%)	95% CI
Overall	232	78 (33.6)	27.5, 39.7
Siti Khadijah Market	116	39 (33.6)	25.1, 43.0
Pasir Mas Market	116	39 (33.6)	25.1, 43.0

Table 4.6 Serovars distribution among positive MAT results on all serovars (n=137)

Serovars	Overall		Siti Khadijah Market		Pasir Mas Market	
	Frequency (n=137)	Percentage (%)	Frequency (n=68)	Percentage (%)	Frequency (n=69)	Percentage (%)
Autumnalis	25	18.2	8	11.8	17	24.6
Sarawak (IMR LEP 175)	21	15.4	13	19.1	8	11.6
Copenhageni (IMR LEP 803/11)	12	8.8	8	11.8	4	5.8
Canicola	10	7.3	8	11.8	2	2.9
Djasiman	9	6.6	5	7.4	4	5.8
Australis	8	5.8	2	2.9	6	8.7
Patoc	8	5.8	3	4.4	5	7.2
Hardjoprajitno	7	5.1	1	1.5	6	8.7
Pyrogenes	7	5.1	4	5.8	3	4.3
Tarassovi	6	4.4	6	8.8	0	0.0
Pomona	6	4.4	4	5.8	2	2.9
Javanica	5	3.6	1	1.5	4	5.8
Icterohaemorrhagiae	4	2.9	0	0.0	4	5.8
Grippotyphosa	3	2.2	2	2.9	1	1.5
Hardjobovis (IMR LEP 27)	2	1.5	1	1.5	1	1.5
Bataviae	2	1.5	0	0.0	2	2.9
Melaka (IMR LEP 1)	1	0.7	1	1.5	0	0.0
Terengganu (IMR LEP 115)	1	0.7	1	1.5	0	0.0

Respondents can be positive to more than one serovars

Total of 137 positive MAT results on all serovars

4.3 Factors Associated with Leptospirosis Seropositivity Among Wet Market Workers

4.3.1 Sociodemographic Factors Associated with Leptospirosis Seropositivity

Multiple logistic regression analysis was carried out to determine the sociodemographic factors associated with leptospirosis seropositivity among wet market workers. The sociodemographic factors tested were age, gender, marital status, monthly income and educational level of respondents. At univariable analysis step, four factors were significant; age, monthly income, marital status and educational level. Table 4.7 showed the univariable analysis for sociodemographic factors associated with leptospirosis among respondents. Preliminary main effect model was obtained after comparing forward and backward likelihood ratio methods. Model was selected based on statistical significant, biological parsimonious and model fitness.

Table 4.7 Simple logistic regression of sociodemographic factors associated with leptospirosis seropositivity among wet market workers (n=232)

Variable	Regression coefficient (b)	Crude OR (95% CI)	Wald statistic (df)	*p*-value
Age	0.02	1.02 (1.004, 1.043)	5.83 (1)	0.016
Gender				
Male	0	1		
Female	0.22	1.24 (0.70, 2.20)	0.55 (1)	0.458
Marital status				
Single	0	1		
Married	0.47	1.60 (0.77, 3.31)	1.61 (1)	0.204
Widower	0.67	1.94 (0.46, 8.08)	0.83 (1)	0.360
Monthly income (RM)				
>940	0	1		
0-580	-0.47	0.62 (0.32, 1.22)	1.88 (1)	0.170
581-940	-0.67	0.51 (0.26, 0.99)	3.93 (1)	0.047
Educational level				
Form 6/Higher education	0	1		
Secondary school	0.63	1.88 (0.85, 4.11)	2.48 (1)	0.115
Primary school	1.28	3.60 (1.32, 9.80)	6.28 (1)	0.012
No formal education	0.51	1.66 (0.50, 5.48)	0.69 (1)	0.405

Table 4.8 Multiple logistic regression of sociodemographic factors associated with leptospirosis seropositivity among wet market workers (n=232)

Variable	Regression coefficient (b)	OR (95% CI)	Wald Statistic (df)	*p*-value
Age	0.02	1.02 (1.004,1.043)	5.83 (1)	0.016

Hosmer Lemeshow Test *p*-value =0.594
Classification table overall correctly classified percentage is 66.8%
Area under ROC curve = 59.1%

The classification table showed that the overall correctly classified percentage was 66.8%. The area under the ROC curve was 59.1% (95% CI: 51.2, 67.1). The model can accurately discriminate 59.1% of the cases. Multiple logistic regression analysis showed that age (*p*-value<0.016) was significantly associated with leptospirosis seropositivity among wet market workers (Table 4.8). A worker with an increase of one year in age has 1.02 times the odds to have leptospirosis seropositivity.

4.3.2 Work-related Factors Associated with Leptospirosis Seropositivity

Simple logistic regression analysis was carried out to determine the work-related factors associated with leptospirosis seropositivity among wet market workers. Duration of employment, type of product sold, usage of mask, glove and boot were factors that have *p*-value <0.25. Table 4.9 showed the univariable analysis for work-related factors associated with leptospirosis among respondents. Preliminary main effect model was obtained after comparing forward and backward likelihood ratio methods. Model was selected based on statistical significant, biological parsimonious and model fitness.

Table 4.9 Simple logistic regression of work-related factors associated with leptospirosis seropositivity among wet market workers (n=232)

Variable	Regression coefficient (b)	Crude OR (95% CI)	Wald statistic (df)	*p*-value
Place of work				
Siti Khadijah Market	0	1		
Pasir Mas Market	0.00	1.00 (0.58, 1.72)	0 (1)	1.000
Duration of employment (month)				
≤5 years	0	1		
>5 years	0.23	1.26 (0.72, 2.20)	0.68 (1)	0.407
No. of days working/week				
≤5 days	0	1		
6 days	-0.42	0.65 (0.24, 1.74)	0.72 (1)	0.395
7 days	-0.17	0.83 (0.33, 2.08)	0.14 (1)	0.705
Type of product sold				
Others	0	1		
Processed food	0.11	1.11 (0.59, 2.07)	0.11 (1)	0.737
Fruits and vegetables	-0.57	0.56 (0.24, 1.33)	1.69 (1)	0.193
Fresh meat	-0.31	0.73 (0.26, 2.05)	0.35 (1)	0.553
Usage of mask at work				
Yes	0	1		
No	1.07	2.92 (0.63, 13.52)	1.88 (1)	0.170
Usage of glove at work				
Yes	0	1		
No	0.89	2.45 (1.02, 5.87)	4.06 (1)	0.044
Usage of boot at work				
Yes	0	1		
No	0.62	1.86 (0.80, 4.31)	2.09 (1)	0.148
Usage of long sleeve at work				
Yes	0	1		
No	-0.14	0.87 (0.48, 1.57)	0.21 (1)	0.644
Rodents sighting at work				
No	0	1		
Yes	-0.22	0.80 (0.41,1.56)	0.40 (1)	0.523
Smoke at work				
No	0	1		
Yes	-0.23	0.79 (0.36, 1.76)	0.31 (1)	0.574
Eat or drink at work				
No	0	1		
Yes	0.26	1.29 (0.74, 2.24)	0.84 (1)	0.357
Washing hand after work				
Yes	0	1		
No	-0.10	0.90 (0.44, 1.82)	0.07 (1)	0.779
Workplace affected by flood				
No	0	1		
Yes	-0.09	0.90 (0.50, 1.62)	0.11 (1)	0.738
Open garbage disposal at workplace				
No	0	1		
Yes	-0.26	0.77 (0.44, 1.33)	0.84 (1)	0.357

Table 4.10 Multiple logistic regression of work-related factors associated with leptospirosis seropositivity among wet market workers (n=232)

Variable	Regression coefficient (b)	OR (95% CI)	Wald Statistic (df)	*p*-value
Usage of glove at work				
Yes	0	1		
No	0.89	2.45 (1.02, 5.87)	4.06 (1)	0.044

Classification table overall correctly classified percentage is 66.4%
Area under ROC curve = 55.3%

The classification table showed that the overall correctly classified percentage was 66.4%. The area under the ROC curve was 55.3% (95% CI: 47.6, 62.9). The model can accurately discriminate 55.3% of the cases. Multiple logistic regression analysis showed that not using glove at work (*p*-value<0.044) was significantly associated with leptospirosis seropositivity among wet market workers (Table 4.10). A worker who do not use glove at work has 2.45 times the odds to have leptospirosis seropositivity compare to worker who use glove.

4.3.3 Recreational Activities Factors Associated with Leptospirosis Seropositivity

The recreational activities factors were not associated with leptospirosis seropositivity among wet market workers. None of the activities had *p*-values<0.25 at univariable analysis. Table 4.11 showed the univariable analysis for recreational activities factors associated with leptospirosis seropositivity among wet market workers.

Table 4.11 Simple logistic regression of recreational activities factors associated with leptospirosis seropositivity among wet market workers (n=232)

Variable	Regression coefficient (b)	Crude Odds Ratio (95% CI)	Wald statistic (df)	*p*-value
Overall risk activities				
No	0	1		
Yes	-0.02	0.98 (0.55, 1.74)	0.01 (1)	0.947
Gardening				
No	0	1		
Yes	0.07	1.07 (0.59, 1.96)	0.06 (1)	0.803
Swimming				
No	0	1		
Yes	0.22	1.24 (0.49, 3.13)	0.21 (1)	0.649
Fishing				
No	0	1		
Yes	-0.25	0.77 (0.23, 2.56)	0.16 (1)	0.681

B. Phase Two

4.4 Descriptive Characteristics of Respondents in Phase Two

All respondents from phase one were included in phase two of this study (n=232). Respondents from Siti Khadijah Market were assigned to intervention group and respondents from Pasir Mas Market were assigned to control group. Only 170 respondents completed the phase two of this study, 88 respondents from control group and 82 respondents from intervention group. Table 4.12 showed the characteristics of respondents in control and intervention groups in the beginning of phase two. Table 4.13 showed the characteristics of respondents who completed the phase two of this study.

Table 4.12 Characteristics of respondents who participated in phase two in control and intervention groups (n=232)

Variables	Frequency (%)		*p*-value
	Control group n=116	Intervention group n=116	
Age	43.0 (13.8)[a]	42.0 (15.5)[a]	0.609[c]
Gender			
Male	51 (44.0)	34 (29.3)	
Female	65 (56.0)	82 (70.7)	0.021[e]
Marital status			
Single/widower			
Married			
Monthly income (RM)			
0-580	48 (41.4)	20 (17.2)	
581-940	28 (24.1)	47 (40.5)	<0.001[e]
>940	40 (34.5)	49 (42.2)	
Educational level			
No formal education	11 (9.5)	8 (6.9)	
Primary school	16 (13.8)	14 (12.1)	
Secondary school	73 (62.9)	64 (55.2)	0.141[e]
Form 6/ Higher education	16 (13.8)	30 (25.9)	
Duration of employment (month)			
≤5 years	53 (45.7)	45 (38.8)	
>5 years	63 (54.3)	71 (61.2)	0.288[e]
Usage of mask at work			
Yes	8 (6.9)	5 (4.3)	
No	108 (93.1)	111 (95.7)	0.392[e]
Usage of gloves at work			
Yes	23 (19.8)	14 (12.1)	
No	93 (80.2)	102 (87.9)	0.107[e]
Usage of boots at work			
Yes	20 (17.2)	15 (12.9)	
No	96 (82.8)	101 (87.1)	0.359[e]
Usage of long sleeve shirt			
Yes	76 (65.5)	83 (71.6)	
No	40 (34.5)	33 (28.4)	0.322[e]
Eat or drink at work			
Yes	57 (49.1)	70 (60.3)	
No	59 (50.9)	46 (39.7)	0.086[e]
Wash hands with soaps after work			
Yes	91 (78.4)	97 (83.6)	
No	25 (21.6)	19 (16.4)	0.315[e]
Prescore Knowledge	75.3 (18.2)[a]	72.0 (17.5)[a]	0.171[c]
Prescore Attitude	87.3 (8.3)[a]	85.3 (10.1)[a]	0.109[c]
Prescore Belief	83.8 (10.3)[a]	81.5 (10.0)[a]	0.082[c]
Prescore Practice	78.1 (10.5)[a]	76.5 (9.6)[a]	0.239[c]

[a]Mean (SD)

[b]Median (IQR)

[c]Independent T-test

[d]Mann-Whitney test

[e]Chi-square

[f]Fisher's Exact Test

Table 4.13 Characteristics of respondents who completed the phase two in intervention and control groups (n=170)

Variables	Frequency (%)		*p*-value
	Control group n=88	Intervention group n=82	
Age	43.90 (13.84)[a]	44.98 (14.89)[a]	0.625[c]
Duration of employment (month)	84 (200)[b]	114 (182)[b]	0.368[d]
Gender			
Male	34 (38.6)	19 (23.2)	
Female	54 (61.4)	63 (76.8)	0.030[e]
Marital status			
Single/widower	14 (15.9)	23 (28.0)	
Married	74 (84.1)	59 (72.0)	0.055[e]
Monthly income (RM)			
0-580	32 (36.4)	15 (18.3)	
581-940	24 (27.3)	32 (39.0)	0.027[e]
>940	32 (36.4)	35 (42.7)	
Educational level			
No formal education	9 (10.2)	6 (7.3)	
Primary school	10 (11.4)	10 (12.2)	
Secondary school	56 (63.6)	47 (57.3)	0.512[e]
Form 6/ Higher education	13 (14.8)	19 (23.2)	
Duration of employment (month)			
≤5 years	39 (44.3)	28 (34.1)	
>5 years	49 (55.7)	54 (65.9)	0.175[e]
Usage of mask at work			
Yes	3 (3.4)	2 (2.4)	
No	85 (96.6)	80 (97.6)	0.533[f]
Usage of gloves at work			
Yes	14 (15.9)	6 (7.3)	
No	74 (84.1)	76 (92.7)	0.082[e]
Usage of boots at work			
Yes	11 (12.5)	9 (11.0)	
No	77 (87.5)	73 (89.0)	0.758[e]
Usage of long sleeve shirt			
Yes	60 (68.2)	62 (75.6)	
No	28 (31.8)	20 (24.4)	0.282[e]
Eat or drink at work			
Yes	48 (54.5)	47 (57.3)	
No	40 (45.5)	35 (42.7)	0.716[e]
Wash hands with soaps after work			
Yes	69 (78.4)	69 (82.9)	
No	19 (21.6)	14 (17.1)	0.457[e]
Prescore Knowledge	78.6 (13.1)[a]	75.2 (13.2)[a]	0.092[c]
Prescore Attitude	88.2 (7.8)[a]	87.3 (7.8)[a]	0.452[c]
Prescore Belief	85.8 (8.7)[a]	83.5 (9.1)[a]	0.088[c]
Prescore Practice	77.0 (10.3)[a]	76.8 (9.7)[a]	0.868[c]

[a]Mean (SD)

[b]Median (IQR)

[c]Independent T-test

[d]Mann-Whitney test

[e]Chi-square

[f]Fisher's Exact Test

4.5 The Effect of Leptospirosis Health Intervention Module in Increasing Knowledge, Attitude, Belief And Practice Score

4.5.1 Knowledge Section

Table 4.14 showed the descriptive statistics of pre-intervention and post-intervention knowledge score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.15. The adjusted mean knowledge score changes for control and intervention group were 3.60 and 16.54 respectively. The adjusted mean difference was 12.93 (95% CI: 8.47, 17.39). Those in intervention group showed significantly higher score compare to control group ($p<0.001$). Gender and monthly income were not significant factors for mean knowledge score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [$F(1,164)=0.99$, $p=0.320$] and groups and monthly income [$F(2,163)=0.60$, $p=0.548$] on knowledge score changes. Figure 4.1 (a) and (b) showed the profile plots for estimated marginal means of knowledge score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.14 Descriptive statistic of pre-intervention and post-intervention knowledge score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	78.65 (13.19)	82.15 (13.13)
Intervention	75.20 (13.29)	92.07 (8.68)

Table 4.15 Effect of intervention on post-pre mean knowledge score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	3.60 (0.57, 6.64)	12.93 (8.47, 17.39)	32.82 (1)	<0.001
Intervention	16.54 (13.09, 19.99)			
Gender				
Male	9.82 (5.87, 13.78)	0.50 (-4.23, 5.23)	0.04 (1)	0.834
Female	10.32 (7.73, 12.91)			
Monthly income (RM)				
0-580	9.03 (4.65, 13.41)	-2.83 (-9.78, 4.11)[c]		
581-940	11.86 (8.06, 15.67)	2.53 (-3.65, 8.72)[d]	0.65 (2)	0.521
>940	9.33 (5.84, 12.81)	0.30 (-6.34, 6.94)[e]		

No significant interaction between groups and gender [F(1,164)=0.99, p=0.320]

No significant interaction between groups and monthly income [F(2,163)=0.60, p=0.548]

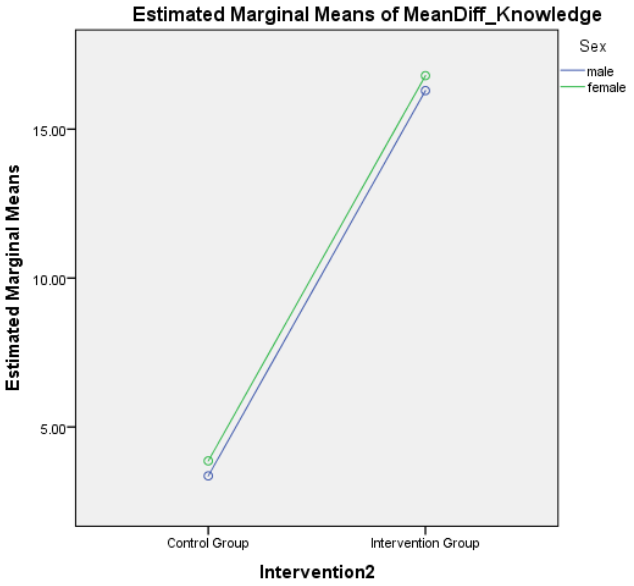
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

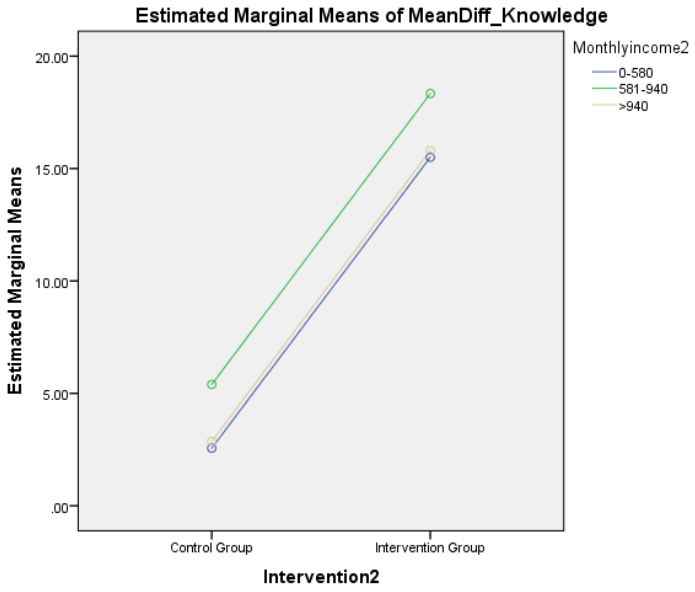
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.1 (a) Comparison of estimated marginal means of knowledge score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of knowledge score changes for control and intervention groups by monthly income

4.5.2 Attitude Section

Table 4.16 showed the descriptive statistics of pre-intervention and post-intervention attitude score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.17. The adjusted mean attitude score changes for control and intervention group were -1.95 and 3.59 respectively. The adjusted mean difference was 5.55 (95% CI: 2.28, 8.81). Those in intervention group showed significantly higher score compare to control group (p=0.001). Gender and monthly income were not significant factors for mean attitude score changes. Multi-way ANOVA analysis showed that there was no significant interaction among groups and gender [F(1,164)=0.11, p=0.733] on attitude score changes. There was significant interaction among groups and monthly income [F(2,163)=5.05, p=0.007] on attitude score changes.

Table 4.18 showed the result for independent T-test for attitude score changes between control and intervention group stratified by monthly income. Monthly income group RM 0-580 and RM >940 showed significant score changes difference between control and intervention groups. Figure 4.2 (a) and (b) showed the profile plots for estimated marginal means of attitude score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. There were also score changes interaction between control and intervention group by monthly income.

Table 4.16 Descriptive statistic of pre-intervention and post-intervention attitude score

Variable	Mean (SD)	
	Preintervention	**Postintervention**
Control	88.27 (7.83)	86.68 (8.79)
Intervention	87.37 (7.84)	92.17 (8.88)

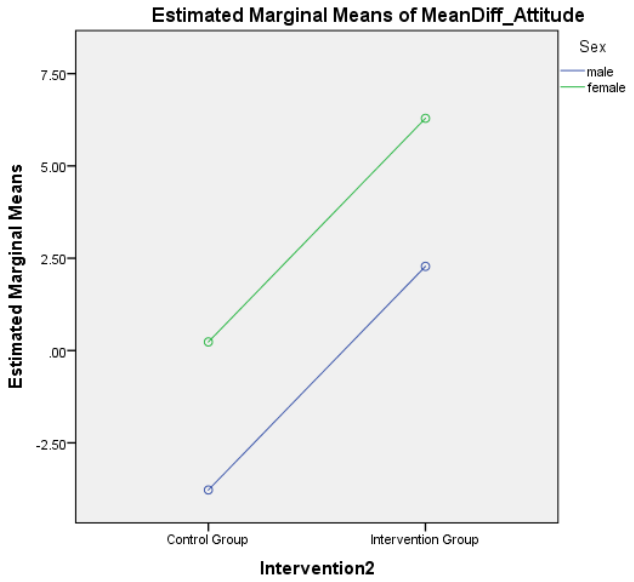
Table 4.17 Effect of intervention on post-pre mean attitude score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-1.95 (-4.17, 0.26)	5.55 (2.28, 8.81)	11.25 (1)	0.001
Intervention	3.59 (1.06, 6.12)			
Gender				
Male	-0.75 (-3.65, 2.14)	3.15 (-0.30, 6.62)	3.23 (1)	0.074
Female	2.40 (0.50, 4.30)			
Monthly income (RM)				
0-580	-0.64 (-3.85, 2.56)	-1.45 (-6.54, 3.63)[c]		
581-940	0.81 (-1.97, 3.59)	-1.49 (-6.03, 3.04)[d]	1.09 (2)	0.337
>940	2.30 (-0.24, 4.85)	2.95 (-1.91,7.82)[e]		

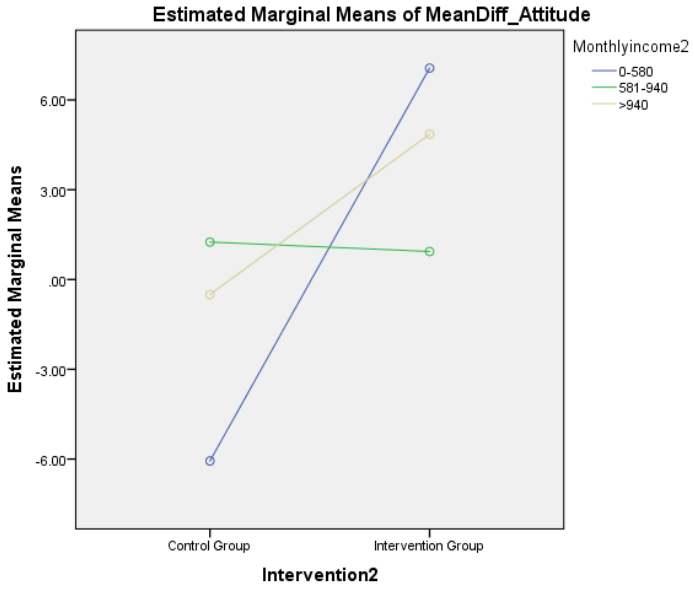
No significant interaction between groups and gender [F(1,164)=0.11, p=0.0.733]
Significant interaction between groups and monthly income [F(2,163)=5.05 , p=0.007]
[a] Adjusted means using Three-way ANOVA analysis
[b] Bonferroni adjustment for 95% CI for difference
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940
[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940
[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580

Table 4.18 Independent T-test for attitude score changes between control and intervention group stratified by monthly income

Monthly income	**Variable**	**Mean (SD)**	**Mean diff. (95% CI)**	**t-statistic (df)**	***p*-value**
0-580	Control	-5.06 (10.64)	13.32		
	Intervention	8.26 (10.16)	(6.71, 19.94)	-4.05 (45)	<0.001
581-940	Control	0.91 (10.53)	1.39		
	Intervention	2.31 (10.09)	(-4.17, 6.96)	-0.50 (54)	0.617
>940	Control	0 (10.10)	5.60		
	Intervention	5.6 (9.91)	(0.71, 10.48)	-2.28 (65)	0.025



(a)



(b)

Figure 4.2 (a) Comparison of estimated marginal means of attitude score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of attitude score changes for control and intervention groups by monthly income

4.5.3 Belief Section

Table 4.19 showed the descriptive statistics of pre-intervention and post-intervention belief score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.20. The adjusted mean belief score changes for control and intervention group were -1.39 and 5.82 respectively. The adjusted mean difference was 7.21 (95% CI: 3.43, 10.99). Those in intervention group showed significantly higher score compare to control group ($p<0.001$). Gender and monthly income were not significant factors for mean belief score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [$F(1,164)=0.51$, $p=0.473$] and groups and monthly income [$F(2,163)=2.91$, $p=0.057$] on belief score changes. Figure 4.3 (a) and (b) showed the profile plots for estimated marginal means of belief score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.19 Descriptive statistic of pre-intervention and post-intervention belief score

Variable	Mean (SD)	
	Preintervention	**Postintervention**
Control	85.86 (8.72)	84.55 (10.97)
Intervention	83.51 (9.14)	90.00 (10.04)

Table 4.20 Effect of intervention on post-pre mean belief score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	**Adj. mean diff. (95% CI)[b]**		
Group				
Control	-1.39 (-3.95, 1.17)	7.21 (3.43, 10.99)	14.22 (1)	<0.001
Intervention	5.82 (2.90, 8.75)			
Gender				
Male	1.86 (-1.48, 5.22)	0.69 (-3.31, 4.71)	0.11 (1)	0.731
Female	2.56 (0.37, 4.76)			
Monthly income (RM)				
0-580	0.26 (-3.44, 3.98)	-2.03 (-7.92, 3.85)[c]		
581-940	2.30 (-0.92, 5.52)	-1.78, (-7.03, 3.46)[d]	1.35 (2)	0.260
>940	4.08 (1.13, 7.04)	3.81 (-1.81, 9.45)[e]		

No significant interaction between groups and gender [F(1,164)=0.51, p=0.473]

No significant interaction between groups and monthly income [F(2,163)=2.91, p=0.057]

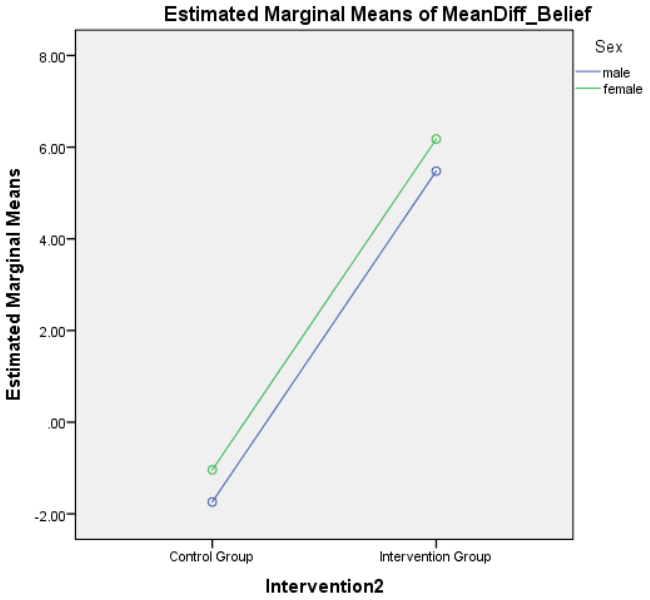
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

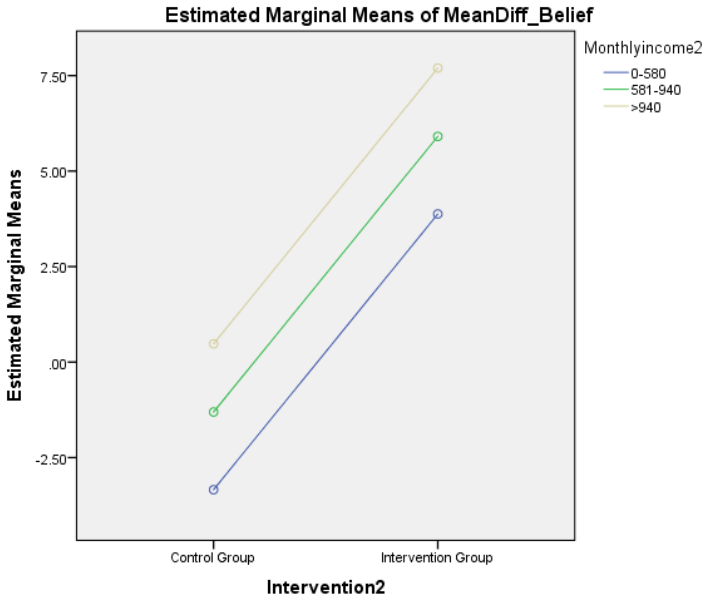
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.3 (a) Comparison of estimated marginal means of belief score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of belief score changes for control and intervention groups by monthly income

4.5.4 Practice Section

Table 4.21 showed the descriptive statistics of pre-intervention and post-intervention practice score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.22. The adjusted mean practice score changes for control and intervention group were 1.06 and 8.41 respectively. The adjusted mean difference was 7.35 (95% CI: 3.64, 11.05). Those in intervention group showed significantly higher score compare to control group ($p<0.001$). Gender and monthly income were not significant factors for mean practice score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [$F(1,162)=0.19$, $p=0.659$] and groups and monthly income [$F(2,161)=0.19$, $p=0.823$] on practice score changes. Figure 4.4 (a) and (b) showed the profile plots for estimated marginal means of practice score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.21 Descriptive statistic of pre-intervention and post-intervention practice score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	77.07 (10.32)	78.28 (12.81)
Intervention	76.81 (9.77)	86.03 (8.93)

Table 4.22 Effect of intervention on post-pre mean practice score different by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	1.06 (-1.47, 3.61)	7.35 (3.64, 11.05)	15.31 (1)	<0.001
Intervention	8.41 (5.55, 11.27)			
Gender				
Male	3.84 (0.54, 7.14)	1.79 (-2.14, 5.74)	0.81 (1)	0.369
Female	5.64 (3.48, 7.79)			
Monthly income (RM)				
0-580	3.52 (-0.12, 7.17)	-0.39 (-6.19, 5.40)[c]		
581-940	3.92 (0.73, 7.11)	-2.86 (-8.01, 2.29)[d]	1.35 (2)	0.260
>940	6.78 (3.89, 9.66)	3.25 (-2.27, 8.78.)[e]		

No significant interaction between groups and gender [F (1, 162)=0.19 , p=0.659]

No significant interaction between groups and monthly income [F (2, 161)=0.19, p=0.823]

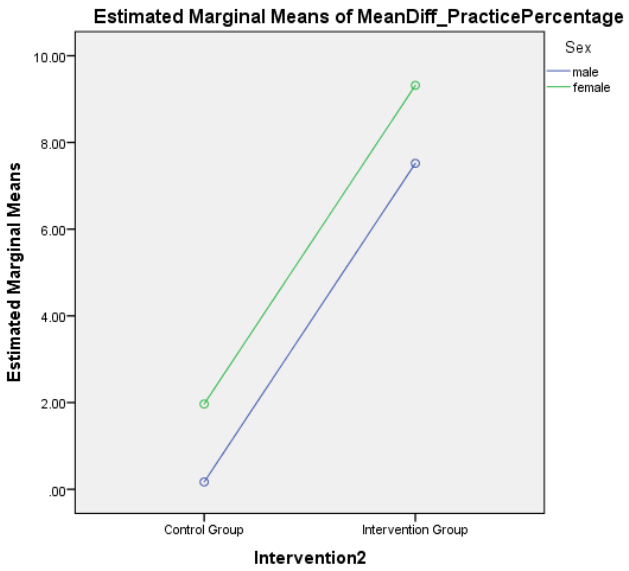
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

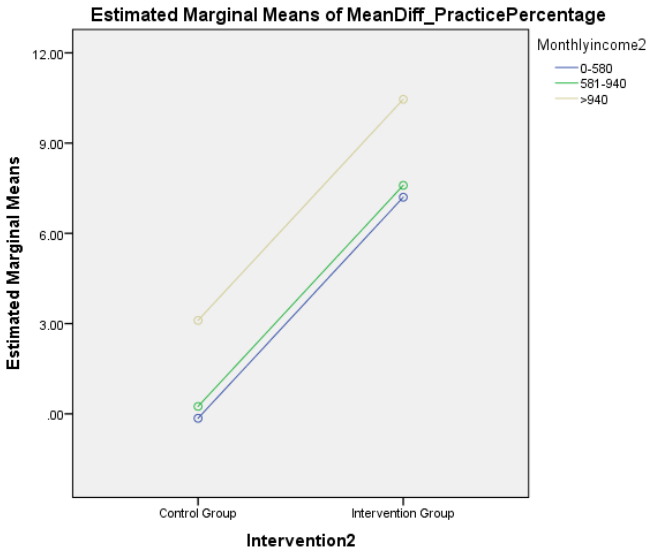
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.4 (a) Comparison of estimated marginal means of practice score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of practice score changes for control and intervention groups by monthly income

4.5.4 Practice P1 Section

P1 was regarding practice of making sure there was no rat in respondent's housing area. Table 4.23 showed the descriptive statistics of pre-intervention and post-intervention P1 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.24. The adjusted mean P1 score changes for control and intervention group were -0.22 and 0.25 respectively. The adjusted mean difference was 0.47 (95% CI: 0.10, 0.84). Those in intervention group showed significantly higher score compare to control group (p=0.013). The adjusted mean P1 score changes for male and female gender were -0.24 and 0.28 respectively. The adjusted mean difference was 0.52 (95% CI: 0.13, 0.92). Female gender showed significantly higher score compare to male (p=0.009). Monthly income were not significant factors for mean P1 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=3.30, p=0.071] and groups and monthly income [F(2,163)=0.36, p=0.699] on P1 score changes. Figure 4.5 (a) and (b) showed the profile plots for estimated marginal means of P1 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.23 Descriptive statistic of pre-intervention and post-intervention P1 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.27 (1.00)	3.10 (0.99)
Intervention	2.95 (1.04)	3.36 (0.90)

Table 4.24 Effect of intervention on pre-post mean P1 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.22 (-0.47, 0.03)	0.47 (0.10, 0.84)	6.31 (1)	0.013
Intervention	0.25 (-0.03, 0.54)			
Gender				
Male	-0.24 (-0.57, 0.08)	0.52 (0.13, 0.92)	6.96 (1)	0.009
Female	0.28 (0.06, 0.98)			
Monthly income (RM)				
0-580	-0.07 (-0.44, 0.29)	-0.20 (-0.78, 0.37)[c]		
581-940	0.13 (-0.18, 0.44)	0.13 (-0.38, 0.65)[d]	0.40 (2)	0.671
>940	-0.00 (-0.29, 0.28)	0.07 (-0.48, 0.62)[e]		

No significant interaction between groups and gender [F(1,164)=3.30, p=0.071]

No significant interaction between groups and monthly income [F(2,163)=0.36, p=0.699]

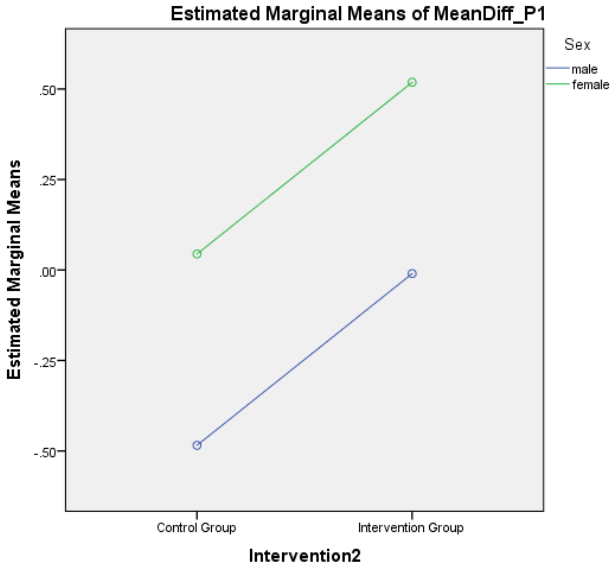
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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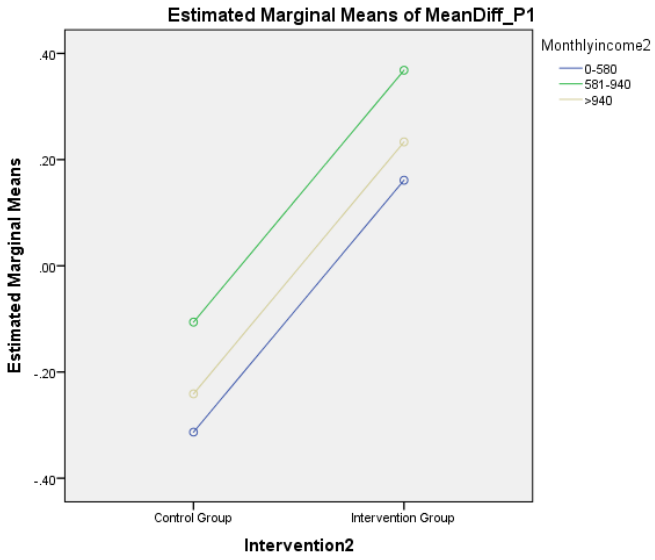


(b)

Figure 4.5 (a) Comparison of estimated marginal means of P1 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P1 score changes for control and intervention groups by monthly income

4.5.5 Practice P2 Section

P2 was regarding practice of recreational activities in area that was declared of leptospirosis outbreak within the past 6 months. Table 4.25 showed the descriptive statistics of pre-intervention and post-intervention P2 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.26. The adjusted mean P2 score changes for control and intervention group were 0.0 and 0.02 respectively. The adjusted mean difference was 0.03 (95% CI: -0.30, 0.36). Those in intervention group were not significantly higher score compare to control group (p=0.848). Gender and monthly income were also not significant factors for mean P2 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.14 , p=0.700] and groups and monthly income [F(2,163)=0.30, p=0.735] on P2 score changes. Figure 4.6 (a) and (b) showed the profile plots for estimated marginal means of P2 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.25 Descriptive statistic of pre-intervention and post-intervention P2 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.70 (0.89)	3.70 (0.69)
Intervention	3.67 (0.86)	3.76 (0.63)

Table 4.26 Effect of intervention on post-pre mean P2 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.00 (-0.23, 0.22)	0.03 (-0.30, 0.36)	0.03 (1)	0.848
Intervention	0.02 (-0.23, 0.28)			
Gender				
Male	-0.07 (-0.36, 0.22)	0.16 (-0.18, 0.51)	0.84 (1)	0.361
Female	0.09 (-0.10, 0.28)			
Monthly income (RM)				
0-580	-0.12 (-0.44, 0.20)	-0.27 (-0.79, 0.24)[c]		
581-940	0.15 (-0.13, 0.43)	0.14 (-0.31, 0.60)[d]	0.82 (2)	0.440
>940	0.00 (-0.25, 0.25)	0.12 (-0.36, 0.62)[e]		

No significant interaction between groups and gender [F(1,164)=0.14 , p=0.700]

No significant interaction between groups and monthly income [F(2,163)=0.30, p=0.735]

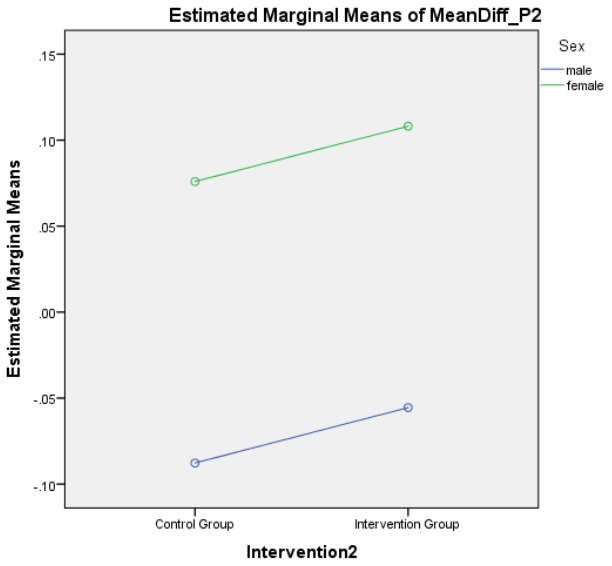
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

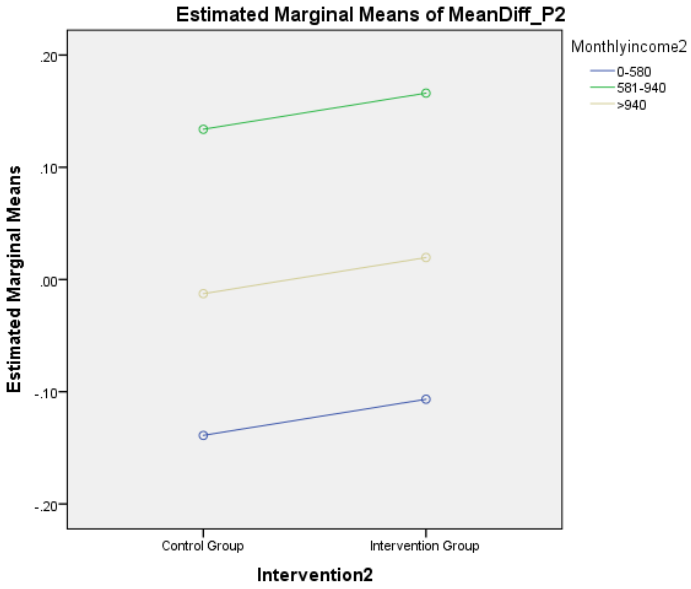
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.6 (a) Comparison of estimated marginal means of P2 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P2 score changes for control and intervention groups by monthly income

4.5.6 Practice P3 Section

P3 was regarding practice of cleaning housing area from garbage. Table 4.27 showed the descriptive statistics of pre-intervention and post-intervention P3 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.28. The adjusted mean P3 score changes for control and intervention group were -0.12 and 0.20 respectively. The adjusted mean difference was 0.33 (95% CI: -0.02, 0.68). Those in intervention group did not showed significantly higher score compare to control group (p=0.070). Gender and monthly income were not significant factors for mean P3 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=2.05 , p=0.154] and groups and monthly income [F(2,163)=0.28, p=0.752] on P3 score changes. Figure 4.7 (a) and (b) showed the profile plots for estimated marginal means of P3 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.27 Descriptive statistic of pre-intervention and post-intervention P3 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.50 (0.78)	3.38 (0.99)
Intervention	3.46 (0.81)	3.67 (0.73)

Table 4.28 Effect of intervention on post-pre mean P3 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.12 (-0.36, 0.12)	0.33 (-0.02, 0.68)	3.31 (1)	0.070
Intervention	0.20 (-0.06, 0.48)			
Gender				
Male	0.04 (-0.27, 0.36)	0.00 (-0.37, 0.38)	0.00 (1)	0.997
Female	0.04 (-0.16, 0.25)			
Monthly income (RM)				
0-580	0.06 (-0.29, 0.41)	0.10 (-0.45, 0.65)[c]		
581-940	-0.04 (-0.34, 0.26)	-0.15 (-0.65, 0.34)[d]	0.28 (2)	0.754
>940	0.11 (-0.16, 0.39)	0.05 (-0.48, 0.58)[e]		

No significant interaction between groups and gender [F(1,164)=2.05 , p=0.154]

No significant interaction between groups and monthly income [F(2,163)=0.28, p=0.752]

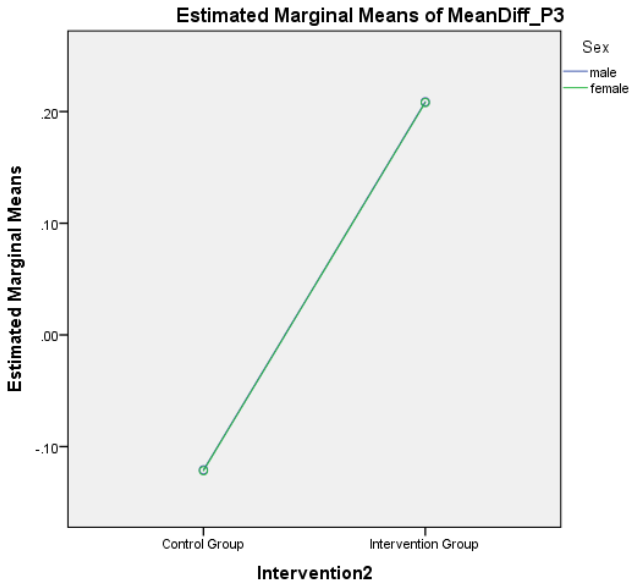
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

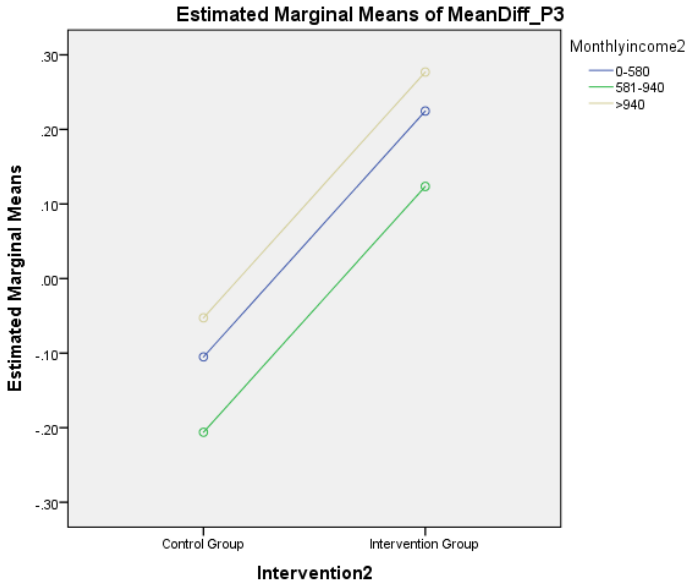
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.7 (a) Comparison of estimated marginal means of P3 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P3 score changes for control and intervention groups by monthly income

4.5.7 Practice P4 Section

P4 was regarding practice of managing garbage when there was a cut on the hand or foot. Table 4.29 showed the descriptive statistics of pre-intervention and post-intervention P4 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.30. The adjusted mean P4 score changes for control and intervention group were -0.46 and 0.02 respectively. The adjusted mean difference was 0.49 (95% CI: 0.08, 0.90). Those in intervention group showed significantly higher score compare to control group (p=0.019). Gender and monthly income were not significant factors for mean P4 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.02 , p=0.876] and groups and monthly income [F(2,163)=0.13, p=0.873] on P4 score changes.

As the assumption for equal variances for multi-way ANOVA was not met, the mean P4 score changes between control and intervention groups were analysed using independent T-test. Table 4.31 showed the result for independent T-test for P4 score changes between control and intervention groups. The intervention group showed significantly higher score compare to control group (p=0.003). Figure 4.8 (a) and (b) showed the profile plots for estimated marginal means of P4 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.29 Descriptive statistic of pre-intervention and post-intervention P4 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.32 (0.86)	2.89 (1.31)
Intervention	3.31 (0.82)	3.48 (0.70)

Table 4.30 Effect of intervention on post-pre mean P4 score different by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.46 (-0.74, -0.18)	0.49 (0.08, 0.90)	5.60 (1)	0.019
Intervention	0.02 (-0.29, 0.34)			
Gender				
Male	-0.14 (-0.78, -0.05)	0.39 (-0.04, 0.82)	3.11 (1)	0.079
Female	-0.02 (-0.26, 0.21)			
Monthly income (RM)				
0-580	-0.39 (-0.80, 0.01)	-0.28 (-0.92, 0.35)[c]		
581-940	-0.11, -0.46, 0.23)	0.03 (-0.54, 0.60)[d]	0.66 (2)	0.515
>940	-0.14 (-0.46, 0.17)	0.25 (-0.36, 0.86)[e]		

No significant interaction between groups and gender [F(1,164)=0.02, p=0.876]

No significant interaction between groups and monthly income [F(2,163)=0.13, p=0.873]

[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

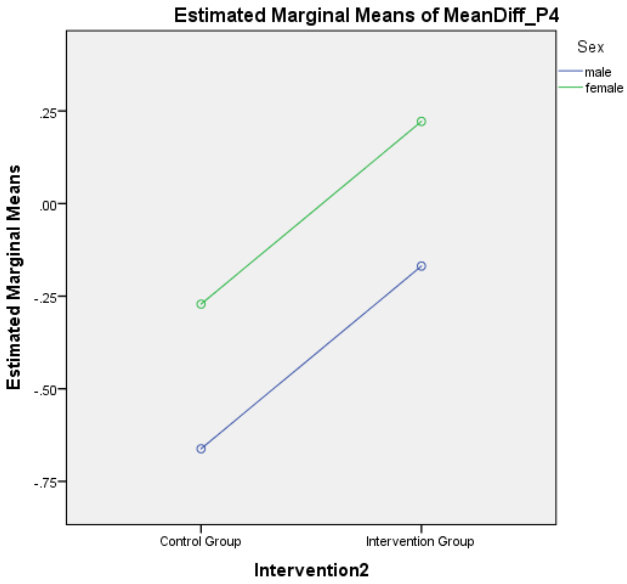
[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580

Table 4.31 Independent T-test for P4 score changes between control and intervention group

Variable	Mean (SD)	Mean diff. (95% CI)	t-statistic (df)	*p*-value
Control	-0.43 (1.54)			
Intervention	0.17 (1.00)	-0.60 (-1.00, -0.21)	-3.04 (150)	0.003*

*equal variances not assumed



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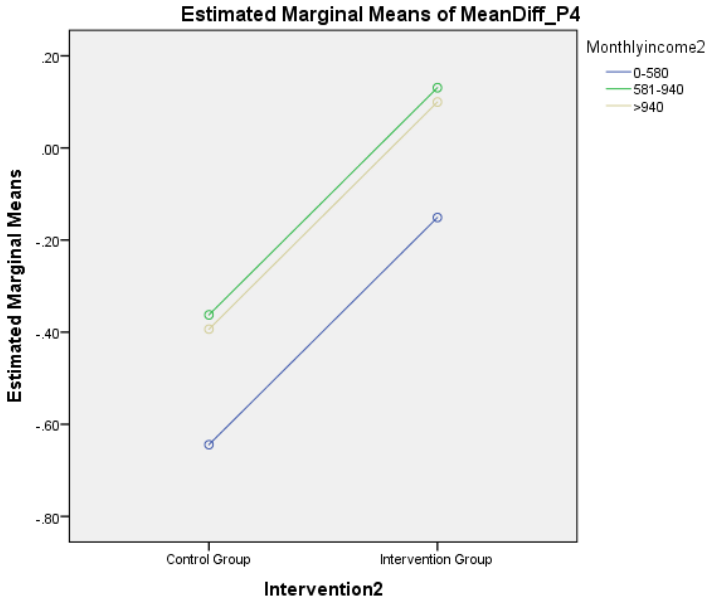


(b)

Figure 4.8 (a) Comparison of estimated marginal means of P4 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P4 score changes for control and intervention groups by monthly income

4.5.8 Practice P5 Section

P5 was regarding practice of eating or drinking when managing garbage. Table 4.32 showed the descriptive statistics of pre-intervention and post-intervention P5 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.33. The adjusted mean P5 score changes for control and intervention group were -0.36 and -0.02 respectively. The adjusted mean difference was 0.34 (95% CI: 0.04, 0.64). Those in intervention group showed significantly higher score compare to control group (p=0.025). Gender and monthly income were not significant factors for mean P5 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=2.20 , p=0.640] and groups and monthly income [F(2,163)=1.76, p=0.174] on P5 score changes.

As the assumption for equal variances for multi-way ANOVA was not met, the mean P5 score changes between control and intervention groups were analysed using independent T-test. Table 4.34 showed the result for independent T-test for P5 score changes between control and intervention groups. The intervention group showed significantly higher score compare to control group (p=0.005). Figure 4.9 (a) and (b) showed the profile plots for estimated marginal means of P5 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.32 Descriptive statistic of pre-intervention and post-intervention P5 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.87 (0.36)	3.50 (1.17)
Intervention	3.86 (0.53)	3.90 (0.46)

Table 4.33 Effect of intervention on post-pre mean P5 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.36 (-0.57, -0.16)	0.34 (0.04, 0.64)	5.10 (1)	0.025
Intervention	-0.02 (-0.25, 0.12)			
Gender				
Male	-0.20 (-0.47, 0.06)	0.01 (-0.30, 0.34)	0.01 (1)	0.908
Female	-0.18 (-0.36, -0.01)			
Monthly income (RM)				
0-580	-0.43 (-0.72, -0.13)	-0.33 (-0.80, 0.13)[c]		
581-940	-0.09 (-0.35, 0.15)	-0.04 (-0.46, 0.37)[d]	2.25 (2)	0.109
>940	-0.05 (-0.29, 0.18)	0.37 (-0.07, 0.82)[e]		

No significant interaction between groups and gender [F(1,164)=2.20 , p=0.640]

No significant interaction between groups and monthly income [F(2,163)=1.76, p=0.174]

[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

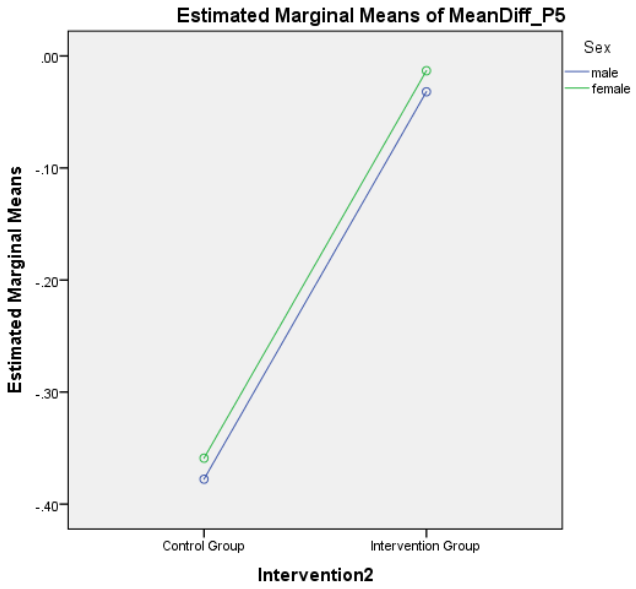
[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580

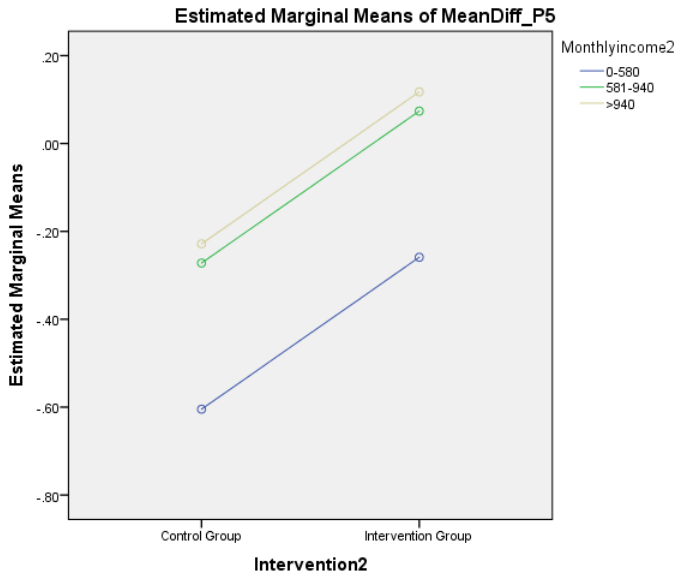
Table 4.34 Independent T-test for P5 score changes between control and intervention group

Variable	Mean (SD)	Mean diff. (95% CI)	t-statistic (df)	*p*-value
Control	-0.38 (1.20)			
Intervention	0.04 (0.62)	-0.41(-0.70, -0.13)	-2.85 (132)	0.005*

*equal variances not assumed



(a)



(b)

Figure 4.9 (a) Comparison of estimated marginal means of P5 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P5 score changes for control and intervention groups by monthly income

4.5.9 Practice P6 Section

P6 was regarding practice of washing hands with soap after managing garbage. Table 4.35 showed the descriptive statistics of pre-intervention and post-intervention P6 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.36. The adjusted mean P6 score changes for control and intervention group were 0.08 and 0.10 respectively. The adjusted mean difference was 0.01 (95% CI: -0.25, 0.28). Those in intervention group did not showed significantly higher score compare to control group (p=0.911). Gender and monthly income were not significant factors for mean P6 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,163)=1.00 , p=0.318] and groups and monthly income [F(2,162)=0.73, p=0.481] on P6 score changes. Figure 4.10 (a) and (b) showed the profile plots for estimated marginal means of P6 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.35 Descriptive statistic of pre-intervention and post-intervention P6 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.65 (0.86)	3.73 (0.66)
Intervention	3.76 (0.65)	3.85 (0.52)

Table 4.36 Effect of intervention on post-pre mean P6 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.08 (-0.09, 0.27)	0.01 (-0.25, 0.28)	0.01 (1)	0.911
Intervention	0.10 (-0.10, 0.31)			
Gender				
Male	0.16 (-0.07, 0.40)	0.14 (-0.14, 0.43)	0.99 (1)	0.320
Female	0.02 (-0.13, 0.17)			
Monthly income (RM)				
0-580	0.02 (-0.24, 0.28)	0.03 (-0.38, 0.45)[c]		
581-940	-0.01 (-0.24, 0.22)	-0.28 (-0.66, 0.08)[d]	2.09 (2)	0.126
>940	0.27 (0.06, 0.48)	0.28 (-0.08, 0.66)[e]		

No significant interaction between groups and gender [F(1,163)=1.00, p=0.318]

No significant interaction between groups and monthly income [F(2,162)=0.73, p=0.481]

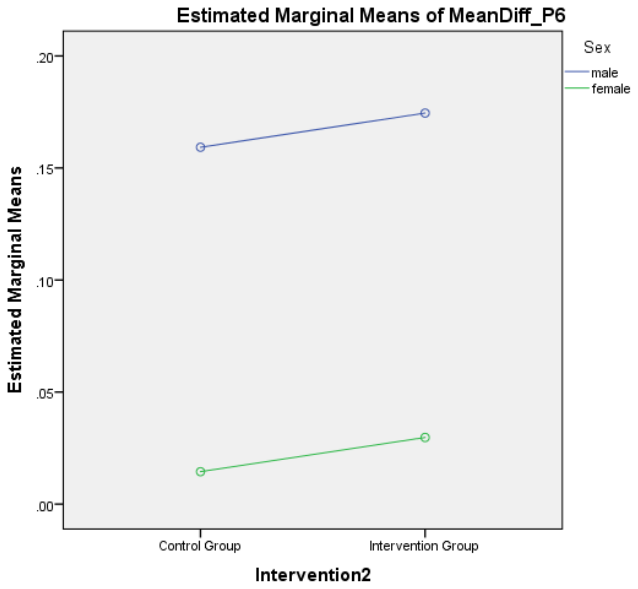
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

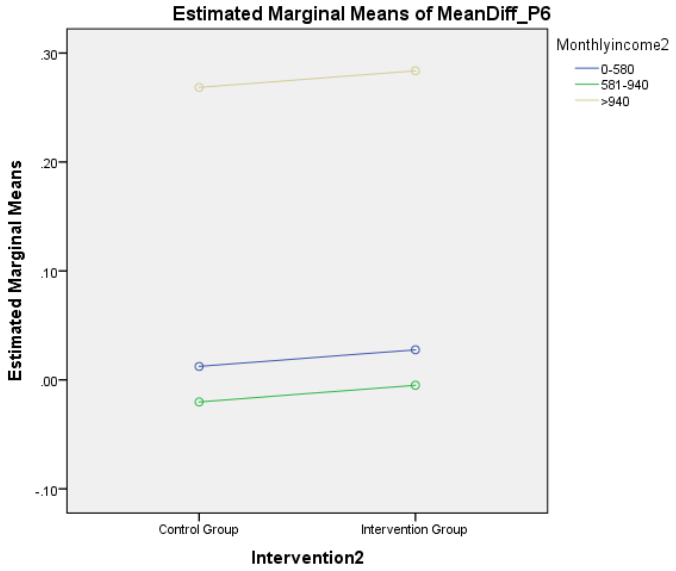
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.10 (a) Comparison of estimated marginal means of P6 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P6 score changes for control and intervention groups by monthly income

4.5.10 Practice P7(i) Section

P7(i) was regarding practice of using glove when managing garbage. Table 4.37 showed the descriptive statistics of pre-intervention and post-intervention P7(i) score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.38. The adjusted mean P7(i) score changes for control and intervention group were 0.55 and 0.80 respectively. The adjusted mean difference was 0.24 (95% CI: -0.20, 0.69). Those in intervention group did not showed significantly higher score compare to control group (p=275). Gender and monthly income were not significant factors for mean P7(i) score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.48, p=0.487] and groups and monthly income [F(2,163)=0.39, p=0.673] on P7(i) score changes. Figure 4.11 (a) and (b) showed the profile plots for estimated marginal means of P7(i) score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.37 Descriptive statistic of pre-intervention and post-intervention P7(i) score

Variable	Mean (SD)	
	Preintervention	**Postintervention**
Control	2.02 (1.25)	2.60 (1.28)
Intervention	2.17 (1.22)	3.02 (1.20)

Table 4.38 Effect of intervention on post-pre mean P7(i) score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.55 (0.25, 0.86)	0.24 (-0.20, 0.69)	1.19 (1)	0.275
Intervention	0.80 (0.46, 1.15)			
Gender				
Male	0.60 (0.20, 0.99)	0.16 (-0.30, 0.64)	0.48 (1)	0.488
Female	0.76 (0.50, 1.02)			
Monthly income (RM)				
0-580	0.68 (0.24, 1.12)	0.01 (-0.68, 0.71)[c]		
581-940	0.67 (0.28, 1.05)	-0.03 (-0.65, 0.59)[d]	0 (2)	0.993
>940	0.70 (0.34, 1.05)	0.01 (-0.65, 0.68)[e]		

No significant interaction between groups and gender [F(1,164)=0.48, p=0.487]

No significant interaction between groups and monthly income [F(2,163)=0.39, p=0.673]

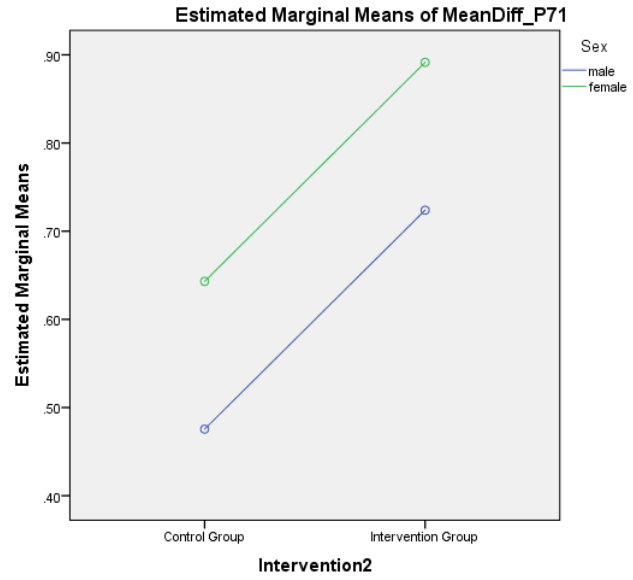
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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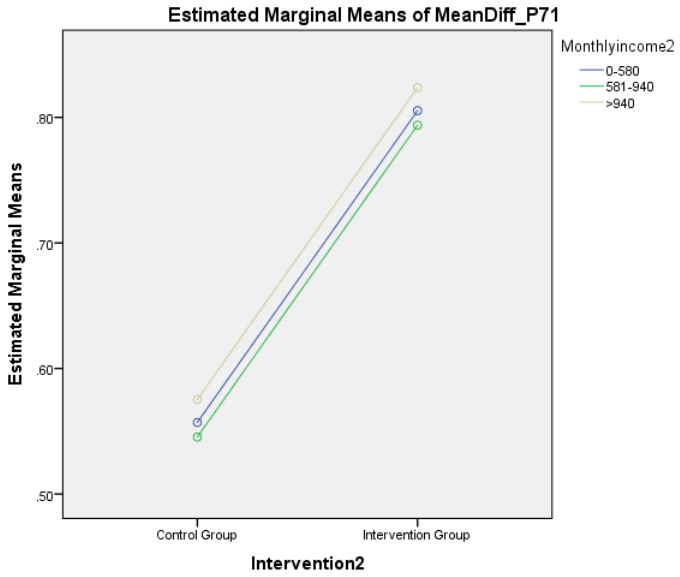


(b)

Figure 4.11 (a) Comparison of estimated marginal means of P7(i) score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P7(i) score changes for control and intervention groups by monthly income

4.5.11 Practice P7(ii) Section

P7(ii) was regarding practice of using boot when managing garbage. Table 4.39 showed the descriptive statistics of pre-intervention and post-intervention P7(ii) score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.40. The adjusted mean P7(ii) score changes for control and intervention group were 0.52 and 1.04 respectively. The adjusted mean difference was 0.52 (95% CI: 0.06, 0.98). Those in intervention group showed significantly higher score compare to control group (p=0.025). Gender and monthly income were not significant factors for mean P7(ii) score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,163)=0.01, p=0.896] and groups and monthly income [F(2,162)=0.01, p=0.991] on P7(ii) score changes. Figure 4.12 (a) and (b) showed the profile plots for estimated marginal means of P7(ii) score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.39 Descriptive statistic of pre-intervention and post-intervention P7(ii) score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	1.96 (1.25)	2.48 (1.32)
Intervention	1.79 (1.03)	2.84 (1.23)

Table 4.40 Effect of intervention on post-pre mean P7(ii) score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.52 (0.21, 0.83)	0.52 (0.06, 0.98)	5.08 (1)	0.025
Intervention	1.04 (0.69, 0.14)			
Gender				
Male	0.82 (0.42, 1.23)	0.08 (-0.40, 0.56)	0.10 (1)	0.742
Female	0.74 (0.47, 1.01)			
Monthly income (RM)				
0-580	0.69 (0.24, 1.15)	-0.08 (-0.79, 0.63)[c]		
581-940	0.77 (0.38, 1.17)	-0.10 (-0.73, 0.53)[d]	0.21 (2)	0.807
>940	0.88 (0.52, 1.24)	0.18 (-0.50, 0.86)[e]		

No significant interaction between groups and gender [F(1,163)=0.01, p=0.896]

No significant interaction between groups and monthly income [F(2,162)=0.01, p=0.991]

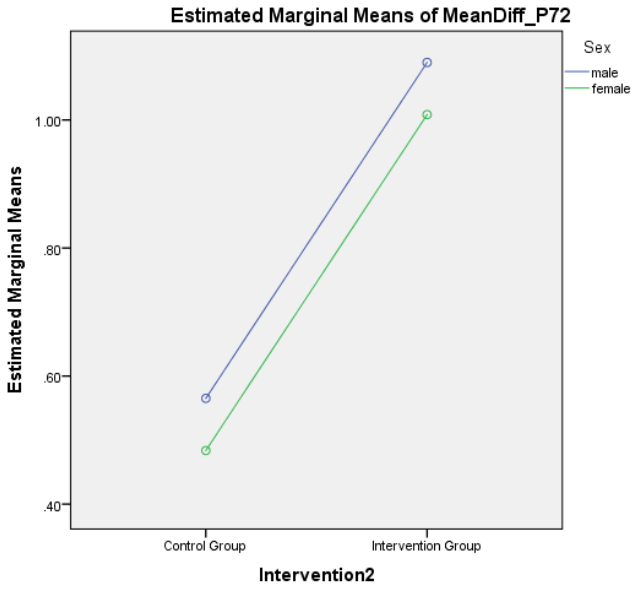
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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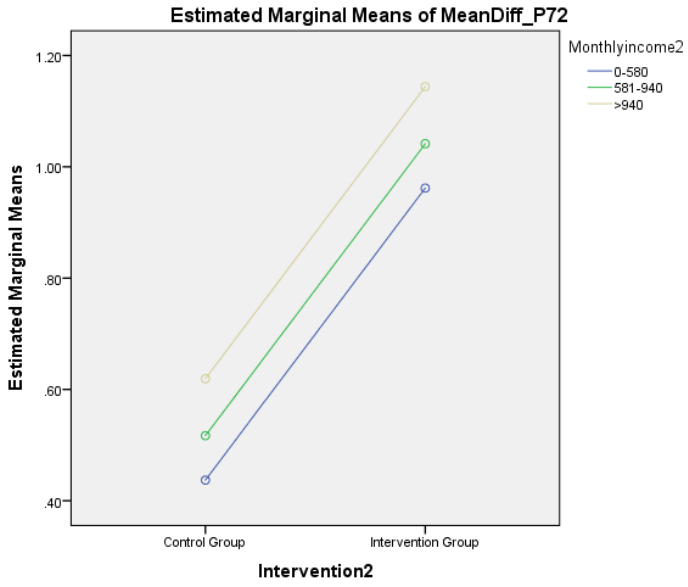


(b)

Figure 4.12 (a) Comparison of estimated marginal means of P7(ii) score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P7(ii) score changes for control and intervention groups by monthly income

4.5.12 Practice P7(iii) Section

P7(iii) was regarding practice of using long sleeve when managing garbage. Table 4.41 showed the descriptive statistics of pre-intervention and post-intervention P7(iii) score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.42. The adjusted mean P7(iii) score changes for control and intervention group were 0.29 and 0.86 respectively. The adjusted mean difference was 0.57 (95% CI: 0.11, 1.04). Those in intervention group showed significantly higher score compare to control group (p=0.015). Gender and monthly income were not significant factors for mean P7(iii) score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,163)=1.52, p=0.218] and groups and monthly income [F(2,162)=1.10, p=0.333] on P7(iii) score changes. Figure 4.13 (a) and (b) showed the profile plots for estimated marginal means of P7(iii) score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.41 Descriptive statistic of pre-intervention and post-intervention P7(iii) score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	2.31 (1.29)	2.60 (1.28)
Intervention	2.46 (1.24)	3.29 (1.07)

Table 4.42 Effect of intervention on post-pre mean P7(iii) score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.29 (-0.02, 0.60)	0.57 (0.11, 1.04)	6.02 (1)	0.015
Intervention	0.86 (0.51, 1.22)			
Gender				
Male	0.64 (0.23, 1.05)	0.13 (-0.36, 0.62)	0.28 (1)	0.596
Female	0.51 (0.24, 0.78)			
Monthly income (RM)				
0-580	0.61 (0.15, 1.07)	0.15 (-0.57, 0.87)[c]		
581-940	0.46 (0.06, 0.85)	-0.20 (-0.84, 0.44)[d]	0.30 (2)	0.741
>940	0.66 (0.30, 1.02)	0.05 (-0.64, 0.74)[e]		

No significant interaction between groups and gender [F(1,163)=1.52, p=0.218]

No significant interaction between groups and monthly income [F(2,162)=1.10, p=0.333]

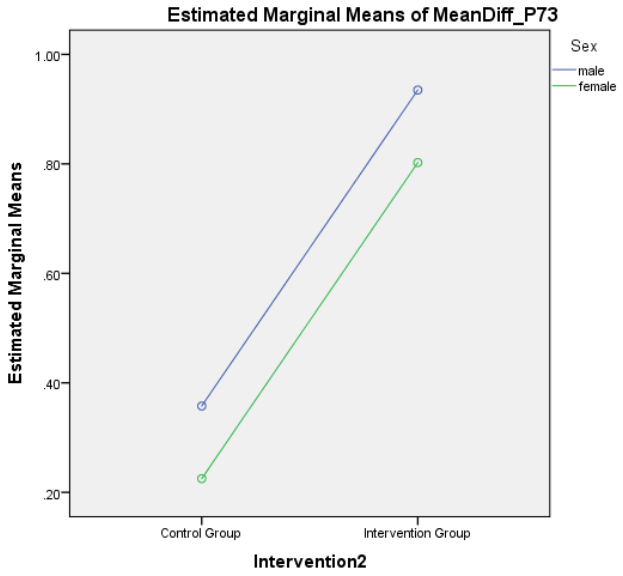
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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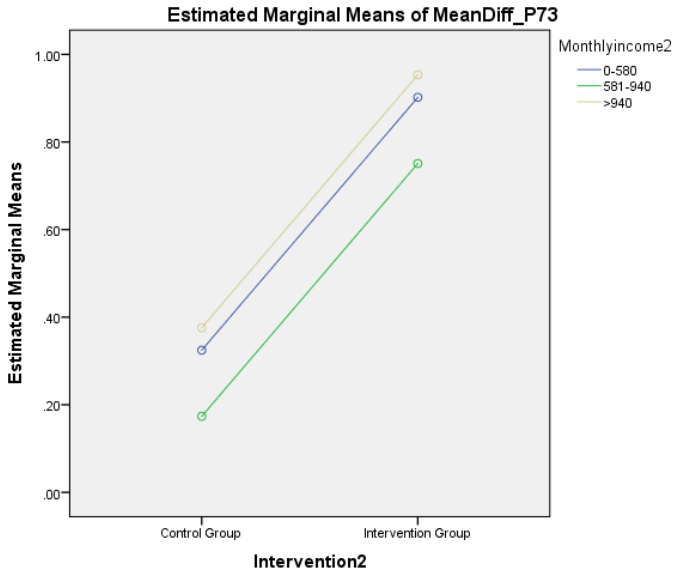


(b)

Figure 4.13 (a) Comparison of estimated marginal means of P7(iii) score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P7(iii) score changes for control and intervention groups by monthly income

4.5.13 Practice P8 Section

P8 was regarding practice of keeping food in covered area. Table 4.43 showed the descriptive statistics of pre-intervention and post-intervention P8 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.44. The adjusted mean P8 score changes for control and intervention group were -0.09 and -0.03 respectively. The adjusted mean difference was 0.06 (95% CI: -0.20, 0.32). Those in intervention group did not showed significantly higher score compare to control group (p=0.660). Gender and monthly income were not significant factors for mean P8 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=1.54, p=0.215] and groups and monthly income [F(2,163)=0.87, p=0.419] on P8 score changes.

As the assumption for equal variances for multi-way ANOVA was not met, the mean P8 score changes between control and intervention groups were analysed using independent T-test. Table 4.45 showed the result for independent T-test for P8 score changes between control and intervention groups. The intervention group score changes were not significantly different compare to control group (p=0.530). Figure 4.14 (a) and (b) showed the profile plots for estimated marginal means of P8 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.43 Descriptive statistic of pre-intervention and post-intervention P8 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.76 (0.75)	3.71 (0.82)
Intervention	3.89 (0.31)	3.92 (0.46)

Table 4.44 Effect of intervention on post-pre mean P8 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.09 (-0.27, 0.09)	0.06 (-0.20, 0.32)	0.19 (1)	0.660
Intervention	-0.03 (-0.24, 0.17)			
Gender				
Male	-0.18 (-0.42, 0.05)	0.25 (-0.03, 0.54)	3.16 (1)	0.082
Female	0.06 (-0.09, 0.22)			
Monthly income (RM)				
0-580	-0.03 (-0.30, 0.22)	0.22 (-0.19,0.64)[c]		
581-940	-0.25 (-0.48, -0.02)	-0.36 (-0.74, 0.01)[d]	2.83 (2)	0.062
>940	0.10 (-0.10, 0.31)	0.14 (-0.25, 0.54)[e]		

No significant interaction between groups and gender [F(1,164)=1.54, p=0.215]

No significant interaction between groups and monthly income [F(2,163)=0.87, p=0.419]

[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

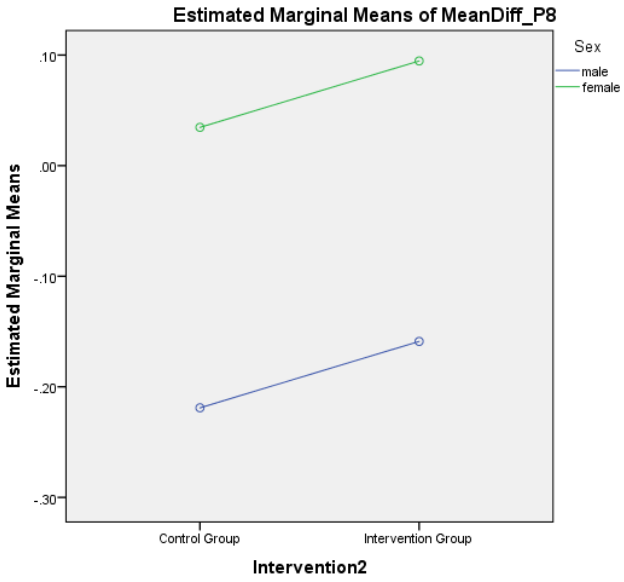
[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580

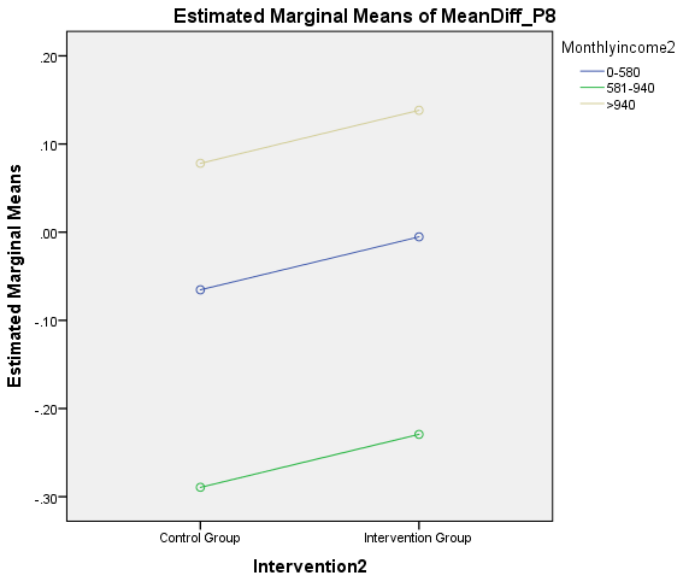
Table 4.45 Independent T-test for P8 score changes between control and intervention group

Variable	Mean (SD)	Mean diff. (95% CI)	t-statistic (df)	*p*-value
Control	-0.05 (1.09)	.		
Intervention	0.04 (0.53)	-0.08 (-0.34, 0.18)	-0.63 (127)	0.530*

*equal variances not assumed



(a)



(b)

Figure 4.14 (a) Comparison of estimated marginal means of P8 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P8 score changes for control and intervention groups by monthly income

4.5.14 Practice P9 Section

P9 was regarding practice of seeking medical treatment when having fever during leptospirosis outbreak. Table 4.46 showed the descriptive statistics of pre-intervention and post-intervention P9 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.47. The adjusted mean P9 score changes for control and intervention group were 0.10 and 0.13 respectively. The adjusted mean difference was 0.03 (95% CI: -0.36, 0.43). Those in intervention group did not showed significantly higher score compare to control group (p=0.876). Gender and monthly income were not significant factors for mean P9 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.45, p=0.503] and groups and monthly income [F(2,163)=0.30, p=0.741] on P9 score changes. Figure 4.15 (a) and (b) showed the profile plots for estimated marginal means of P9 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.46 Descriptive statistic of pre-intervention and post-intervention P9 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.38 (1.13)	3.51 (0.85)
Intervention	3.52 (1.00)	3.71 (0.82)

Table 4.47 Effect of intervention on post-pre mean P9 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.10 (-0.16, 0.37)	0.03 (-0.36, 0.43)	0.02 (1)	0.876
Intervention	0.13 (-0.17, 0.44)			
Gender				
Male	0.10 (-0.24, 0.46)	0.03 (-0.38, 0.45)	0.02 (1)	0.871
Female	0.14 (-0.09, 0.37)			
Monthly income (RM)				
0-580	-0.04 (-0.43, 0.34)	-0.02 (-0.64, 0.60)[c]		
581-940	-0.02 (-0.36, 0.31)	-0.46 (-1.02, 0.08)[d]	2.86 (2)	0.060
>940	0.44 (0.13, 0.75)	0.48 (-0.10,1.08)[e]		

No significant interaction between groups and gender [F(1,164)=0.45, p=0.503]

No significant interaction between groups and monthly income [F(2,163)=0.30, p=0.741]

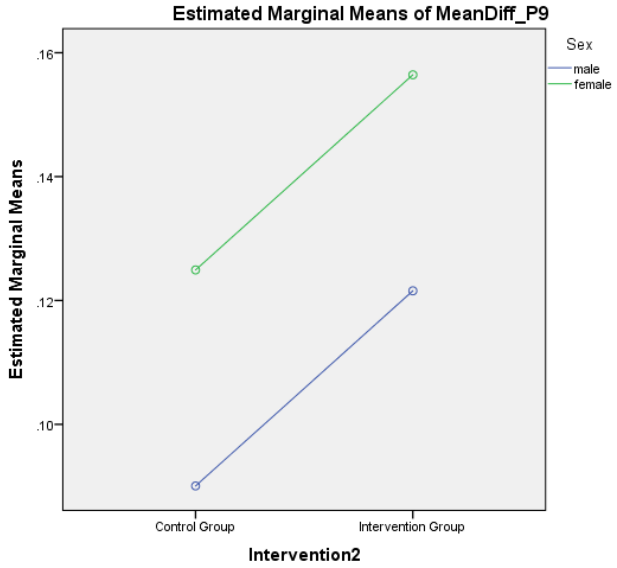
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

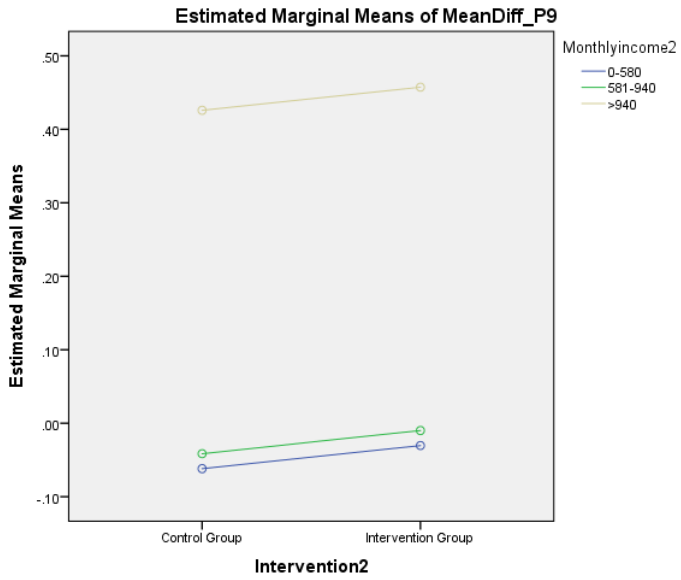
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.15 (a) Comparison of estimated marginal means of P9 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P9 score changes for control and intervention groups by monthly income

4.5.15 Practice P10 Section

P10 was regarding practice of keeping the dustbin closed to avoid rodents. Table 4.48 showed the descriptive statistics of pre-intervention and post-intervention P10 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.49. The adjusted mean P10 score changes for control and intervention group were -0.01 and 0.19 respectively. The adjusted mean difference was 0.21 (95% CI: -0.07, 0.50). Those in intervention group did not showed significantly higher score compare to control group (p=0.146). Gender and monthly income were not significant factors for mean P10 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.35, p=0.551] and groups and monthly income [F(2,163)=0.85, p=0.426] on P10 score changes. Figure 4.16 (a) and (b) showed the profile plots for estimated marginal means of P10 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.48 Descriptive statistic of pre-intervention and post-intervention P10 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.63 (0.83)	3.63 (0.83)
Intervention	3.64 (0.80)	3.95 (0.21)

Table 4.49 Effect of intervention on post-pre mean P10 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.01 (-0.21, 0.18)	0.21 (-0.07, 0.50)	2.13 (1)	0.146
Intervention	0.19 (-0.02, 0.42)			
Gender				
Male	-0.02 (-0.28, 0.23)	0.23 (-0.07, 0.54)	2.18 (1)	0.141
Female	0.20 (0.03, 0.37)			
Monthly income (RM)				
0-580	-0.10 (-0.39, 0.17)	-0.28 (-0.74, 0.16)[c]		
581-940	0.17 (-0.07, 0.42)	-0.01 (-0.42, 0.38)[d]	1.65 (2)	0.194
>940	0.19 (-0.03, 0.42)	0.30 (-0.13, 0.74)[e]		

No significant interaction between groups and gender [F(1,164)=0.35, p=0.551]

No significant interaction between groups and monthly income [F(2,163)=0.85, p=0.426]

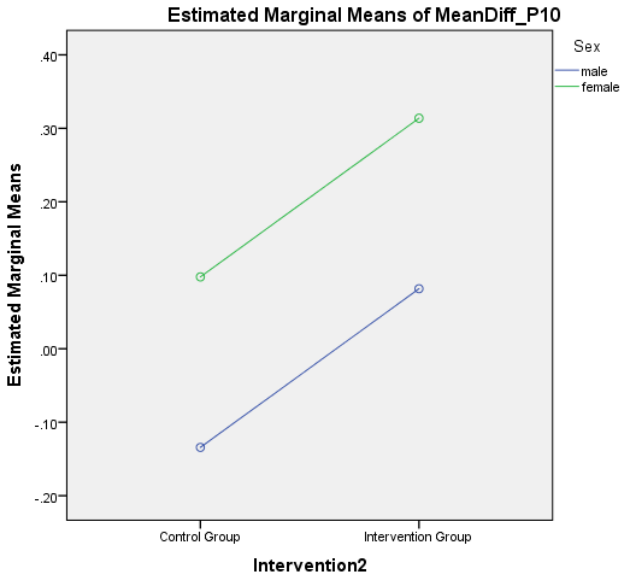
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

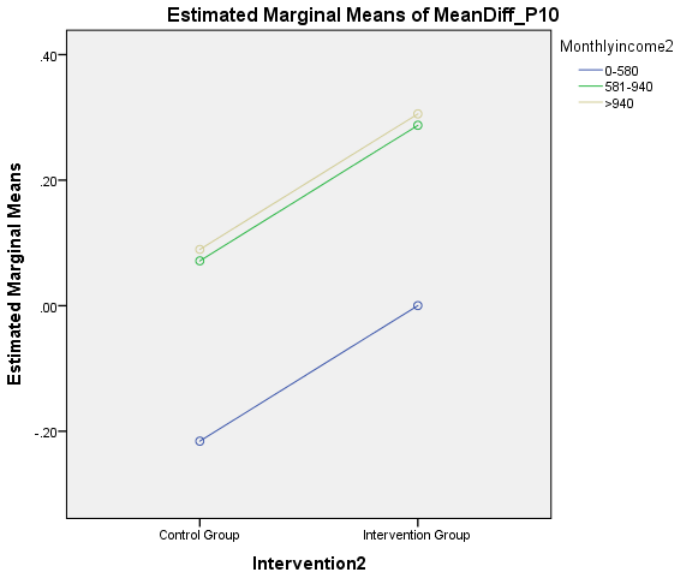
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.16 (a) Comparison of estimated marginal means of P10 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P10 score changes for control and intervention groups by monthly income

4.5.16 Practice P11 Section

P11 was regarding practice of washing the soda cans before drinking. Table 4.50 showed the descriptive statistics of pre-intervention and post-intervention P11 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.51. The adjusted mean P11 score changes for control and intervention group were 0.41 and 1.22 respectively. The adjusted mean difference was 0.80 (95%: CI: 0.30, 1.31). Those in intervention group showed significantly higher score compare to control group (p=0.002). Gender and monthly income were also significant factors for mean P11 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.32, p=0.320] and groups and monthly income [F(2,163)=0.33, p=0.714] on P11 score changes. Figure 4.17 (a) and (b) showed the profile plots for estimated marginal means of P11 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income.

Table 4.50 Descriptive statistic of pre-intervention and post-intervention P11 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	2.45 (1.46)	2.82 (1.35)
Intervention	2.41 (1.53)	3.37 (1.14)

Table 4.51 Effect of intervention on post-pre mean P11 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.41 (0.07, 0.76)	0.80 (0.30, 1.31)	9.89 (1)	0.002
Intervention	1.22 (0.83, 1.61)			
Gender				
Male	1.19 (0.74, 1.64)	0.74 (0.20, 1.27)	7.40 (1)	0.007
Female	0.45 (0.15, 0.74)			
Monthly income (RM)				
0-580	1.15 (0.65, 1.64)	0.76 (-0.02, 1.55)[c]		
581-940	0.38 (-0.05, 0.81)	-0.55 (-1.25, 0.15)[d]	3.12 (2)	0.046
>940	0.93 (0.53, 1.33)	-0.21 (-0.97, 0.53)[e]		

No significant interaction between groups and gender [F(1,164)=0.32, p=0.567]

No significant interaction between groups and monthly income [F(2,163)=0.33, p=0.714]

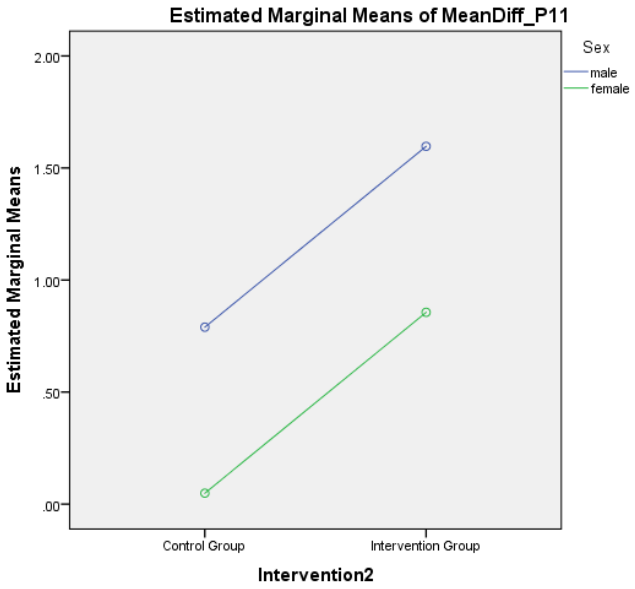
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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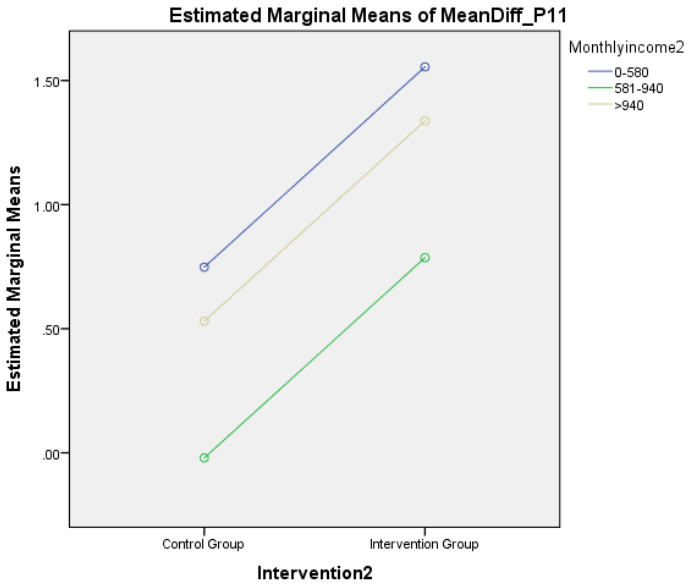


(b)

Figure 4.17 (a) Comparison of estimated marginal means of P11 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P11 score changes for control and intervention groups by monthly income

4.5.17 Practice P12 Section

P12 was regarding practice of washing kitchen utensils before using. Table 4.52 showed the descriptive statistics of pre-intervention and post-intervention P12 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.53. The adjusted mean P12 score changes for control and intervention group were 0.05 and 0.12 respectively. The adjusted mean difference was 0.07 (95% CI: -0.16, 0.31). Those in intervention group did not showed significantly higher score compare to control group (p=0.554). Gender and monthly income were not significant factors for mean P12 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.03, p=0.845] and groups and monthly income [F(2,163)=0.05, p=0.944] on P12 score changes. Figure 4.18 (a) and (b) showed the profile plots for estimated marginal means of P12 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.52 Descriptive statistic of pre-intervention and post-intervention P12 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.69 (0.76)	3.73 (0.59)
Intervention	3.76 (0.65)	3.89 (0.38)

Table 4.53 Effect of intervention on post-pre mean P12 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.05(-0.10, 0.22)	0.07 (-0.16, 0.31)	0.35 (1)	0.554
Intervention	0.12 (-0.05, 0.31)			
Gender				
Male	0.13 (-0.08, 0.34)	0.07 (-0.17, 0.32)	0.35 (1)	0.553
Female	0.05 (-0.08, 0.19)			
Monthly income (RM)				
0-580	0.03 (-0.20, 0.26)	-0.09 (-0.47, 0.27)[c]		
581-940	0.13 (-0.07, 0.33)	0.01 (-0.31, 0.34)[d]	0.23 (2)	0.793
>940	0.11 (-0.07, 0.30)	0.08 (-0.27, 0.43)[e]		

No significant interaction between groups and gender [F(1,164)=0.03, p=0.845]

No significant interaction between groups and monthly income [F(2,163)=0.05, p=0.944]

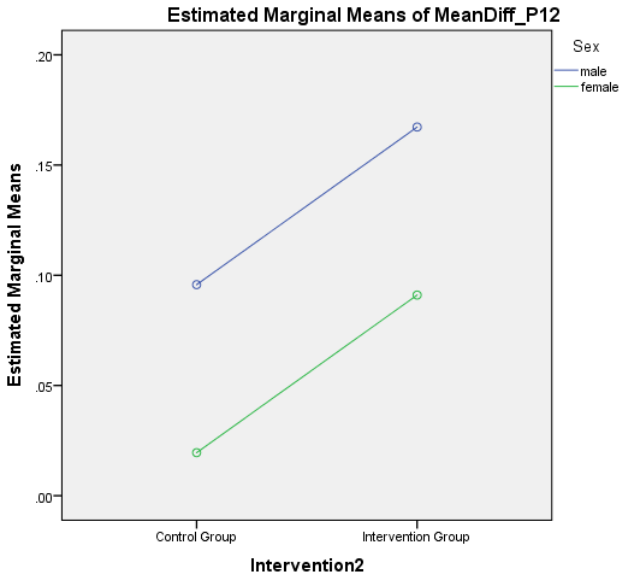
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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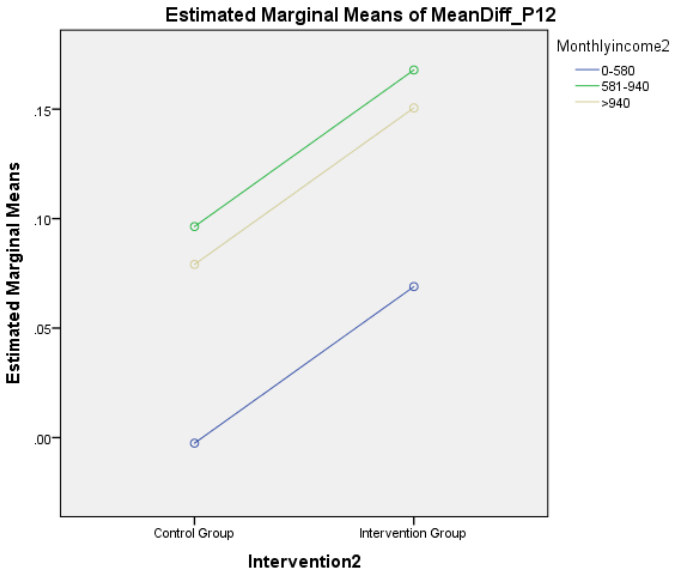


(b)

Figure 4.18 (a) Comparison of estimated marginal means of P12 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P12 score changes for control and intervention groups by monthly income

4.5.18 Practice P13 Section

P13 was regarding practice of choosing clean restaurants. Table 4.54 showed the descriptive statistics of pre-intervention and post-intervention P13 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.55. The adjusted mean P13 score changes for control and intervention group were -0.08 and 0.18 respectively. The adjusted mean difference was 0.10 (95% CI: -0.14, 0.17). Those in intervention group did not showed significantly higher score compare to control group (p=0.185). Gender and monthly income were not significant factors for mean P13 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=1.73, p=0.190] and groups and monthly income [F(2,163)=0.09, p=0.910] on P13 score changes. Figure 4.19 (a) and (b) showed the profile plots for estimated marginal means of P13 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.54 Descriptive statistic of pre-intervention and post-intervention P13 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	3.80 (0.58)	3.72 (0.63)
Intervention	3.87 (0.39)	3.91 (0.32)

Table 4.55 Effect of intervention on post-pre mean P13 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	-0.08 (-0.18, 0.01)	0.10 (-0.04, 0.25)	1.76 (1)	0.185
Intervention	0.18 (-0.09, 0.13)			
Gender				
Male	-0.03 (-0.17, 0.09)	0.01 (-0.14, 0.17)	0.02 (1)	0.879
Female	-0.02 (-0.11, 0.06)			
Monthly income (RM)				
0-580	-0.09 (-0.23, 0.05)	-0.04 (-0.27, 0.18)[c]		
581-940	-0.04 (-0.17, 0.08)	-0.08 (-0.29, 0.12)[d]	1.13 (2)	0.326
>940	0.04 (-0.07, 0.15)	0.13 (-0.09, 0.35)[e]		

No significant interaction between groups and gender [F(1,164)=1.73, p=0.190]

No significant interaction between groups and monthly income [F(2,163)=0.09, p=0.910]

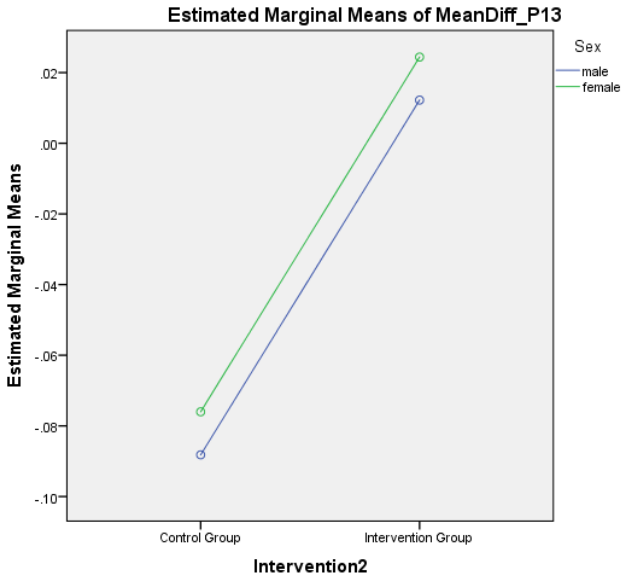
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



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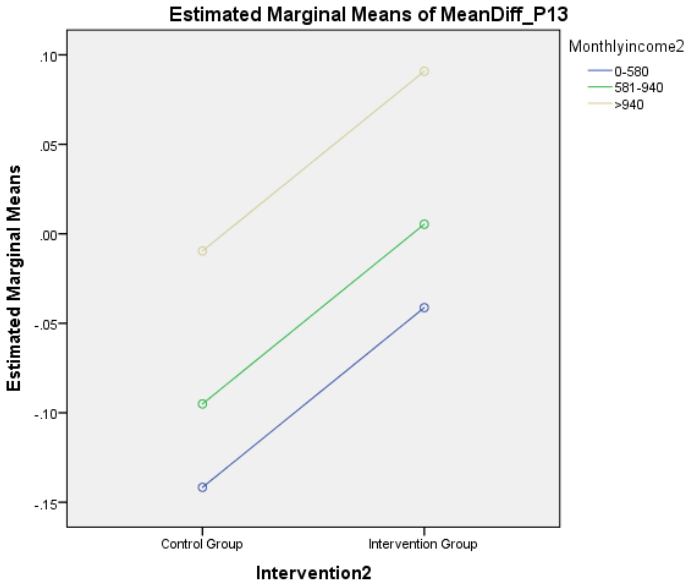


(b)

Figure 4.19 (a) Comparison of estimated marginal means of P13 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P13 score changes for control and intervention groups by monthly income

4.5.19 Practice P14 Section

P14 was regarding practice of covering wound/cut when managing garbage. Table 4.56 showed the descriptive statistics of pre-intervention and post-intervention P14 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.57. The adjusted mean P14 score changes for control and intervention group were 0.36 and 0.22 respectively. The adjusted mean difference was 0.13 (95% CI: -0.29, 0.55). Those in intervention group did not showed significantly higher score compare to control group (p=0.534). Gender and monthly income were not significant factors for mean P14 score changes. Multi-way ANOVA analysis showed that there were no significant interaction among groups and gender [F(1,164)=0.07, p=0.788] and groups and monthly income [F(2,163)=0.73, p=0.483] on P14 score changes. Figure 4.20 (a) and (b) showed the profile plots for estimated marginal means of P14 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups both by gender and monthly income. However, the difference was not statistically significant.

Table 4.56 Descriptive statistic of pre-intervention and post-intervention P14 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	2.65 (1.14)	3.04 (1.15)
Intervention	3.15 (1.04)	3.46 (0.94)

Table 4.57 Effect of intervention on post-pre mean P14 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.36 (0.07, 0.65)	0.13 (-0.29, 0.55)	0.38 (1)	0.534
Intervention	0.22 (-0.09, 0.55)			
Gender				
Male	0.19 (-0.18, 0.56)	0.21 (-0.23, 0.66)	0.86 (1)	0.353
Female	0.40 (0.15, 0.64)			
Monthly income (RM)				
0-580	0.21 (-0.19, 0.63)	-0.07 (-0.73, 0.58)[c]		
581-940	0.29 (-0.06, 0.65)	-0.07 (-0.66, 0.51)[d]	0.18 (2)	0.835
>940	0.37 (0.04, 0.70)	0.15 (-0.47, 0.78)[e]		

No significant interaction between groups and gender [F(1,164)=0.07, p=0.788]

No significant interaction between groups and monthly income [F(2,163)=0.73, p=0.483]

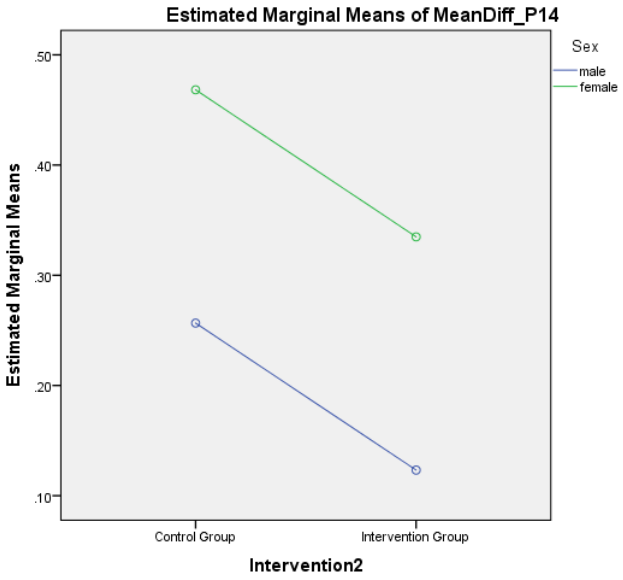
[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

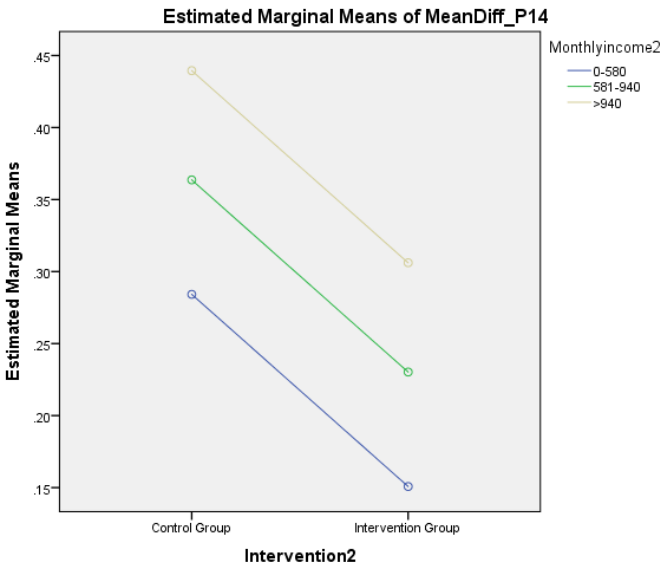
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580



(a)



(b)

Figure 4.20 (a) Comparison of estimated marginal means of P14 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P14 score changes for control and intervention groups by monthly income

4.5.20 Practice P17 Section

P17 was regarding practice of smoking when managing garbage. Table 4.58 showed the descriptive statistics of pre-intervention and post-intervention P17 score. The effect of LHIM intervention after adjusting for the effect of gender and monthly income was presented in Table 4.59. The adjusted mean P17 score changes for control and intervention group were 0.10 and 0.37 respectively. The adjusted mean difference was 0.26 (95% CI: -0.42, 0.95). Those in intervention group did not showed significantly higher score compare to control group (p=0.451). Gender were significant factors for mean P16 score change (p=0.048). Monthly income were not significant factors for mean P17 score changes. Multi-way ANOVA analysis showed that there was no significant interaction among groups and gender [F(1,164)=0.02, p=0.866]. There was significant interaction between groups and monthly income [F(2,163)=5.32, p=0.006] on P17 score changes.

Table 4.60 showed the result for independent T-test for attitude score changes between control and intervention group stratified by monthly income. Monthly income group RM 0-580 and RM >940 showed significant score changes difference between control and intervention group. Figure 4.23 (a) and (b) showed the profile plots for estimated marginal means of P17 score changes for control and intervention groups by gender and monthly income respectively. The score changes were higher in intervention group compared to control groups by gender. For monthly income groups, there were interaction of P17 score changes between control and intervention groups.

Table 4.58 Descriptive statistic of pre-intervention and post-intervention P17 score

Variable	Mean (SD)	
	Preintervention	Postintervention
Control	1.90 (1.89)	2.07 (1.91)
Intervention	1.41 (1.87)	2.01 (1.96)

Table 4.59 Effect of intervention on post-pre mean P17 score changes by adjusting for gender and monthly income (n=170)

Variable	Post-pre mean score different		F-stat (df)	*p*-value
	Adj. mean (95% CI)[a]	Adj. mean diff. (95% CI)[b]		
Group				
Control	0.10 (-0.36, 0.57)	0.26 (-0.42, 0.95)	0.57 (1)	0.451
Intervention	0.37 (-0.16, 0.90)			
Gender				
Male	-0.13 (-0.74, 0.48)	0.74 (0.01, 1.42)	3.97 (1)	0.048
Female	0.60 (0.20, 1.00)			
Monthly income (RM)				
0-580	0.08 (-0.59, 0.76)	-0.36 (-1.44, 0.70)[c]		
581-940	0.45 (-0.13, 1.04)	0.27 (-0.68, 1.23)[d]	0.39 (2)	0.675
>940	0.17 (-0.36, 0.71)	0.09 (-0.93, 1.12)[e]		

No significant interaction between groups and gender [F(1,164)=0.02, p=0.866]

Significant interaction between groups and monthly income [F(2,163)=5.32, p=0.006]

[a] Adjusted means using Three-way ANOVA analysis

[b] Bonferroni adjustment for 95% CI for difference

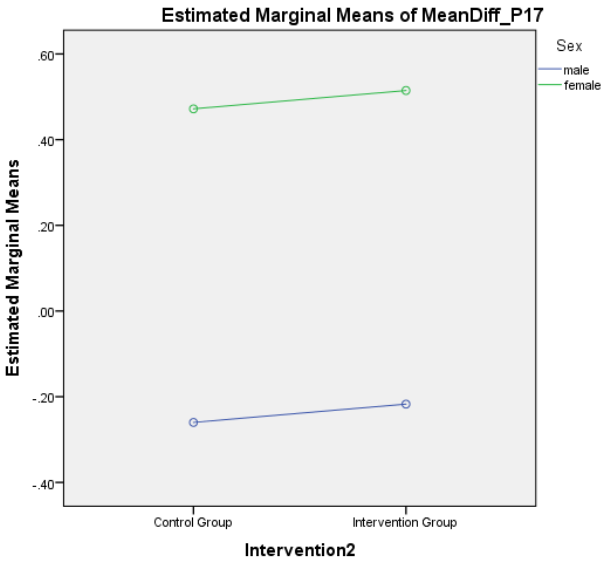
[c] Mean for monthly income RM 0-580 - mean for monthly income RM 581-940

[d] Mean for monthly income RM 581-940 - mean for monthly income RM >940

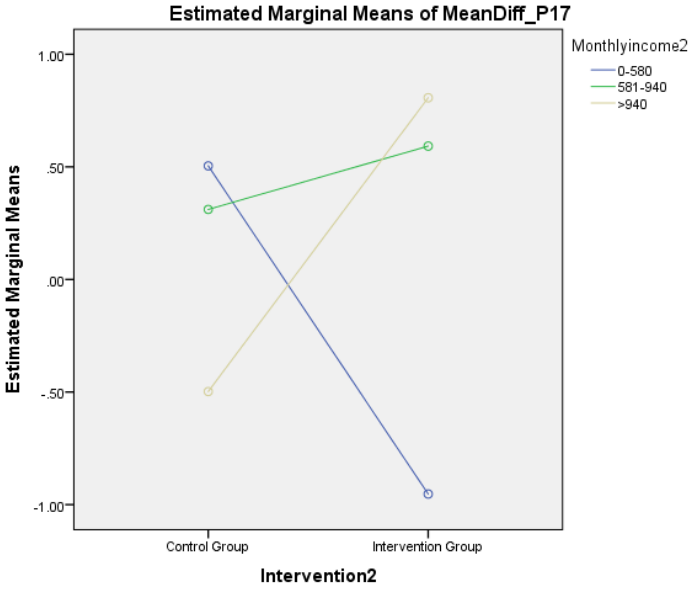
[e] Mean for monthly income RM >940 - mean for monthly income RM 0-580

Table 4.60 Independent T-test for P17 score changes between control and intervention group stratified by monthly income

Monthly income	Variable	Mean (SD)	Mean diff. (95% CI)	t-statistic (df)	*p*-value
0-580	Control	0.68 (2.14)	1.42		
	Intervention	-0.73 (2.15)	(0.06, 2.77)	2.11 (45)	0.040
581-940	Control	0.25 (1.98)	-0.59		
	Intervention	0.84 (2.37)	(-1.79, 0.60)	-0.99 (54)	0.325
>940	Control	-0.40 (1.98)	-1.34		
	Intervention	0.94 (2.19)	(-2.37, -0.32)	-2.63 (65)	0.011



(a)



(b)

Figure 4.21 (a) Comparison of estimated marginal means of P17 score changes for control and intervention groups by gender (b) Comparison of estimated marginal means of P17 score changes for control and intervention groups by monthly income

4.5.23 Summary of Effect of Leptospirosis Health Intervention Module On Practice Items

Table 4.61 Summary of effect of intervention on practice items

Item	Description	Mean difference	*p*-value
P1	Practice of making sure there was no rat in respondent's housing area	0.47 (0.10, 0.84)	0.013
P2	Practice of recreational activities in area that was declared of leptospirosis outbreak	0.03 (-0.30, 0.36)	0.848
P3	Practice of cleaning housing area from garbage	0.33 (-0.02, 0.68)	0.070
P4	Practice of managing garbage when there were cut on the hand or foot	0.49 (0.08, 0.90)	0.019
P5	Practice of eating or drinking when managing garbage.	0.34 (0.04, 0.64)	0.025
P6	Practice of washing hands with soap after managing garbage	0.01 (-0.25, 0.28)	0.911
P7(i)	Practice of using glove when managing garbage	0.24 (-0.20, 0.69)	0.275
P7(ii)	Practice of using boot when managing garbage	0.52 (0.06, 0.98)	0.025
P7(iii)	Practice of using long sleeve when managing garbage	0.57 (0.11, 1.04)	0.015
P8	Practice of keeping food in covered area	0.06 (-0.20, 0.32)	0.660
P9	Practice of seeking medical treatment when having fever during leptospirosis outbreak	0.03 (-0.36, 0.43)	0.876
P10	Practice of keeping the dustbin closed to avoid rodents	0.21 (-0.07, 0.50)	0.146
P11	Practice of washing the soda cans before drinking	0.80 (0.30, 1.31)	0.002
P12	Practice of washing kitchen utensils before using	0.07 (-0.16, 0.31)	0.554
P13	Practice of choosing clean restaurants	0.10 (-0.04, 0.25)	0.185
P14	Practice of covering wound/cut when managing garbage	0.13 (-0.29, 0.55)	0.534
P17	Practice of smoking when managing garbage	0.26 (-0.42, 0.95)	0.451

CHAPTER 5

DISCUSSION

5.1 Sociodemographic Characteristics of Respondent

Leptospirosis is endemic in Malaysia. The wet and humid climate all year round favour the transmission of the disease. Certain groups are at risk of infection due to exposure at their workplace. Two main wet markets in Kota Bharu and Pasir Mas districts were involved in this study where 232 workers participated. Majority of the respondents were female with mean age of 42.6 years old. This is not surprising as many women in Kelantan involved in micro and small businesses (Azmi, 2012). The median (IQR) of monthly income in this study was RM 800 (500) and 59.1% of respondents attained secondary school education. This is consistent with a study on women entrepreneurs in Malaysia by Arshad *et al.* (2015). The researchers noted that majority of workers in Siti Khadijah Market were women age 30 to 50 years old. Majority of women entrepreneurs in the study had secondary school education. As a comparison, a study on seroprevalence of leptospirosis was conducted among market workers and food handlers in urban area of Selangor (Suhailah *et al.*, 2018). In the study, the mean age of respondents were 34 years old and majority were male with 65.8%. However, 56.7% of the respondents in the study were immigrants which might explain the difference.

In contrast, many previous local studies focusing on high-risk occupational groups involved male dominated occupations (Azfar *et al.*, 2014; Ridzuan *et al.*, 2016a; Shafei *et al.*, 2012). This is because high-risk occupations were related to outdoor activities and manual labour. For example, in a seroprevalence study among town

service workers in Kelantan by Shafei *et al.* (2012), all the study respondents were Malay male with mean age of 42.1 years old. Similarly, study by Hafiz *et al.* (2017) among cattle farmers in Kelantan reported majority of the respondents were male with mean age of 50.5 years old. Both type of works were high risk as the workers were exposed to urine of infected animals in the environment.

5.2 Seroprevalence of Leptospirosis Among Wet Market Workers

In the first phase of this study, cross sectional study design was used to determine the seroprevalence of leptospirosis among wet market workers in Kelantan and its associated factors. Cross sectional study design is an observational study design. It is suitable to study prevalence of disease or risk factors in population. Using this design, researchers measures the exposure and outcome at the same time. This study design is appropriate to assess relationship between exposure and disease although causal relationship cannot be established. Furthermore, this design is relatively easy and can be conducted relatively faster than cohort study (Mann, 2003; Setia, 2016). Thus, cross sectional study design is suitable to study the seroprevalence of leptospirosis among wet market workers and its associated factors.

Kota Bharu and Pasir Mas districts were selected as the location for this study. These two districts were reported to have the highest number of confirmed leptospirosis cases in 2014. Out of 620 leptospirosis cases in Kelantan in 2014, 153 (24.7%) cases were reported in Pasir Mas district and 94 (15.2%) cases were reported in Kota Bharu district. The cases in these two districts represent almost 40.0% of all cases of leptospirosis in Kelantan in 2014 (Azimullah *et al.*, 2016). Pasir Mas Market and Siti Khadijah Market were the main wet market in both districts, thus were selected as

study location to examine the seroprevalence of leptospirosis among wet market workers.

Microscopic agglutination test (MAT) was used to identify antibodies against leptospiral in blood samples in this study. Antibodies against leptospiral develop after an infection occur even in asymptomatic and mild disease. The antibodies persist for long period of time after infection subsided. Serosurveillance provide information regarding infection itself rather than the disease, thus reflect on the risk of exposure. By using MAT to detect antibodies, the result provides seroprevalence data on leptospirosis which reflect the risk of the disease in population and high-risk groups. Furthermore, MAT can provide information on circulating serovars compare to ELISA test which give no information on infective serovars. ELISA test use genus-specific antigen that reactive broadly to check for IgM antibodies (WHO, 2003).

In this study, the samples from the respondents were tested against 20 different leptospiral serovars that are common in Malaysia which include six local strains and 14 WHO strains. Other studies in Malaysia were observed using similar serovars for MAT analysis (Ridzuan *et al.*, 2016b; Sakinah *et al.*, 2015; Samsudin *et al.*, 2015; Suhailah *et al.*, 2018). Cut-off point of 1≥100 were used in this study to indicate seropositive MAT analysis (Haake and Levett, 2015). This cut-off point was selected to measure the prevalence of exposure to leptospirosis as compared to higher cut-off point for clinical disease. Several local studies used similar cut-off point for MAT (Ridzuan *et al.*, 2016b; Shafei *et al.*, 2012). However, there are studies which used different cut-off point for seropositive MAT (Dreyfus *et al.*, 2014; Sakinah *et al.*,

2015; Samsudin *et al.,* 2015). Comparison between studies should be done with cautious as different serovars and cut-off point for MAT were used.

Malaysia is a tropical country with warm climate and heavy rainfall. This provide a suitable niche for leptospiral to survive in the environment. Tropical climate also provide home for wide variety of animals that can become a reservoir for leptospiral. Previously, leptospirosis was known to be the disease of the rural and occupation which related to outdoor activities and animals. However recent information showed that the disease has become a public health challenges in urban areas in many countries (Johnson *et al.,* 2004; Ko *et al.,* 1999b). Risk of leptospirosis had been reported at several urban areas in Malaysia including wet markets. Despite the possibility for leptospirosis infection among wet market workers, little information with respect to epidemiology of leptospirosis among this groups available (Benacer *et al.,* 2013a; Benacer *et al.,* 2013b). Thus, this study was carried out to look for evidence of risk for leptospirosis among wet market workers.

The overall seroprevalence of leptospirosis found in this study was 33.6%. The finding was similar between respondents from both Siti Khadijah Market and Pasir Mas Market where 39 out of 116 workers participated in this study were positive for antibodies against leptospiral. A study on seroprevalence of leptospirosis among market workers and food handlers in Selangor was carried out by Suhailah *et al.* (2018). The researchers found that the overall seroprevalence of 46.3% among the participants. As for market workers alone, 52 out of 120 (43.3%) were found positive for antibodies against leptospiral based on MAT analysis. The study used cut-off point titre of 1≥100 for positive MAT. The researchers suggested that rat infestation at

workplace as the reason for the high positive results among participants (Suhailah *et al.*, 2018).

In this study, 184 (79.3%) of the respondents reported sighting of rats or rodents at their workplace. Rats and rodents are highly adaptable animals and live commensally with human (Raj *et al.*, 2009; Zain *et al.*, 2012). They are known as the major reservoirs for leptospiral that contribute to human infection. Places such as wet markets provide source of food and suitable condition for the rats and rodents to breed and populate (Benacer *et al.*, 2013b). The rodents excrete their urine that contain the leptospiral into the surroundings at the wet markets. Workers can get infected when they are in contact with the urine of infected animals (Haake and Levett, 2015).

The evidence that rats and small rodents carry leptospiral have been documented in certain urban area including wet markets in Malaysia. The study by Benacer *et al.* (2013b) found that 6.7% of rodents captured were positive for leptospiral. Serovars Javanica and Batavie were the predominant groups positive in the study. Another study done on samples of water and soils at selected urban sites also found present of leptospiral. The analysis of the samples showed 35 out of 151 samples (23.2%) showed leptospiral isolates (Benacer *et al.*, 2013a). In addition, the results demonstrated that samples of effluent waters from night and wet markets showed presence of more leptospiral compared to samples of lake waters. The authors suggested improper waste disposal attract animals' carrier to market areas which contaminated the environment with leptospiral. This study also yield higher positive results compare to environment samples from rural area of Terengganu and Kelantan by Ridzlan *et al.* (2010). These findings support the risk exposure to leptospirosis at wet market areas.

Beside rodents, stray animal such as cats were also observed at both wet markets during the study. Reports regarding leptospirosis among domestic animals and pets as source of transmission to human are abundant. These stray animals get infected from the contaminated water and soils around the wet markets. In addition, cats prey on rodents as their source of food which can get them infected (Hartmann *et al.*, 2013; Markovich *et al.*, 2012; Rodriguez *et al.*, 2014). Furthermore, the humid and wet condition found at wet markets are suitable for survival of leptospiral in the environment after excreted by the carrier animals (Benacer *et al.*, 2013a).

Another study on high risk occupational group by (Shafei *et al.*, 2012) showed that the seroprevalence of leptospirosis among town service workers in Kelantan was 24.7%. The blood samples were only tested with 18 representing serovars compared to 20 serovars in this study which might explained the lower seropositive results. Town service workers are high risk group as their tasks expose them to contaminated environment. The study also documented higher positive results among garbage collectors compared to town cleaners, landscapers and lorry drivers. This suggested different job tasks resulted in different degree of exposure to leptospirosis (Shafei *et al.*, 2012).

Ridzuan *et al.* (2016b) found seroprevalence level of 28.6% among oil palm plantations workers in Malaysia. The workers were considered high risk groups as they involved in agricultural sector and exposed to infected animal urine (Mohamed-Hassan *et al.*, 2012). Similarly, the study noted different amount of exposure between job categories as fruit collectors had the highest seroprevalence compared to harvesters, pesticide applicators and pruners. Fruit collectors had most contact with

soil and water at the plantations and prone to injury while doing their work which explained the finding.

As for comparison, seroprevalence study on non high-risk population by Sakinah *et al.* (2015) in Ampang Jaya, Selangor noted positive leptospiral antibodies of 27.0%. The researchers in the study used cut-off point of 1≥50 for MAT positive result as compared to 1≥100 in this study. This resulted in high seropositive MAT analysis for leptospiral antibodies among the study respondents. Another community study on seroprevalence of leptospirosis was conducted in four villagers in Kuching, Sarawak by Thayaparan *et al.* (2015). The study used cut-off point of 1≥100 for positive MAT result. The researchers noted that the respondents in the study had high seroprevalence level for leptospirosis (35.9%). This result was expected as the villages selected for the study were located near the wildlife habitation which might explain the high seropositive result (Thayaparan *et al.*, 2015).

Previous studies in Malaysia had successfully isolated 37 serovars of leptospiral from human and animal samples (El Jalii and Bahaman, 2004). The respondents in this study were found to be positive to 18 different types of leptospiral serovars. The predominant serovars were serovars Autumnalis, Sarawak (IMR LEP 175) and Copenhageni (IMR LEP 803/11). The results also showed that market workers in this study can developed different antibodies specific to each serovars in the same samples. This indicate that varies leptospiral serovars circulate in wet market environment and human can get infected repeatedly by different serovars as these serovars-specific antibodies will not necessarily protect infection of other serovars (Izurieta *et al.*, 2008).

Similarly, Ridzuan *et al.* (2016b) found that workers at oil palm plantations were exposed to 9 different serovars with predominant serovars were Sarawak (IMR LEP 175), followed by Patoc, Celledoni, Javanica, Australis, Pyrogenes, Copenhageni (IMR LEP 803/11) and Terengganu (IMR LEP 115). There were also workers tested positive to more than one serovars. Suhailah *et al.* (2018) reported positive leptospiral antibodies against serovars Sarawak (IMR LEP 115), Patoc, Hardjobovis (IMR LEP 27), Terengganu (IMR LEP 115), Australis and Grippotyphosa. Respondents in Kajang were more exposed to local strain of leptospiral compared to Subang Jaya respondent which were more exposed to WHO strain. In this study, different predominant serovars were noted between Siti Khadijah Market and Pasir Mas Market. This indicate different circulating serovars in the environment depending on local epidemiology (Suhailah *et al.*, 2018). They also noted 22 study respondents who had antibodies against more than one serovars. Beside multiple exposure to different serovars, cross-reaction between serovars can happen as reported by Chirathaworn *et al.* (2014).

5.3 Associated Factors for Leptospirosis Among Wet Market Workers

WHO has emphasized on prevention and control of leptospirosis among the community especially the risk groups. These measures depend on the local epidemiological setting of the area due to different source of infection, mechanism of disease transmission, type of animal carriers, environment and human factors (Haake and Levett, 2015; WHO, 2003). Currently there is lack of information regarding transmission determinants among wet market workers. Thus, it is important to examine these factors to provide information for effective preventive and control measures.

5.3.1 Sociodemographic Factors

Sociodemographic factors were examined in this study to determine the associated factors for leptospirosis seropositivity among wet market workers in Kelantan. The factors included in the analysis were age, gender, marital status, monthly income and educational level. At univariate analysis, three factors were found to be associated with leptospirosis. The factors were age, monthly income and educational level. However, at multivariate analysis, only factor age was found to be associated with leptospirosis. The mean (SD) age of respondents in this study was 42.6 (14.68) years old ranging from 18 to 79. The multiple logistic analysis showed that with one-year increase in age increase the odds for leptospirosis by 1.02.

A person can get infected by leptospirosis due to direct or indirect exposure to infected animal urine. This exposure can happen during daily activities which include household chores, occupational and recreational activities. The degree of exposure depends on duration and frequency of the activities. As the age of a person increased, the duration of exposure to contaminated environment will increased. In this study, the wet market workers work in environment which infested by rodents and contaminated environment. The workers were also exposed to leptospiral at home or during recreational activities. With increasing age, the duration and frequency of exposure to the pathogens increase thus increasing the risk of infection. Another possible explanation was relationship between age and development of chronic disease such as diabetes mellitus and weakening of body immune system. Host immune system is one of the host factor that determine development of infection disease in individual beside agent and environmental factors. Chronic disease patient such as diabetes mellitus prone to develop ulcer, delayed wound recovery and reduce immune

defence system. This predispose the patient to infection such as leptospirosis. Dreyfus *et al.* (2014) suggested change of immune system as a possible reason for increasing risk of infection with age in abattoir workers in New Zealand. Studies also documented that increasing age predisposed to more severe leptospirosis infection due to similar circumstances (Haake and Levett, 2015; Ko *et al.*, 1999a; Lopes *et al.*, 2004).

Similar finding was noted in a study on risk factors of human leptospirosis in Argentina by Vanasco *et al.* (2008). The study was a case control study based on laboratory-based surveillance in Argentina. The study found that age more than 30 years old was one of the significant factors associated with leptospirosis. The risk of infection was doubled in age group more than 30 years old compared to younger age group. Beside age, occupation in rural setting, contact with contaminated surface water and flooding were also associated with leptospirosis in the study. In addition, Alavi *et al.* (2013) also reported that age more than 35 years old was significantly associated leptospirosis infection. This study was a cross sectional study done in Khuzestan, Iran where the respondents were living in rural rice farming areas. The authors suggested that the reason for this was young people tend to migrate to urban areas to stay and work thus reducing the risk of infection in the group (Alavi *et al.*, 2013).

In contrast, several studies have reported that age was not a significant factor for leptospirosis infection. Kawaguchi *et al.* (2008) studied the risk factors for leptospirosis in flood prone areas in Lao People's Democratic Republic. Although age was not significantly associated with leptospiral infection, age groups 15 to 24 and 25 to 34 years old had the highest seroprevalence for leptospirosis compared to older age groups. These age groups were prone to risk activities compared to older age group.

In a study among abattoir workers in Nigeria, younger age group was found to be at risk for leptospirosis infection. Age 18 to 25 years old were found to have seven times the risk of leptospirosis infection compared to other groups. In the study, age 18 to 25 years old were the majority of the work force in the abattoir which might explained the finding (Abiayi *et al.*, 2015).

In term of gender, majority of respondents in this study were female (63.4%). Gender was not significantly associated with leptospirosis seropositivity in this study. From the observation during the study, both gender had similar task and job description at the wet market workers. Furthermore, wet markets are confined area where all the workers had similar exposure to contaminated environment. This might explain the non-significant result in this study

Many previous studies on high-risk groups were done in male dominated jobs. As mention before, many of the high-risk occupations involve outdoor tasks and heavy manual jobs. Men are also prone to engage in outdoor risk activities which predispose them to leptospirosis infection compare to women. This pattern explains the finding of male gender associated with leptospirosis in previous studies (Costa *et al.*, 2015; Felzemburgh *et al.*, 2014; Garba *et al.*, 2017b; Lau *et al.*, 2016).

Ethnicity and citizenship were not examined in current study. All respondents in this study were Malaysian with Malay race. In a study on Kelantan leptospirosis cases in 2014, Azimullah *et al.* (2016) reported that majority of the reported cases were Malay ethnicity (88.7%) and Malaysian citizen (92.1%). This finding was based on population study which does not reflect high-risk group. However, a local study on

determinant of leptospirosis seropositivity among municipal service workers in Selangor found that nationality was significantly associated with seropositive result. Foreigners were noted to have higher seroprevalence compared to Malaysian citizens. The finding was probably due to communication barriers which lead to improper work practice and their living condition which exposed them to leptospirosis infection (Samsudin *et al.*, 2015).

Monthly income is related to socioeconomic of the family. There have been several studies in the literature reporting on association between income and socioeconomic level with exposure to leptospirosis. Low socioeconomic status was significantly associated with leptospirosis. This finding was related to living condition where cases of leptospirosis are more common in slum area, poor sanitation, crowding, poor garbage disposal system which provide habitat for stray animals and rodents (Escandon-Vargas *et al.*, 2017; Haake and Levett, 2015). However, monthly income was not significantly associated with leptospirosis seropositivity in this study. This study respondents were from almost similar socioeconomic status and had the same occupational exposure to contaminated environment at the workplace.

Another sociodemographic factor which was found not significant in this study was educational level. More than half (78.9%) of study respondents had secondary school and higher education. However, the level of education was not reflected on preventive practices among the workers. Other than long sleeve shirt which are norm for Malay female, less than 20.0% of them used mask, gloves or boots. In contrast, lower educational level was found to be significantly associated with leptospirosis seropositivity in other studies. Higher educational level was associated with increase

awareness regarding diseases and better health practice that protect them from leptospirosis infection (Dias *et al.*, 2007; Johnson *et al.*, 2004).

5.3.2 Work-related Factors

Leptospirosis has long been known to be associated with outdoor activities and occupations with contact to contaminated environment and water. Usage of suitable personal protective equipment such as glove, mask and boot have been proposed for prevention of infection to leptospirosis during recreational and work activities. Personal protective equipment reduces exposure of mucous membranes and skin to infecting pathogens including leptospiral (Haake and Levett, 2015). In this study workers who did not use glove during work was found to have higher odds to get leptospirosis among wet market workers. However, usage of mask, boots and long sleeves were not associated with leptospirosis infection. In wet markets, the main task of workers is to prepare and trade goods. The activities of wet market workers involved using hands which frequently in contact with environment which could be contaminated with urine of infected animals. The leptospiral can enter the body if there were cuts or wound on the skin where they get into contact with. Moreover, majority of the workers used their hands for eating and drinking at workplace which can transmit the leptospiral into their body system. Thus, usage of glove during work provide protection to hand and wound against leptospirosis infection.

This is supported by a study by Ridzuan *et al.* (2016a) regarding work related factors for leptospirosis among oil palm plantation workers. The researchers found that not using rubber glove, present of hand wound and did not wash hand after working increase the risk for leptospirosis for the workers. Workers at oil palm plantations used

their hand for manual works which require direct contact with environment thus enable transmission of leptospiral. Similarly, the study also noted that usage of other type of personal protective equipment such as boots, long pants and long sleeve shirt were not associated with leptospirosis infection among the workers (Ridzuan *et al.*, 2016a).

In contrast, Dreyfus *et al.* (2014) reported that usage of personal protective equipment did not protect against leptospirosis among abattoir workers in New Zealand. Furthermore, personal protective equipment usage noted to increase the risk of getting infected in the study. The researchers proposed that workers lift their glasses and masks during work to remove fog and sweat. Additionally, accumulation of water in gloves humidifies skin and reduce the natural protection of the skin. These lead to increase infection of leptospirosis among workers who used PPE (Dreyfus *et al.*, 2014). However, the finding was only significant in one subgroup in the study.

Majority of respondents in this study have been working for more than five years at wet markets (57.8%). Surprisingly, analysis on duration of employment demonstrated that it was not significantly associated with leptospirosis seropositivity. Initially it was thought that duration of employment was related to duration and frequency of exposure at workplace. This finding showed that the risk of infection was not due to time factor of exposure but more toward mechanism of the exposure. For example, not using glove during work was found to be significant factor due to direct exposure of body part to the contaminated environment (WHO, 2003). Similarly, number of days working per week was found to be insignificant factor for leptospirosis seropositivity.

Rodents were the main animal carriers for human leptospirosis. Rodents sighting at workplace was investigated for association with leptospirosis seropositivity among wet market workers in this study. More than half of the respondents had reported sighting of rats at their workplace. However, the factor was found to be insignificant. This might be due to wet markets are confined place. Sighting of rodents and rats reflect the present of animal carriers in the area. Since the sighting were reported in both wet markets, the exposure to leptospirosis due to present of animal carriers were insignificant. Although the result of statistical analysis was not significant, the report of rodents sighting was important to identify animal reservoirs for leptospiral at wet market areas. One of the strategy recommended by WHO for prevention and control of leptospirosis include reduction of animal carriers population (WHO, 2003).

Two work related risk behaviours were also examined in this study. Only 14.7% of the respondents reported smoking at work and 54.7% reported eating or drinking at work. Habits of smoking, eating or drinking at work were shown to be associated with leptospirosis seropositivity in previous studies (Campagnolo *et al.*, 2000; Cook *et al.*, 2016). These actions can introduce leptospiral into the oral cavity where it can infect the person. However, these behaviours were found not to be significantly associated with leptospirosis seropositivity among wet market workers in this study. Workers should be educated regarding modifiable risk behaviours to reduce risk of leptospirosis infection.

One preventive behaviour examined in this study was practice of hand washing with soap after work. More than 80.0% of the respondents admit practicing this behaviour. This is important as leptospiral can be transmitted to workers' hand during work.

However, the factor was found to be insignificant to leptospirosis seropositivity in this study. Other studies had reported that this behaviour was protective against leptospirosis infection among workers in high risk groups (Campagnolo *et al.*, 2000; Sulong *et al.*, 2011).

5.3.3 Recreational Activity

Leptospirosis cases due to recreational activities are on the rise. This is due to increasing popularity of recreational water and endurance sports. Exposure to water body and soil near the wildlife habitation during the activities exposed the person to leptospiral in the environment. Activities such as gardening and fishing were also associated with leptospirosis (Haake and Levett, 2015; Monahan *et al.*, 2009). In this present study, recreational activities of the wet market workers were examined to see association with leptospirosis seropositivity. Total of 78 workers (33.6%) reported engaging in recreational activities such as gardening, swimming and fishing. However, no significant association between these activities with leptospirosis seropositivity were observed. This might be due to the target population in this study involved risk group which were exposed mainly at the workplace. Nevertheless, public including the risk groups need to be educate regarding preventive practices when engaging in these activities (WHO, 2003).

5.4 Effect of Leptospirosis Health Intervention Program on Knowledge, Attitude, Belief and Practice Among Wet Market Workers

For the second phase of this study, non-randomised control trial design was used to study the effect of Leptospirosis Health Intervention Module on change of knowledge, attitude, belief and practice score. One of the anticipated threat to internal validity in

this study was issue of contamination of intervention program between intervention and control groups. Contamination happens when intervention is accidentally received by a non-intervention or control group. This will change the outcome of the control group towards the effect of intervention in intervention group causing underestimation of the result of the intervention (Keogh-Brown *et al.*, 2007).

To reduce contamination of intervention in this study, participants were assigned to control and intervention groups based on their workplace. Participants from Siti Khadijah Market were assigned to intervention group and participants from Pasir Mas Market were assigned to control group. This is a study design method to reduce contamination by separating intervention and control groups geographically (Keogh-Brown *et al.*, 2007). However, using this type of design exposed the study to confounders as the characteristics of the groups can differs at baseline. In this study baseline characteristics of respondents were compared between both groups and identified possible confounders were controlled during statistical analysis of data (Axelrod and Hayward, 2006).

In this study, only data from respondents who completed the intervention were included for analysis to assess the effectiveness of the intervention program. All respondents from control and intervention groups who were selected at initial phase of the study answered the preintervention questionnaire. Respondents in the intervention group were explained regarding the protocol of the study including the intervention program. Only respondents who agreed to fully attended the program were selected in this study. The venue for the program located about 100 metres from Siti Khadijah Market. Before the program, the respondents were given reminders in

form of short message service (sms), invitation letter and phone call regarding the program. However, only 92 respondents from intervention group attended the intervention program. During post intervention follow up, only 88 respondents from control group and 82 respondents from intervention group completed the post intervention questionnaire. These figures make up 24.1% and 29.3% loss to follow up in control and intervention groups respectively. The overall loss to follow up in this study was 26.7%. Among the reason for loss to follow up were the rainy monsoon season which coincident with the intervention program. The rainy season affect the attendants to the program due to transportation issue. Respondents also informed of health issues as the reason for absenteeism. There were also workers who transfer to other places or switch job to other area during the study.

For the second phase of this study, data from the respondents from intervention and control groups were collected twice, before the intervention program (pre-intervention) and six weeks after the completion of the program (post-intervention). Since the data were repeated in both groups, Repeated Measures Analysis of Variance (RM ANOVA) was planned to be used for analysing the effect of Leptospirosis Health Intervention Program on knowledge, attitude, belief and practice between intervention and control group. However, the assumptions for normality and homogeneity of variance for RM ANOVA were not met. Violation of assumptions for statistical analysis can lead to inaccurate test statistic and cause invalid inferences from the results (Nimon, 2012). Hence, multi-way ANOVA was used in this study to compare the knowledge, attitude, belief and practice score changes after the intervention between intervention and control groups.

Possible confounders were anticipated in this study as respondents' assignment to intervention and control groups were not randomized. Respondents from Siti Khadijah Market were assigned to intervention group and respondents from Pasir Mas Market were assigned to control group. Without randomization, the characteristics of respondents can be unbalanced and produced confounders. Confounders are variables that correlate with both independent and dependent variables. Confounding variables can affect the true relationship between independent and dependent variables resulting in overestimate or underestimate of the true association. Confounders can be control using randomization, restriction and matching at study design stage. Confounders can also be controlled using statistical methods (Pourhoseingholi *et al.,* 2012).

In this study, multi-way ANOVA analysis was used to control possible confounders (gender and monthly income). Baseline characteristics between control and intervention groups were compared to detect possible confounders. Gender and monthly income were found to be significantly difference between control and intervention groups. As gender and income were influential on health attitude and behaviours, both variables were treated as confounders in this study (Arbiol *et al.,* 2016; Denton *et al.,* 2004). By including gender and monthly income as independent variables in multi-way ANOVA analysis, the adjusted parameters were provided for mean difference between control and intervention groups (McNamee, 2005; Pourhoseingholi *et al.,* 2012).

5.4.1 Effect of Leptospirosis Health Intervention Program on Knowledge

Leptospirosis has long been known as a zoonotic disease. Human usually get infected when they get in contact directly or indirectly with urine of infected animal. The

disease can be prevented and controlled using strategies focusing on controlling source of infection, interrupting route of transmission and protection of human from infection. Source of infection can be controlled by measures such as reduction of animals' reservoir, separation of human from animal reservoirs habitats and immunization of pets and livestock. Transmission of infection can be interrupted by minimizing contact with contaminated environment such as using protective clothing and covering wound where exposure is expected. Human can be protected from infection and disease by increasing awareness regarding leptospirosis among the public especially those who involved in high risk activities. Public can protect themselves from infection by taking necessary measures and recognize the disease at early stage to get treatment. Vaccine for human are available but the use of vaccination in the population is limited due to serovars-specific protection provided by the vaccine (WHO, 2003).

Leptospirosis Health Intervention Module was developed as a health educational module to increase awareness of public especially risk groups regarding leptospirosis. Health education is defined as "activities which raise an individual's awareness, giving the individual the health knowledge required to enable him or her to decide on a particular health action" (Whitehead, 2004). This is to empower the public to practice preventive measures which is in line with WHO target for health promotion which is to enable people to have control upon their own health and their determinants (Kumar and Preetha, 2012). Specifically, the aim of LHIM is to improved knowledge, attitude, belief and practice regarding prevention and control of leptospirosis among public especially the risk groups.

The changes in knowledge, attitude, belief and health practice among respondents in this study can be explained using Health Belief Model (HBM) theory. HBM theory explained that actions toward health are taken by people when they perceive they are susceptible to a disease, they perceive the severity of the disease, they perceive benefits of taking action to prevent the disease outweigh the perceive barriers toward action and they perceive self-efficacy (Glanz *et al.*, 2008). Perceived susceptibility means that the people belief that they are exposed and can contract the disease while perceived severity refers to the consequences of having the disease including clinical and social effects. Both perceived susceptibility and perceived severity are known as perceived threat. For action to be taken, the decision is influence by perceived benefits which are belief regarding benefits of the actions that can be taken. Perceived barriers are negative aspect of the actions such as cost, side effects and time spend to do the action. People also need to belief that they can successfully do the required actions to prevent the disease which is the perceived self-efficacy.

The result in this study showed that LHIM intervention given to intervention group were able to increase the knowledge of the respondents. The mean score of knowledge significantly increase 3.59 points in intervention group compared to -1.95 points in control group after six weeks post intervention after adjusting for gender and monthly income. In the module of LHIM, respondents in intervention group were educate regarding general information of leptospirosis which include the aetiological agent, carrier animals, transmission of the disease, local distribution of the disease, risk groups and risk areas of leptospirosis, symptoms and signs of the disease, clinical staging and severity of the disease. The respondents were also introduced to simple preventive measures that can be taken to avoid infection. These measures include hand

washing technique, proper use of personal protective equipment, wound covering during risk activities and hygienic practices at home and workplace. The barriers to taking preventive steps were also discussed during the intervention program. For example, respondents were informed regarding various types of gloves and mask available in the market which were affordable. Simple measures such as hygienic care at home and workplace, hand wash practice and wound care can prevent from leptospirosis infection.

The contents of the LHIM intervention which was developed by specialists from various field were adequate to educate the respondents in this study regarding basic knowledge about leptospirosis. The contents cover all the aspects of Health Belief Model theory of behavioural changes including perceived susceptibility, perceived severity, perceived benefits, perceived barriers and perceived self-efficacy. The LHIM was developed in Malay language using simple terms to convey medical information to the population. This is important as majority of Malaysian especially in Kelantan speak in Malay language.

The LHIM used varies methods in the intervention program to deliver the health education to the respondents. Lectures, video presentations, role play, small groups discussions, demonstrations and games were used as a medium to convey the messages. Each method has its own advantages and limitations. For example, lecture is effective at presenting facts material to large groups of people, but it is a one-way communication and the degree of acceptance is difficult to measure. Video presentation is an entertaining educational session. It can supplement the content of a lecture, but it has similar limitation as a lecture. As for small groups discussion, it

allows everyone to participate and give their opinions on the subject. However, good facilitator is needed as the discussion can get side tracked. Demonstrations such as hand washing technique and proper use of personal protective equipment can give a better understanding of practical preventive actions compare to lecture or video presentation. However, demonstration is more effective in small group compare to large audience (Allender *et al.*, 2013). By combining multiple methods in the LHIM program, the delivery of health message can be more effective.

As a comparison, an intervention study by Azfar *et al.* (2018) using Leptospirosis Interactive Health Promotion Modul (LIHPM) significantly improved the knowledge score of respondents in intervention group. The study was conducted among town service workers in Kelantan. The mean knowledge score among respondents in intervention group increased from 54.95 to 89.26 compared to 49.67 to 53.57 in control group. In the study, LIHPM was a two days program which includes animation show, interview, mind mapping, practical session, games and role play. Similarly, an educational intervention study by Bipin *et al.* (2010) was conducted in Navsari district, India. Educational messages regarding leptospirosis were given to residents of villages in the district using street plays and poster exhibition. The street plays were performed twice in the villages followed by poster presentation regarding cause, transmission, symptoms and measures to prevent leptospirosis. The study found that the knowledge of the residents was significantly increase after the intervention. The researchers suggested that educational intervention such as plays and posters in local language can be an effective tools to increase awareness in community (Bipin *et al.*, 2010).

5.4.2 Effect of Leptospirosis Health Intervention Program on Attitude

Attitude is define as "a feeling or opinion about something or someone, or a way of behaving" (Online Cambrige Dictionary, 2018). In this study, the effect of LHIM on knowledge, attitude, belief and practice regarding leptospirosis was evaluated. The attitude of risk groups toward leptospirosis was investigated in previous studies (Arbiol *et al.*, 2016; Azfar *et al.*, 2018). Azfar *et al.* (2018) reported that 48.0% of respondents had unsatisfactory attitude towards leptospirosis among town service workers in Kelantan. The study also found that the positive attitude at workplace was lower than positive attitude during off work. In another study by Arbiol *et al.* (2016) on knowledge, attitude and practice toward leptospirosis among Lakeshore Communities of Calamba and Los Banos, Philippines found that the attitude score toward leptospirosis was higher compared to knowledge and practice score among the respondents. The researchers proposed that attitude alone is not adequate to transform to good health practices and need to be complement by sufficient knowledge to be translated to good preventive actions. Beside knowledge and attitude toward the disease, limited fund and low income were cited as possible explanation regarding poor practices of using protective clothing and rats control measures in the study (Arbiol *et al.*, 2016). Study by Azfar *et al.* (2018) showed that with effective intervention program, knowledge regarding the disease improved which also lead to improvement of attitude among the town service workers. In the study, the attitude score of intervention group increased significantly compared to control group after six weeks of intervention. The study demonstrated an increase of attitude score from 66.02 to 93.36 and a decrease of attitude score from 70.61 to 67.18 in intervention and control groups respectively.

In this current study, there was a significant increase in attitude score among respondents in intervention group compared to the control group. This change was attributed to the increase of knowledge after the intervention program. The knowledge score regarding leptospirosis increased significantly in intervention group which indicate the LHIM program were able to successfully convey the health messages to respondents. With better understanding regarding aetiology, transmission, risk factors and severity of the disease, the attitude of respondents toward leptospirosis improved. The finding in this study indicate that attitude of the workers toward leptospirosis is influenced by the knowledge regarding the disease. (Fabrigar *et al.*, 2006; Smedley and Syme, 2001).

5.4.3 Effect of Leptospirosis Health Intervention Program on Belief

In this study, the belief score increased significantly among the intervention group compared to control group after six weeks of intervention program. The belief score regarding leptospirosis among wet market workers increased from 83.51 to 90.00 in intervention group compared to a decreased from 85.86 to 84.55 in control group. This result showed that the belief regarding leptospirosis was congruence with the knowledge and attitude of the workers toward leptospirosis. Evidence from literature suggested that retrieval, formation and modification in beliefs are influenced by attitudes (Marsh and Wallace, 2005). With relevant knowledge given to the workers regarding threat (susceptibility and severity) of leptospirosis and measures for prevention and control of the disease through the LHIM intervention program, the attitude and belief of the respondents can be improved. To our best knowledge, there were lack of literatures on evaluation of belief domain in relation to effect of health education on leptospirosis. Thus, direct comparisons were difficult.

However, in a study on educational program among glaucoma patients by Mohamed *et al.* (2011) demonstrated the intervention improved belief regarding incorrect caused of glaucoma. The study used educational program content include information regarding glaucoma, misconceptions on glaucoma and demonstrations on using eyedrop and eye exercise. The program used local language to deliver the health message. The researchers found that the knowledge, attitude, belief and practice regarding glaucoma improved significantly after the intervention program. These finding showed that good educational health intervention can increased knowledge and improved attitude and belief of the respondents.

5.4.4 Effect of Leptospirosis Health Intervention Program on Practice

The results in this study showed a significant improvement in overall score of practice among respondents in intervention group compare to control group. The practice score increased from 76.81 to 86.03 in intervention group compare to 77.07 to 78.28 in control group. However, when analysed by each practice item, only some aspect of practice scores increased significantly among the respondents in the intervention group compared to control group. The improvement of practice scores were seen in practice of making sure there was no rat in respondent's housing area, practice of managing garbage when there were cut on the hand or foot, practice of eating or drinking when managing garbage, practice of using boot when managing garbage, practice of using long sleeve when managing garbage, practice of washing the cans before drinking and practice of covering wound or cut during flood.

The LHIM intervention program incorporated the knowledge regarding risk activities that exposed to leptospirosis with measure for prevention and control that should be taken to reduce risk of exposure and infection to the disease. These include hygienic practice at home and workplace, practice of managing garbage, practice of using of personal protective equipment, practice of seeking medical treatment and practice of covering wound in risk activities. Demonstration regarding hand washing technique and various use of PPE including mask, glove, boot and long sleeve were integrated into the intervention program. These activities increase awareness of respondents to healthy preventive practice toward leptospirosis. Improvement of knowledge, attitude and belief of the workers resulted in improvement of some aspect of the practice score.

However, there were several practices that show no significant difference of score changes between intervention and control groups after the intervention. These include practice of having recreational activities in area that was declared of leptospirosis outbreak within the past six months, practice of cleaning housing area from garbage, practice of washing hands with soap after managing garbage, practice of using glove when managing garbage, practice of keeping food in covered area, practice of seeking medical treatment when having fever during leptospirosis outbreak, practice of keeping the dustbin closed to avoid rodents, practice of washing kitchen utensils before using, practice of choosing clean restaurants, practice of covering wound and cut when managing garbage, practice of using personal protective equipment during flood and practice of smoking when managing garbage.

Although the score changes in intervention group were not significant when compared to control group, all these practice items showed improvement in score after the

intervention. Many of these practice items already had high baseline preintervention mean score. Out of maximum score of 4.00, these practice items had preintervention mean score of 3.67 for P2 (having recreational activities in area that was declared of leptospirosis outbreak within the past six months), 3.46 for P3 (practice of cleaning housing area from garbage), 3.76 for P6 (practice of washing hands with soap after managing garbage), 3.89 for P8 (practice of keeping food in covered area), 3.52 for P9 (practice of seeking medical treatment when having fever during leptospirosis outbreak), 3.64 for P10 (practice of keeping the dustbin closed to avoid rodents), 3.76 for P12 (practice of washing kitchen utensils before using) and 3.87 for P13 (practice of choosing clean restaurants). Thus, improvement in these practice items were not significantly detectable by statistical analysis. Table 5.1 showed the summary of preintervention and postintervention mean score of the above practice items. Although there was an increase in mean score of practice item P7(i) regarding practice of using glove when managing garbage from 2.17 to 3.02, the control group also had an increase mean score from 2.02 to 2.60 making the difference between changes not significant. As for practice item P17 regarding practice of smoking when managing garbage, majority of respondents in control and intervention group were not smokers thus making the mean score low.

Table 5.1 Summary of preintervention and postintervention mean score of practice items

Item	Group	Preintervention	Postintervention
P2	Control	3.70 (0.89)	3.70 (0.69)
	Intervention	3.67 (0.86)	3.76 (0.63)
P3	Control	3.50 (0.78)	3.38 (0.99)
	Intervention	3.46 (0.81)	3.67 (0.73)
P6	Control	3.65 (0.86)	3.73 (0.66)
	Intervention	3.76 (0.65)	3.85 (0.52)
P8	Control	3.76 (0.75)	3.71 (0.82)
	Intervention	3.89 (0.31)	3.92 (0.46)
P9	Control	3.38 (1.13)	3.51 (0.85)
	Intervention	3.52 (1.00)	3.71 (0.82)
P10	Control	3.63 (0.83)	3.63 (0.83)
	Intervention	3.64 (0.80)	3.95 (0.21)
P12	Control	3.69 (0.76)	3.73 (0.59)
	Intervention	3.76 (0.65)	3.89 (0.38)
P13	Control	3.80 (0.58)	3.72 (0.63)
	Intervention	3.87 (0.39)	3.91 (0.32)

Maximum score of 4.00

The significant improvement in overall mean score of practice this study was similar to study by Azfar *et al.* (2018). In the study, the researchers found that there was significant difference in practice score between intervention and control group of the town service workers after the intervention program. The mean practice score increased from 58.81 to 85.55 and decreased from 60.19 to 59.75 in intervention and control groups respectively. Similarly, the intervention program used in the study included activities such as personal protective equipment hands on, hand washing technique with soap, hand rub technique with sanitizer and role play (Azfar *et al.*, 2018). This supported the evident that effective health education program can promote

positive health behavior. Positive attitude and belief complemented with relevant knowledge will improve the individuals ability to translate prevention measures into action (Arbiol *et al.,* 2016). These findings were also supported by studies on other infectious diseases. Significant association were demonstrated between knowledge, attitude and health related behaviour in study on dengue and rabies (Ali *et al.,* 2013; Dhimal *et al.*, 2014; Sambo *et al.*, 2014). These evidence emphasis on the education as an important tool to improve knowledge, attitude and prevention practices against leptospirosis among risk groups.

5.5 Limitations of Study

Microscopic agglutination test (MAT) was used to detect antibodies against leptospiral in blood samples in this study. This method reflects the exposure that wet market workers had to leptospiral. The exposure to leptospiral can occur not only during work but also during daily activities and recreational activities. Thus, the seroprevalence level measured in this study cannot be specifically assumed to be due to occupational exposure alone. Using other non-high risk group to compare occupational exposure will give a better understanding on occupational risk of wet market workers. However, due to time and budget limitation of this study, no control group was recruited.

This study used validated questionnaire on knowledge, attitude, belief and practice on leptospirosis to collect information from wet market workers. Interviewer guided method was used to collect the information. There was possibility of social desirability bias when respondents reported their information to the researchers. Social desirability bias is a response bias when respondents give answers which are socially acceptable to others. This can lead to over estimating of good behaviour or under estimating of

unfavourable behaviour. Although respondents were informed of confidentiality of information, the possibility of social desirability bias cannot be fully excluded.

For the second phase of this study, health education was used as a method of intervention program for the wet market workers. One of the issue of health education program is contamination to the control group. In this study, the control and intervention groups were assigned according to their respective workplace. This separated the groups geographically as Siti Khadijah Market and Pasir Mas Market located in two different districts. However, the possibility of contamination cannot be fully eliminated as there might still be some communication between workers from both markets.

In the second phase of this study, the effect of intervention program was assessed six weeks after the completion of the program. The results showed the assessment of short term effect of the program on knowledge, attitude, belief and practice on wet market workers. A longer follow up evaluation will give a better perspective on sustainability or retention of intervention program effect especially on modification of behaviour. However, due to limitation of study period, this was not assessed in this study.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The findings from this study showed that the seroprevalence level of leptospirosis among wet market workers were 33.6%. The workers in wet markets were exposed to varies leptospiral serovars and repeated infection can happen with different serovars. This indicate that wet market workers are high risk occupational group for exposure to leptospirosis. Increasing age and not using glove during work were found to increase the odds for leptospirosis seropositivity among the wet market workers in this study. The identification of occupational factor in this study can help authority to focus their intervention in preventing leptospirosis among the workers. For the second phase of this study, the Leptospirosis Health Intervention Program was found to be effective in improve knowledge, attitude, belief and practice among the wet market workers after six weeks of intervention. This program is a good tool for health education among the workers to increased awareness and promote preventive behaviour against leptospirosis infection.

6.2 Recommendation

The findings regarding seroprevalence and distribution of serovars among wet market workers in this study indicate that there is a need to increased awareness and promote preventive behaviour among the workers. These will enable the workers to take control of their own health. Local authorities should take action to reduce the exposure at wet market areas. Hygienic control and rodents' reduction at wet market area can reduce the exposure to leptospirosis at workplace. Currently there is a compulsory course and

typhoid vaccination program for food handler implemented by health and local authority to prevent typhoid outbreak in the community. Similar approach can be used to prevent leptospirosis by conducting program to increase awareness regarding the disease and promote good preventive behaviour among wet market workers. Leptospirosis Health Intervention Module can be used as a tool for education program as it was effective in improving the knowledge, attitude, belief and practice of the workers.

Further research on rodents and environment samples at wet market areas should be conducted to ascertain the epidemiological link between aetiological agent, animal carrier and transmission of leptospiral at wet market areas. This will support the evidence of occupational risk exposure at wet market areas. Long term study on effect of Leptospirosis Health Intervention Program should be carried out to examine sustainability and retainment of improvement in knowledge, attitude, belief and practice among the workers.

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APPENDICES