HUMAN INTESTINAL PARASITES ON BODY SURFACES OF COCKROACHES AT UNIVERSITI SAINS MALAYSIA (USM) HEALTH CAMPUS

by

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"In the name of Allah, Most Gracious, Most Merciful"

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	Percent
°C	Degree celsius
µL	Microliter
µm	Micrometer
µM	Micro molar
mg	Milligram
mL	Milliliter
mm	Millimeter
g	Gram
bp	Base pair
cm	Centimeter
Min/mins	Minute/minutes
Sec/s	Second/seconds
x *g*	Relative centrifugal force
rpm	Revolutions per minute
w/v	Weight/volume
B. germanica	*Blattella germanica*
P. americana	*Periplaneta americana*
spp.	Species
SSP2	Sporozoite Surface Protein 2
PBS buffer	Phosphate-buffered saline buffer
TAE buffer	Tris-acetate-EDTA buffer

RNA	Ribonucleic Acid
DNA	Deoxyribonucleic acid
SSU-rRNA	Small Subunits ribosomal RNA
dNTP	Deoxyribonucleoside triphosphate
IC/IAC	Internal Control/Internal Amplification Control
PCR	Polymerase Chain Reaction
PVPP	Polyvinylpolypyrrolidone
Ct value	Cycle treshold value
DPX	Di-n-butyl phthalate in Xylene
NTC	Non Template Control
a.m	Ante meridiem
p.m	Post meridiem

PARASIT USUS MANUSIA PADA PERMUKAAN BADAN LIPAS DI UNIVERSITI SAINS MALAYSIA (USM) KAMPUS KESIHATAN

ABSTRAK

Di negara membangun, parasit usus manusia biasanya dijumpai. Walau bagaimanapun, kepentingan vermin seperti lipas, penular mekanikal parasit tersebut seringkali diabaikan oleh masyarakat. Kajian ini bertujuan untuk menyaring parasit usus manusia yang ditemui pada permukaan badan lipas di asrama Desasiswa Nurani, USM Kampus Kesihatan, Kubang Kerian, Kelantan. Sejumlah 127 lipas ditangkap di empat lokasi yang berbeza dengan menggunakan balang kosong yang diubahsuai, perangkap lipas komersial dan sarung tangan getah. Di antara dua spesies lipas yang ditangkap, *Periplaneta americana* (125/127; 98.4%) adalah lebih dominan daripada *Blattella germanica* (2/127; 1.6%). Empat atau lima ekor lipas telah dikumpulkan di dalam setiap tiub 50 ml untuk mendapatkan sejumlah 26 kumpulan. Larutan 0.9% salin normal digunakan untuk menanggalkan parasit daripada permukaan badan lipas dengan menggunakan vorteks. Selepas itu lipas tersebut dikeluarkan daripada tiub dan tiub tersebut diemparkan untuk mendapatkan pelet, yang selanjutnya diwarnakan berasingan dengan pewarna *Trichrome* atau pewarna *Modified Acid-Fast*. Selepas itu, parasit disaring menggunakan mikroskop cahaya. Di samping itu, pengesanan DNA *Entamoeba histolytica* dilakukan menggunakan tindak balas rantai polymerase (PCR) dupleks. Asai PCR diinkorporasi dengan gen *Plasmodium falciparum* sebagai kawalan dalaman untuk menolak keputusan DNA negatif palsu. Keputusan mikroskopi menunjukkan tiada ova dan larva cacing atau sista protozoa di dalam semua sampel. Walau bagaimanapun, 34.6% (9/26) kumpulan lipas daripada sejumlah 26 kumpulan didapati mengandungi larva *Strongyloides stercoralis*. Menariknya, analisis PCR

menunjukkan bahawa semua sampel adalah negatif untuk gen *E. histolytica*. Kesimpulannya, tiada ova cacing mahupun sista protozoa atau trofozoit protozoa ditemui di dalam sampel yang dikaji tetapi beberapa sampel didapati mengandungi larva rhabditiform dan dewasa *S. stercoralis*.

HUMAN INTESTINAL PARASITES ON BODY SURFACES OF COCKROACHES AT UNIVERSITI SAINS MALAYSIA (USM) HEALTH CAMPUS

ABSTRACT

In developed countries, human intestinal parasites are commonly found yet; the importances vermins such as cockroaches, mechanical transmitters of the parasite are often neglected by the communities. This study aimed to screen for human intestinal parasites found on body surfaces of cockroaches at Desasiswa Nurani hostel, USM Health Campus, Kubang Kerian, Kelantan. A total of 127 cockroaches were caught at four different locations using modified empty jar, commercial cockroach trap and hand gloves. Between the two species of cockroaches, the dominant species was *Periplaneta americana* (125/127; 98.4%) followed by *Blattella germanica* (2/127; 1.6%). Four or five cockroaches were grouped in each 50 ml tube to obtain a total of 26 groups. A solution of 0.9% normal saline was used to dislodge the parasites from body surfaces of the cockroaches by using vortex. After removing the cockroaches, the solution was centrifuged to obtain the pellet, which was then stained separately with trichrome or modified acid-fast stain. Subsequently, the parasites were screened using a light microscope under low and high magnification. In addition, DNA detection of *Entamoeba histolytica* was performed using an in-house duplex polymerase chain reaction (PCR) assay. The assay was incorporated with *Plasmodium falciparum* gene as the internal control to rule out false negative results. The microscopy results showed that neither helminth ova and larva nor protozoa cysts and trophozoites were observed in the samples. However, 34.6% (9/26) groups of cockroaches were found to harbour *Strongyloides stercoralis*. Interestingly, PCR analysis revealed that all the samples were

negative for *E. histolytica.* In conclusion, neither helminth ova nor protozoa cyst or trophozoite were found in the samples. However, some samples contained rhabditiform larvae and free-living adult of *S. stercoralis*.

CHAPTER 1

INTRODUCTION

1.1 Study Background

Parasite is an organism that lives in or on another organism. It obtains nourishment and protection from its host while offering no benefit in return. Human parasites are often harmful to the body and can cause serious diseases. Human intestinal parasites are organisms that live in the intestinal tract. The two main groups of intestinal parasites are helminths and protozoa. Parasitic helminths and protozoa are commonly found in areas where the personal hygiene and socio-economic status are low (Ajero *et al.*, 2014; Chamavit *et al.*, 2011; El-Sherbini and El-Sherbini, 2011). These unhygienic areas are often infested with cockroaches.

It was reported that intestinal parasites can cause significant morbidity and mortality throughout the world, particularly in undeveloped countries and in persons with co-morbidities (Kucik *et al.*, 2004). However, parasitic infections caused by intestinal helminths and protozoa parasites are also reported among the most prevalent infections in humans in developing countries (Haque, 2007). In developed countries, protozoal parasites are the more common cause of gastrointestinal infections compared to helminths.

There are four common species of intestinal helminthic parasites which also known as geohelminths and soil-transmitted helminths; *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale* and *Necator americanus* (hookworms) while the most common intestinal protozoal parasites are *Giardia* *intestinalis*, *Entamoeba histolytica*, *Cyclospora cayetanensis* and *Cryptosporidium spp.* (Haque, 2007).

Numerous studies have been carried out to determine the prevalences of intestinal parasites among inhabitants of both urban and rural areas in Malaysia (Jamaiah and Rohela, 2005). However, most of the studies were usually targeted at special groups. It were reported that these enteric parasitic diseases are highly prevalent and widely distributed among all ethnic groups and age groups in many parts of Malaysia (Kan, 1988).

It has been estimated that approximately 50 million people worldwide suffer from invasive amoebic infection each year, resulting in 40-100 thousand deaths annually (Haque, 2007). Spread of these protozoal parasites in developing countries mostly occurs through faecal contamination as a result of poor sewage management and poor quality of water (Haque, 2007).

The overall infection rate of parasitic infection among the public in Kuala Lumpur was 6.9% (Jamaiah and Rohela, 2005). *Trichiuris trichiura* was reported to be the most common parasite (4.5%), followed by *A. lumbricoides* (0.8%), *Clonorchis sinensis* (0.8%), hookworm (0.4%) and *E. histolytica* (0.4%) (Jamaiah and Rohela, 2005). The overall prevalence of soil-transmitted helminthic infection was 56.0% (Zulkifli *et al.*, 2000). Previous study reported the predominant helminth found in Kelantan was *A. lumbricoides* while the most common type of infection was a mixed infection of *A. lumbricoides* and *T. trichiura* (Zulkifli *et al.*, 1999). This study reported that the prevalence rate of *A. lumbricoides*, *T. trichiura* and hookworm infections were 47.5%, 33.9% and 6.2% respectively. Interestingly, besides *T. trichiura, A. lumbricoides* and hookworm, *E. histolytica* was also reported to be endemic in Kelantan (Zeehaida *et al.*, 2009). The endemicity of these faecal, soil and water-borne diseases is an indication of the environmental sanitation and socioeconomic status of the community.

Cockroaches are distributed throughout the world and they are among the most notorious insects inhabiting apartments, food handling establishments and health care facilities (Hamu *et al.*, 2014). They have the ability to breed all year long in a suitable environmental conditions. These features of cockroaches, together with their nocturnal activity probably make them widespread. Moreover, adult cockroaches also have the ability to survive without food for several weeks (Hamu *et al.*, 2014).

Cockroaches are likely to be encountered in environments which provide favourable environmental conditions and a ready source of food (Fotedar *et al.*, 1991). Cockroaches generally like warm, moist environments with abundant food (Chamavit *et al.*, 2011). It was stated that sewers and wet, decaying areas are the natural habitats of cockroach (Jirage, 2012). Cockroaches are omnivores, they eat anything organic but prefer food sources such as sweets, cheese, meat products, starches and grease (Alzain, 2013). Besides, they also feed on plants, vegetables and fruits. Cockroaches frequently feed on human faeces, garbage and sewage which provide copious opportunity for them to disseminate pathogenic agents (Al-bayati *et al.*, 2011; Hamid and Shahnaz, 2012).

Many reports revealed that cockroaches are responsible for serious health problems, provoke allergic reactions and even act as vector of human enteric parasites (Fakoorziba *et al.*, 2010; Tatfeng *et al.*, 2005; Thyseen *et al.*, 2004). It was found that cockroaches are known vectors of human enteropathogens as there are reports of the isolation of various human pathogens from these insects (Fotedar *et al.*, 1991). Cockroaches eating and living habits, body structures and mobility make them well adapted for mechanically transmitting diseases.

Cockroaches may disseminate these organisms in many ways, predominantly by depositing them along with their excrement on human food (Robinson, 2005; Uneke, 2007). When they run over food, they will contaminate the food by leaving an oily liquid that has offensive odour or contain bacteria that can cause food poisoning (Ojianwuna, 2014). Some of the diseases caused by these pathogenic helminths and protozoan are amoebiasis, giardiasis and ascariasis which may also be responsible for chronic diarrhea, liver failure, intestinal disturbances and stunted growth in the affected individuals (El-Sherbini and El-Sherbini, 2011).

1.2 Problem Statement and Rationale of The Study

In the last five years, there were neither report nor survey done on human parasitic infections in big towns and cities, although numerous studies were carried out in rural and remote districts of Malaysia. A better sanitary facilities in big towns have reduced the transmission of intestinal parasitic infections from human faeces to uncovered food by cockroaches. However, cockroaches are still able to reach septic tanks in houses, restaurants, canteens and kitchens located in big towns. Hence, these pests have the potential to spread the parasites via their body surfaces to food consumed by urban folks. Therefore, this pilot observational study attempts to screen the presence of human intestinal parasites on external body surfaces of cockroaches found in USM Health Campus. In addition, the endemicity of *E. histolytica* reported in Kelantan in 2009 make it more interesting to further screen the samples for *E. histolytica* gene.

1.3 Study Objectives

General Objective:

To screen human intestinal parasites on external body surfaces of cockroaches found at Desasiswa Nurani, USM Health Campus.

Specific Objectives:

1. To identify the cockroach species at Desasiswa Nurani, USM Health Campus.
2. To identify the species of protozoa and helminths found on the external body surfaces of cockroaches caught at kitchen and toilets of Desasiswa Nurani Cafetaria and from rooms and drains of Desasiswa Nurani.
3. To further screen for *E. histolytica* in the samples collected by using in-house duplex polymerase chain reaction (PCR) assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Human Intestinal Parasites Transmitted by Cockroaches

2.1.1 Common Human Intestinal Helminth

2.1.1(a) *Strongyloides stercoralis*

Morphology

The global prevalence of *S. stercoralis* is unknown, but experts estimate that there are between 30–100 million infected persons worldwide (CDC, 2014). *S. stercoralis* is found more frequently in the socioeconomically disadvantaged, in institutionalized populations and in rural areas. It is often associated with agricultural activities. The most common way of becoming infected with *S. stercoralis* is by contacting soil that is contaminated with *S. stercoralis* larvae.

Adult male is shorter and broader than female *S. stercoralis*. Female adult is translucent and very small (Length: 2.5 mm; Breadth: 0.4–0.5 mm). The lifespan is around 1 year. Each female lays 30–40 partially embryonated eggs per day in the mucosal epithelium of the intestine. Eggs of *S. stercoralis* are rarely found in stool. The eggs resemble eggs of hookworms but it is much smaller, measuring 50-80 μm in length and 30-35 μm in width. It is oval, transparent and has a very thin shell. There are two types of *S. stercoralis* larvae which are rhabditiform larvae and filariform larvae.

Life cycle

The *S. stercoralis* life cycle is more complex than that of most nematodes with its alternation between free-living and parasitic cycles and its potential for autoinfection and multiplication within the host (CDC, 2015). Two types of cycles exist.

In free-living cycle, the rhabditiform larvae passed in the stool can either become infective filariform larvae (direct development) or free living adult males and females, that mate and produce eggs from which rhabditiform larvae hatch (CDC, 2015). Eventually, it will become infective filariform larvae. The filariform larvae penetrate the human host skin to initiate the parasitic cycle.

In parasitic cycle, filariform larvae in contaminated soil penetrate the human skin and migrate into the small intestine. Historically, it was believed that the L3 larvae migrate via the bloodstream to the lungs, where they are eventually coughed up and swallowed (CDC, 2015). However, there is also evidence that L3 larvae can migrate directly to the intestine via connective tissues. In the small intestine, they molt twice and become adult female worms. The females live threaded in the epithelium of the small intestine and by parthenogenesis produce eggs, which yield rhabditiform larvae (CDC, 2015).

The rhabditiform larvae can either be passed in the stool or can cause autoinfection. In an autoinfection, the rhabditiform larvae become infective filariform larvae, which can penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection) (CDC, 2015). In either case, the filariform larvae may disseminate throughout the body.

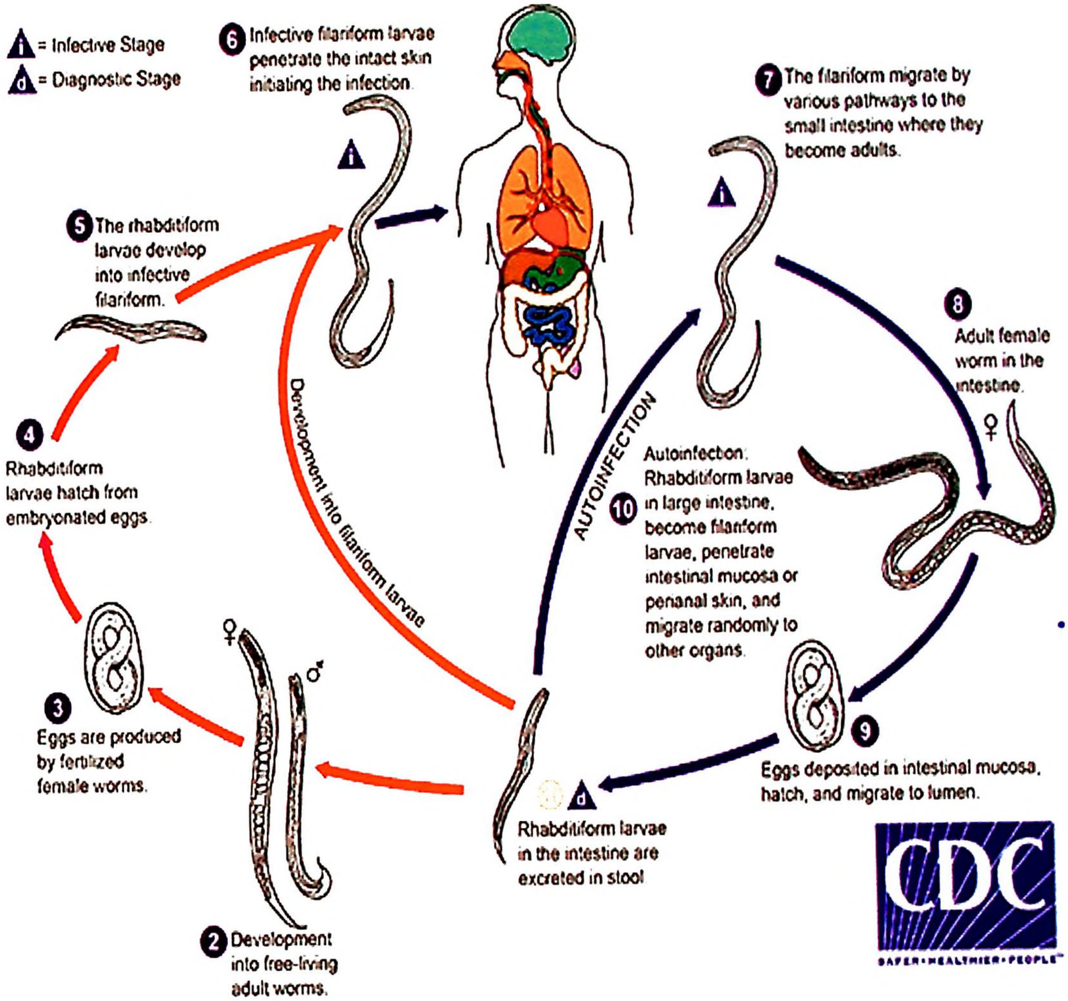

Figure 2.1: Life cycle of *S. stercoralis*

Source: http://www.cdc.gov/parasites/strongyloides/biology.html

2.1.1(b) *Ascaris lumbricoides*

Morphology

Ascaris lumbricoides is the largest nematode (roundworm) parasitizing the human intestine. Adult females measure 20 to 35 cm while adult males measure 15 to 30 cm (CDC, 2013). Male adults have a curved tail with 2 copulatory spicules while female adults have a straight tail. There are unfertilised egg, fertilised egg and embryonated egg. Unfertilised egg has an ellipsoidal shape, brown thin shell and do not float in saturate salt solution (Length: 78-105 μm; Width: 38-55 μm). Fertilised egg has an oval to subspherical shape, thick shell without light brown outer coat and float in saturated salt solution (Length: 45-70 μm; Width: 35-50 μm). However, only embryonated egg with larvae is infective to human.

Figure 2.2: Adult female *A. lumbricoides*

Source: http://www.cdc.gov/dpdx/ascariasis/index.html

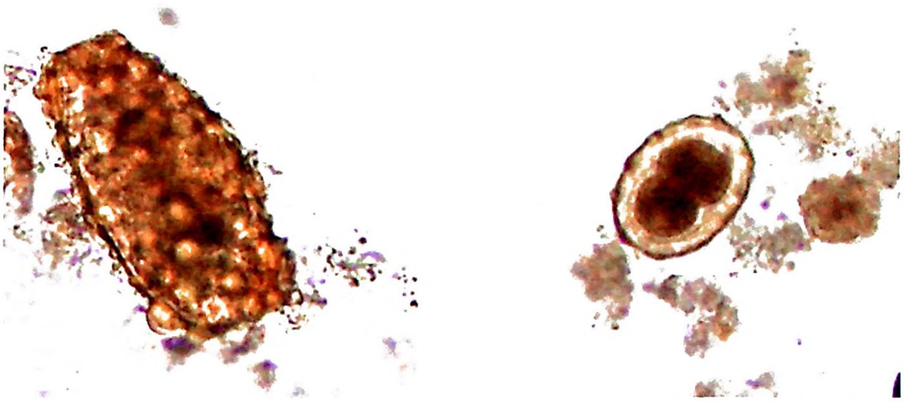

Figure 2.3: Unfertilized egg of *A. lumbricoides* in an unstained wet mount, 200x magnification (left) and fertilized egg of *A. lumbricoides* in an unstained wet mount of stool, with embryos in the early stage of development.

Source: http://www.cdc.gov/dpdx/ascariasis/index.html

Life cycle

Adult worms live in the lumen of the small intestine. A female may produce approximately 200,000 eggs per day, which are passed with the faeces. Unfertilized eggs may be ingested but are not infective. Fertile eggs embryonate and become infective after 18 days to several weeks, depending on the environmental conditions. After infective eggs are swallowed, the larvae hatch, invade the intestinal mucosa and are carried via the portal, then systemic circulation to the lungs. The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat and are swallowed. Upon reaching the small intestine, they develop into adult worms. It requires between 2 and 3 months from ingestion of the infective eggs to oviposition by the adult female. Adult worms can live 1 to 2 years. The eggs are passed through the faeces and the cycle continues.

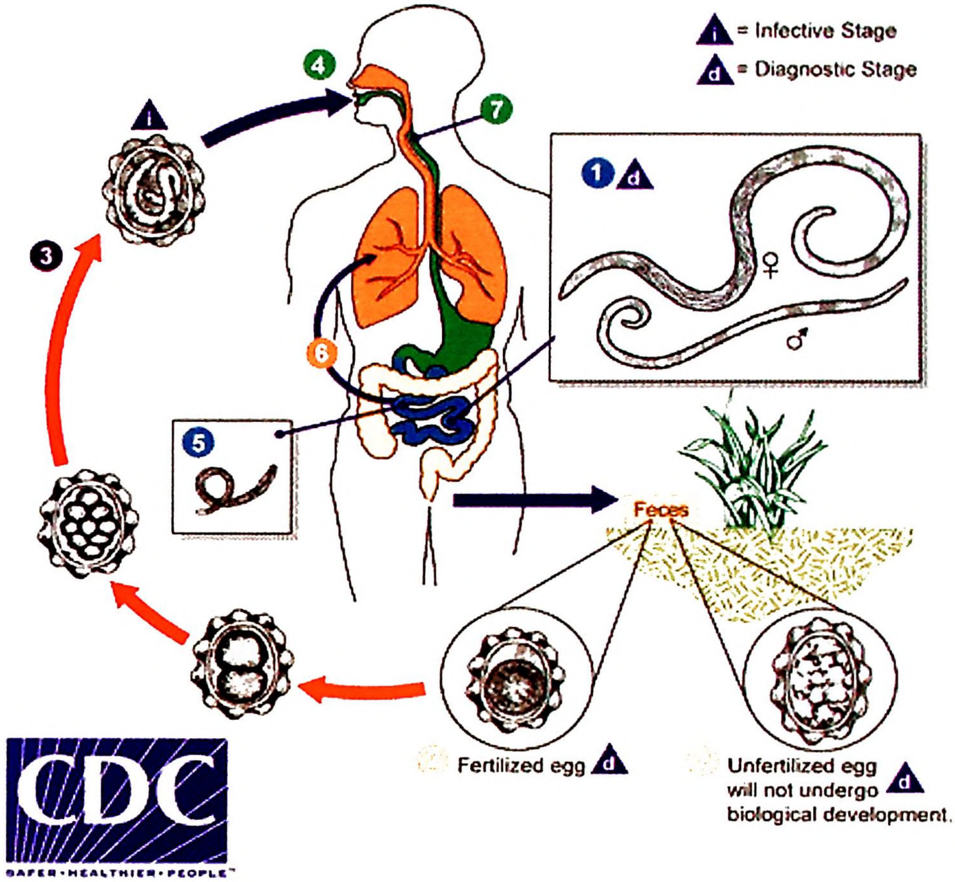

Figure 2.4: Life cycle of *A. lumbricoides*

Source: http://www.cdc.gov/dpdx/ascariasis/index.html

2.1.1(c) *Trichiuris trichiura*

Morphology

The nematode (roundworm) *T. trichiura*, also called the human whipworm (CDC, 2013). The egg of *T. trichiura* will float in saturated salt solution, smooth outer shell and has a distintive bipolar plugs (Length: 50-54 µm; width: 22-23 µm).

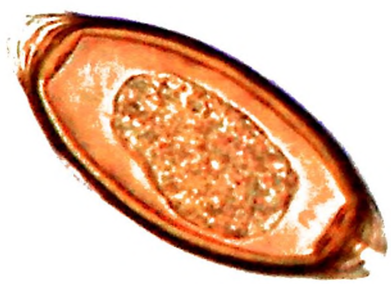

Figure 2.5: Egg of *T. trichiura* in an unstained wet mount.

Source: http://www.cdc.gov/dpdx/trichuriasis/index.html

Life cycle

The unembryonated eggs are passed with the stool. In the soil, the eggs develop into a 2-cell stage, an advanced cleavage stage and then they embryonate. The eggs become infective in 15 to 30 days. After ingestion, the eggs hatch in the small intestin and release larvae that mature and establish themselves as adults in the colon. The adult worms approximately 4 cm in length, live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year.

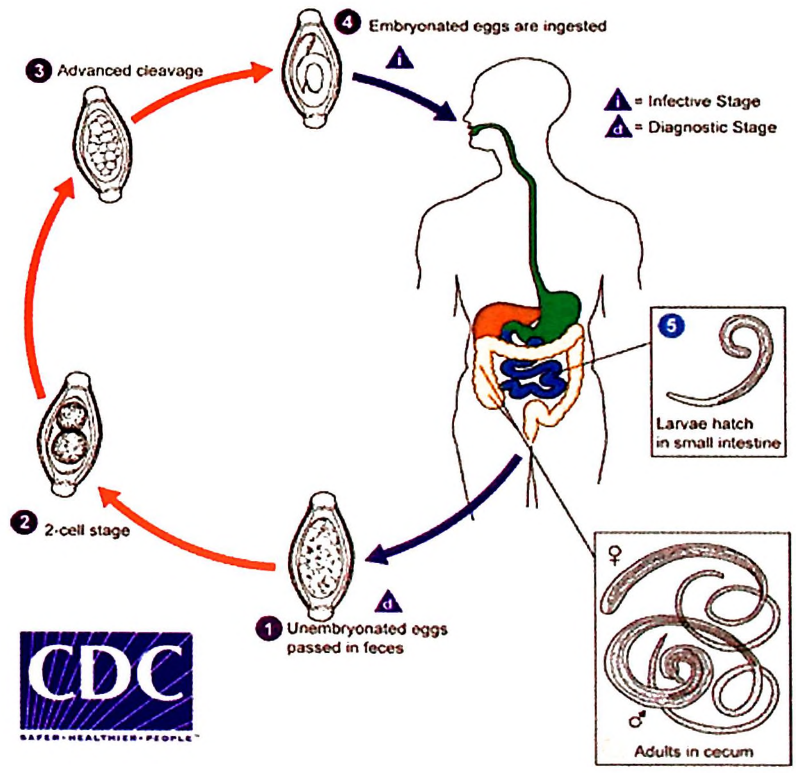

Figure 2.6: Life cycle of *T. trichiura*

Source: http://www.cdc.gov/dpdx/trichuriasis/index.html

2.1.1(d) *Taenia spp.*

Morphology

The cestodes (tapeworms) *Taenia saginata* (beef tapeworm) and *T. solium* (pork tapeworm). *T. solium* eggs can also cause cysticercosis. Length of adult worms is usually 5 cm or less for *T. saginata* (however it may reach up to 25 cm) and 2 to 7 cm for *T. solium* (CDC, 2013). The adults produce proglottids which mature, become gravid, detach from the tapeworm and migrate to the anus or are passed in the stool approximately 6 per day (CDC, 2013). *T. saginata* adults usually have 1,000 to 2,000 proglottids, while *T. solium* adults have an average of 1,000 proglottids. The egg of *Taenia spp.* is sperical in shape. It is brown to dark yellow in colour (31-34 mm in diameter). It also has a thick embryophore (shell wall) which is radially striated.

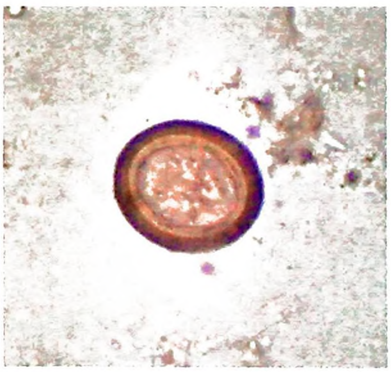

Figure 2.7: *Taenia spp.* egg in unstained wet mount.

Source: http://www.cdc.gov/dpdx/taeniasis/

Life cycle

Taeniasis is the infection of humans with the adult tapeworm of *T. saginata* or *T. solium*. Humans are the only definitive hosts for *T. saginata* and *T. solium*. Eggs or gravid proglottids are passed with faeces. The eggs can survive for days to months in the environment. Cattle (*T. saginata*) and pigs (*T. solium*) become infected by ingesting vegetation contaminated with eggs or gravid proglottids (CDC, 2013). In the animal's intestine, the oncospheres hatch, invade the intestinal wall and migrate to the striated muscles, where they develop into cysticerci. A cysticercus can survive for several years in the animal. Humans become infected by ingesting raw or undercooked infected meat. In the human intestine, the cysticercus develops over 2 months into an adult tapeworm, which can survive for years.

The adult tapeworms attach to the small intestine by their scolex and reside in the small intestine. The eggs contained in the gravid proglottids are released after the proglottids are passed with the faeces. *T. saginata* may produce up to 100,000 and *T. solium* may produce 50,000 eggs per proglottid respectively.

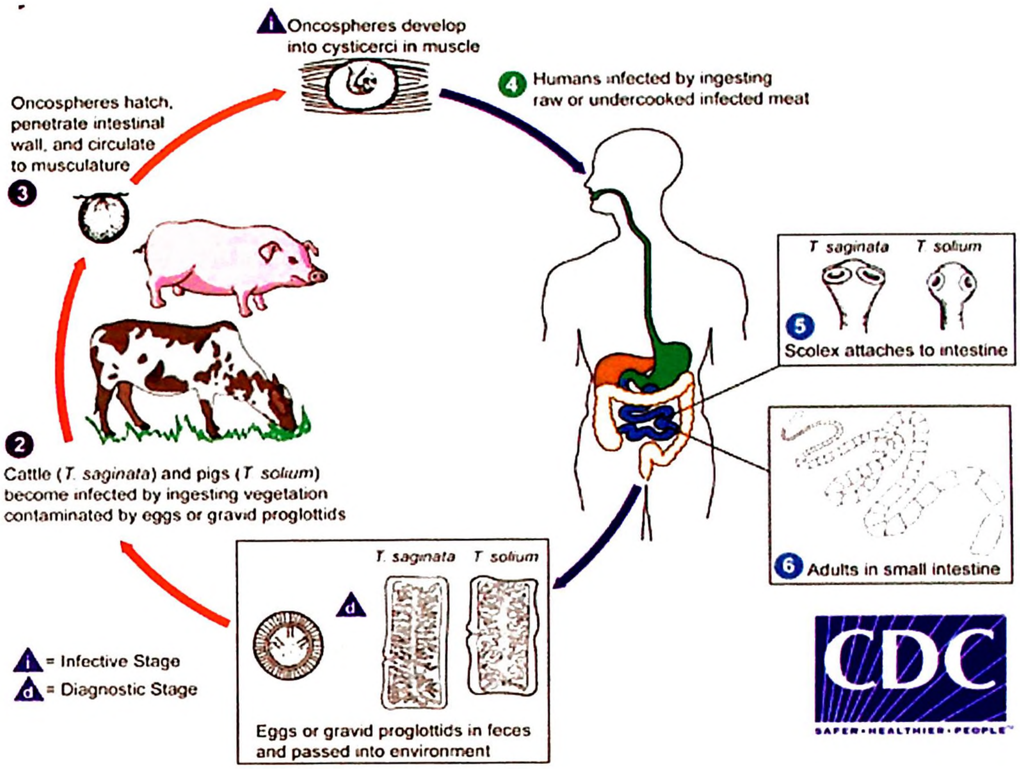

Figure 2.8: Life cycle of *Taenia spp.*

Source: http://www.cdc.gov/parasites/taeniasis/biology.html

2.1.1(e) Hookworm

Morphology

The human hookworms include the nematode species, *Ancylostoma duodenale* and *Necator americanus* (CDC, 2013). Adult hookworm have an anterior end that is bent slightly dorsally. They are about 1 cm in length and usually, male is smaller than female. Male adult has a copulatory bursta at the posterior end. Egg of hookworms has a thin colourless shell. It is oval in shape ang has about 2 to 8 cells. The length of the egg is 60 μm and 40 μm in width.

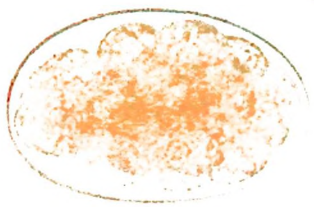

Figure 2.9: Hookworm egg in unstained wet mount.

Source: http://www.cdc.gov/dpdx/hookworm/gallery.html

Life cycle

Eggs are passed in the stool and under favorable conditions, larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the faeces and/or the soil and after 5 to 10 days (and two molts) they become filariform (third-stage) larvae that are infective. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the blood vessels to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx and are swallowed. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host. Most adult worms are eliminated in 1 to 2 years, but the longevity may reach several years.

Some *A. duodenale* larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by *A. duodenale* may probably also occur by the oral and transmammary route. *N. americanus*, however, requires a transpulmonary migration phase.

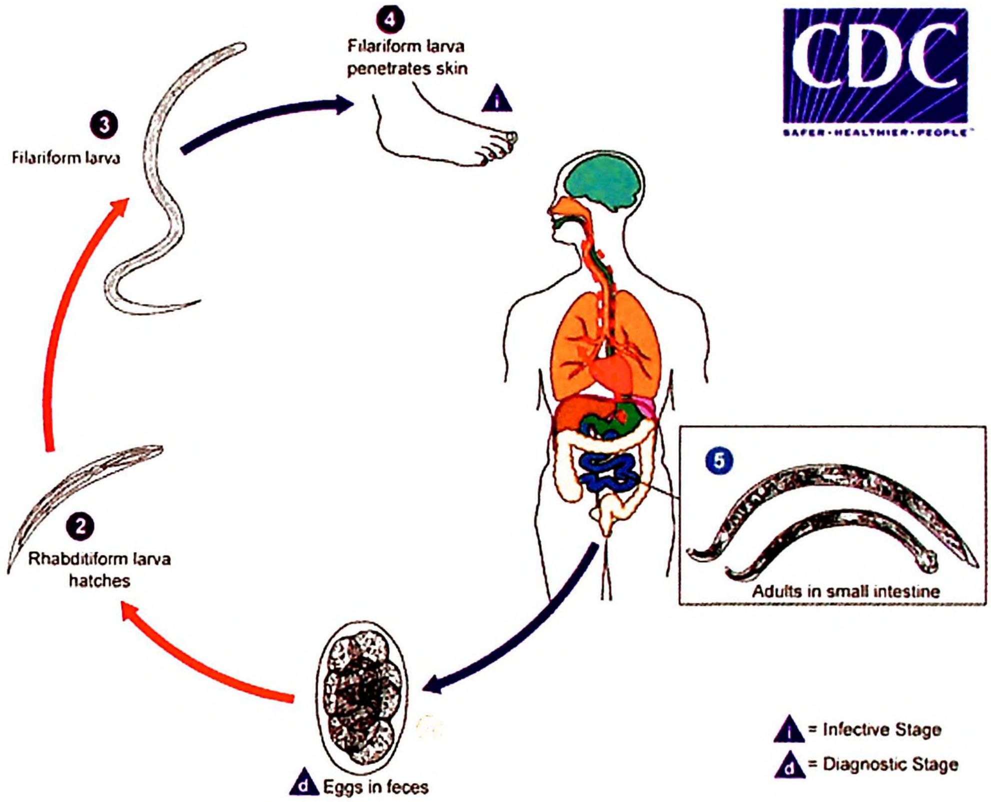

Figure 2.10: Life cycle of hookworms.

Source: http://www.cdc.gov/parasites/hookworm/biology.html

2.1.2 Common Human Intestinal Protozoa

2.1.2(a) *Cryptosporidium parvum*

Morphology

Many species of *Cryptosporidium spp.* exist, that infect humans and a wide range of animals. *C. parvum* and *Cryptosporidium hominis* (formerly known as *C. parvum* anthroponotic genotype or genotype 1) are the most prevalent species causing disease in humans (CDC, 2010).

C. parvum may exist in 6 morphological stages which are oocyst, sporozoite, trophozoite, meront, microgamont and macrogamont. Oocyst is the diagnostic and infective stage. The oocyst is colourless, spherical or oval shape which measures 4.5-6 µm in diameter. Each oocyst contains up to four slender and crescent-shaped sporozoites (no sporocysts). The oocyst sporulates inside the single host. There are two types of oocyst which are thick-walled oocyst and thin-walled oocyst (has a single unit membrane covering).

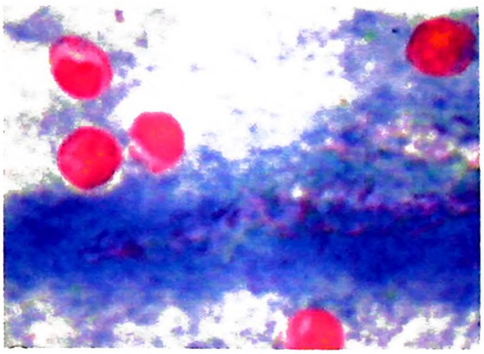

Figure 2.11: Oocyst of *C. parvum* stained with modified acid-fast stain measuring 4-8 µm in diameter.

Source: http://parasitol.kr/journal/view.php?number=1963

Life cycle

Sporulated oocysts, containing 4 sporozoites are excreted by the infected host through faeces and possibly other routes such as respiratory secretions. Transmission of *C. parvum* and *C. hominis* occurs mainly through contact with contaminated water. Occasionally food sources, such as chicken salad, may serve as vehicles for transmission. Many outbreaks in the United States have occurred in waterparks, community swimming pools and day care centers. Zoonotic and anthroponotic transmission of *C. parvum* and anthroponotic transmission of *C. hominis* occur through exposure to infected animals or exposure to water contaminated by faeces of infected animals.

Following ingestion (and possibly inhalation) by a suitable host, excystation occurs. The sporozoites are released and parasitize epithelial cells of the gastrointestinal tract or other tissues such as the respiratory tract. In these cells, the parasites undergo asexual multiplication (schizogony or merogony) and then sexual multiplication (gametogony) producing microgamonts (male) and macrogamonts (female). Upon fertilization of the macrogamonts by the microgametes, oocysts develop that sporulate in the infected host. Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host and the thin-walled oocyst, which is primarily involved in autoinfection. Oocysts are infective upon excretion, thus permitting direct and immediate fecal-oral transmission.

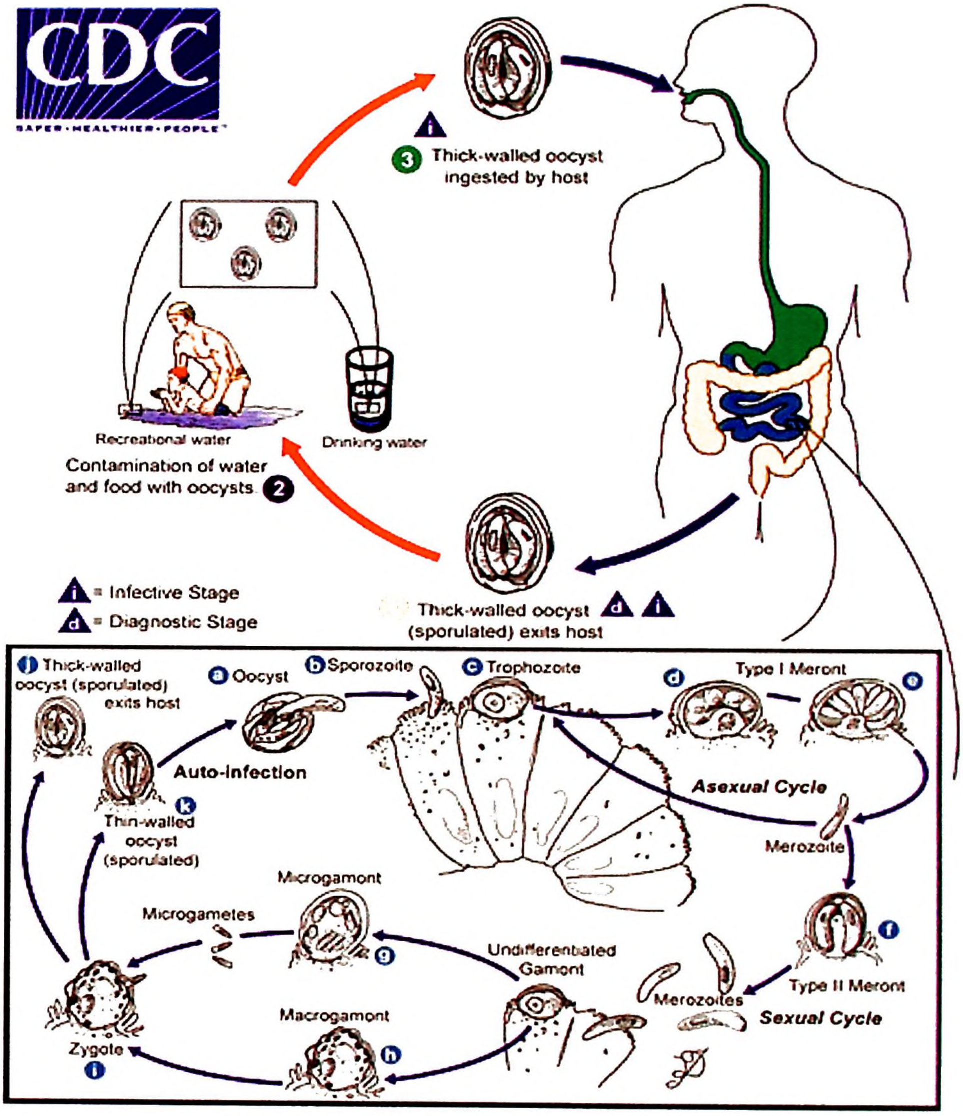

Figure 2.12: Life cycle of *C. parvum*.

Source: http://www.cdc.gov/parasites/crypto/biology.html

2.1.2(b) *Isospora belli*

Morphology

The coccidian parasite, *Cystoisospora belli* or also known as *Isospora belli* infects the epithelial cells of the small intestine and is the least common of the three intestinal coccidia that infect humans (CDC, 2013). The oocysts of *I. belli* are large (25 to 30 μm) and have a typical ellipsoidal shape. When excreted, they are immature and contain one sporoblast. The oocyst matures after excretion. The single sporoblast divides in two sporoblasts, which develop cyst walls, becoming sporocysts, which eventually contain four sporozoites each (CDC, 2013).

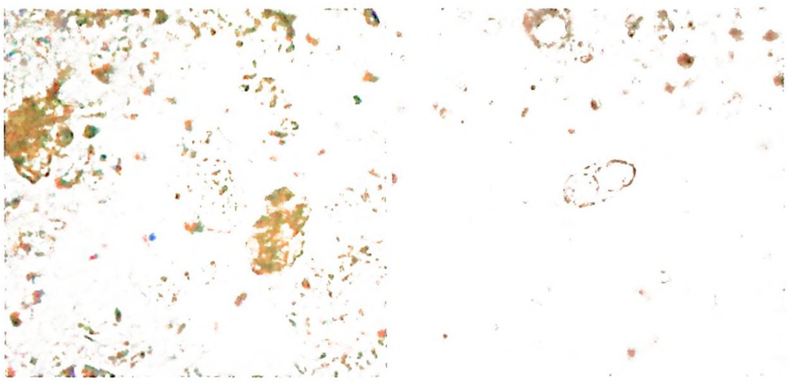

Figure 2.13: Immature oocyst of *C. belli* in an unstained wet mount, containing a single sporoblast (left) and immature oocyst of *C. belli* in an unstained wet mount showing two sporoblasts (right)

Source: http://www.cdc.gov/dpdx/cystoisosporiasis/gallery.html

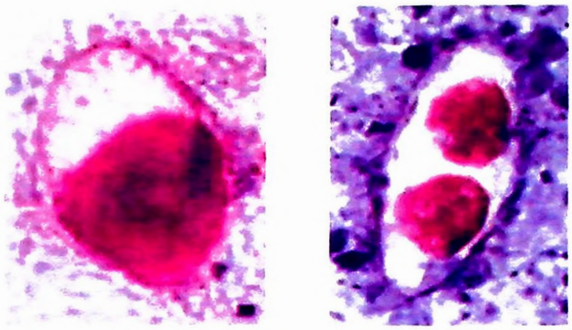

Figure 2.14: Microscopic photograph of *I. belli* sporulate oocyst stained with modified acid-fast stain (left) and immature oocyst of *I. belli* stained with modified acid-fast stain measuring 20-30 µm long by 10-19 µm wide.

Source: http://www.cmpt.ca/photo_album_parasitology/parasitology_photos_6_coc.htm

Life cycle

The oocyst is immature and usually contains just one sporoblast at the time of excretion in stool. During further maturation, the sporoblast divides in two which make the oocyst now contains two sporoblasts. Then, the sporoblasts secrete a cyst wall, thus becoming sporocysts and the sporocysts divide twice, resulting in four sporozoites per each of two sporocysts. Infection occurs by ingestion of mature (fully sporulated) oocysts. The sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony. When the schizonts are ruptured, merozoites are released, which invade epithelial cells and continue the cycle of asexual multiplication. Trophozoites develop into schizonts, which contain multiple merozoites. After a minimum of one week, the sexual stage begins with the development of male and female gametocytes. Fertilization results in the development of oocysts, which are excreted in the stool and the cysle continues.