DETECTION OF ANTI-*GIARDIA* ANTIBODIES AMONG SELECTED KELANTAN CATTLE FARMERS: AN OCCUPATIONAL RISK SURVEILLANCE

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A STUDY ON ANTI-*GIARDIA* ANTIBODY AMONG SELECTED KELANTAN CATTLE FARMERS: AN OCCUPATIONAL RISK SURVEILLANCE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

~	About
%	Percentage
>	More than
°C	Degree Celsius
µg	Microgram
µL	Microliter
CBB	Coomassie brilliant blue
CDC	Centers for Disease Control and Prevention
cm	Centimeter
mm	Millimeter
CSA	Crude soluble antigen
dH_2O	Distilled water
DNA	deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
et al.	*et alii* – ‘and others’
× *g*	Gravity
g	Gram

IgG	Immunoglobulin G
IHA	Indirect hemagglutination assay
kDa	Kilodalton
L	Litre
mA	MiliAmpere
mL	Milliliter
mM	Milimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
OD_{450nm}	Optical density at the wavelength of 450 nm
TBS	Tris-Buffered Saline
TBST	TBS-Tween 20
PBS	Phosphate Buffered Saline
PBST	PBS-Tween 20
s	Second
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SPSS	Statistical Product and Service Solutions
TMB	3,3’,5,5’-Tetramethylbenzidine
rpm	Revolutions per minute

LB	Luria Bertani
NDI	Neglected Diseases Initiative
WHO	World Health Organization

PENGESANAN ANTIBODI ANTI-GIARDIA DALAM KALANGAN LADANG LEMBU KELANTAN TERPILIH: PENGAWASAN RISIKO PEKERJAAN

ABSTRAK

Giardiasis, yang disebabkan oleh *Giardia lamblia* telah dikenalpasti sebagai salah satu jangkitan protozoa usus di seluruh dunia. Oleh kerana penyakit ini disebarkan melalui laluan dubur-mulut dan berpotensi disebarkan secara zoonosis, pekerjaan seperti penternak lembu merupakan salah satu pekerjaan yang berisiko tinggi. Di Malaysia, prevalens giardiasis berkisar antara 0.2 hingga 20% dan jangkitan sering dikaitkan dengan kekurangan zat makanan terutama di kalangan kanak-kanak. Prevalens yang dilaporkan adalah disimpulkan berdasarkan kes-kes yang masih aktif, tanpa mengira mereka yang pernah terdedah kepada jangkitan dalam masa yang terdekat itu. Kajian keratan rentas ini dilakukan untuk menyiasat prevalens giardiasis dan kaitannya dengan pendedahan pekerjaan di kalangan petani lembu di Kelantan dan membandingkannya dengan kumpulan kajian lain, iaitu Orang Asli dan penderma darah. Sampel serum yang diperoleh daripada 90 peserta menjalani ujian imunosorben berkaitan enzim (ELISA) dalaman untuk pengesanan antibodi IgG anti-*Giardia*. Data sosiodemografi telah dianalisis menggunakan SPSS v24.0 (Microsoft Corporation, USA). ELISA dalaman dioptimumkan dengan parameter piawai seperti kepekatan antigen pelapisan sebanyak 10 µg/mL, pencairan serum 1:50 dan antibodi sekunder terkonjugasi HRP 1:6000. Seroprevalens Orang Asli, penderma darah dan petani masing-masing adalah 68.7%, 1% dan 27.7%. Terdapat perbezaan yang signifikan ($P < 0.001$) antara seroprevalens Orang Asli dan petani. Tidak ada penemuan yang signifikan yang ditunjukkan antara pemboleh ubah demografi subjek dan bacaan

ELISA OD_{450nm}. Kesimpulannya, seropositiviti giardiasis lebih tinggi pada populasi endemik berbanding dengan sera populasi bukan endemik. Kajian lebih lanjut diperlukan untuk mengenal pasti factor-faktor yang berkemungkinan kepada risiko seropositiviti tersebut.

DETECTION OF ANTI-*GIARDIA* ANTIBODIES AMONG SELECTED KELANTAN CATTLE FARMERS: AN OCCUPATIONAL RISK SURVEILLANCE

ABSTRACT

Giardiasis, caused by *Giardia lamblia*, has been recognized as one of the most common intestinal protozoan infections worldwide. As the disease is spread by the faecal-oral route and potentially zoonotic transmitted, occupations such as cattle farmers are one of the high-risk jobs. In Malaysia, the prevalence of giardiasis ranges between 0.2 to 20%, and infection is often associated with malnutrition particularly among children. The reported prevalence inferred only to active cases, but not the people who are exposed to the infection recently. This cross-sectional study was conducted to investigate the prevalence of giardiasis in relation to occupational exposure among cattle farmers in Kelantan and to compare it with other study groups, Orang Asli and blood donor. Achieved serum samples from 90 participants were subjected to an in-house enzyme-linked immunosorbent assay (ELISA) for the detection of anti-*Giardia* IgG antibody. Sociodemographic data were analysed using SPSS v24.0 (Microsoft Corporation, USA). An in-house ELISA was optimised with standardised parameters as such coating antigen concentration of 10 µg/mL, serum dilution of 1:50, and HRP-conjugated secondary antibody of 1:6000. The seroprevalence of Orang Asli, blood donors, and farmers were 68.7%, 1%, and 27.7% respectively. There were significant ($P<0.001$) difference between the seroprevalence of Orang Asli and farmers. There were no significant findings shown between subjects' demographic variables and ELISA OD_{450nm} reading. In conclusion, the seropositivity of giardiasis is higher in the endemic population as compared to the sera

of non-endemic population. Further study is required to identify the possible risk factors of the seropositivity.

CHAPTER 1

INTRODUCTION

1.1 Research background

Giardia lamblia, also known as *Giardia intestinalis* and *Giardia duodenalis,* is the only known species from the genus *Giardia* that can infect humans and a variety of animals including the domestic and wildlife animals (Li, Wang, Wang, & Zhang, 2017). Giardiasis has been recognized as one of the most common intestinal protozoan infections worldwide, with over 280 million reported cases per year. Since 2006, human giardiasis has been included in the World Health Organization's (WHO) Neglected Diseases Initiative (Perrucci et al., 2019). Giardiasis is prevalent worldwide with most of the reported cases are from Asia, Africa, and Latin America. In United State, most cases are among returning travellers from Caribbean, Middle East, Eastern Europe, Central America, South America, North Africa, sub-Saharan Africa, and South-Central Asia (Benedict & Roellig, 2020; Rumsey & Waseem, 2018).

The pathological outcomes of *Giardia* infection range from villus- and crypt-atrophy, enterocyte apoptosis, to severe damage of epithelial barrier function. Some clinical studies revealed that there is an association between *Giardia* infection and the development of irritable bowel syndrome and chronic fatigue (Cernikova, Faso, & Hehl, 2019). Long-term recurrent exposure to giardia infection is correlated with malabsorption among children. Chronic *Giardia* infection in children has been a major health concern as the infection may interrupt their cognitive development and physical growth. Poorly developed future generation may hamper the improvement of the local socioeconomic (Choy et al., 2014; Trelis et al., 2019). Hence, it is pertinent to discover

and eliminate giardiasis in the high-risk populations. This could be done via mass surveillance and prompt treatment.

Risk factors of *Giardia* infection include low socioeconomic factors, limited access to clean water system, poor environmental and personal sanitation (Choy et al., 2014). Giardiasis is transmitted via faecal-oral route. Although human-to-human transmission could happen, humans get infected more commonly upon the ingestion of cysts contaminated food or water (Al-Mekhlafi et al., 2013). Transmission via direct contact is associated with animal handlers such as farmers and veterinarians because domestic livestock are the reservoir of the protozoan, as well. The infection could be accidentally acquired as farmers are dealing with the handling of animal faeces (Di Piazza et al., 2013). Studies conducted in Bangladesh and in West Bengal, India manifested a significant association between *Giardia* infection in calves and their handlers (Ehsan et al., 2015).

Giardiasis is an endemic protozoal disease in Malaysia. Despite its widespread, majority of local studies conducted were limited to aborigines' population. The figure regarding the prevalence and associated risk factors of occupational exposure to *Giardia* infection remain unclear.

Most of the prevalence studies conducted for giardiasis in developing countries are either based on microscopy alone or culture and microscopy as the screening tools. However, microscopy method is not preferable to be used for prevalence studies due to low sensitivity and detection is limited to current infection. Prevalence study conducted by Naz *et al*., (2018) using microscopic examination was claimed to have an inaccurate result due to the low sensitivity of the method. Therefore, the present study aimed to investigate the prevalence of *Giardia* infection among selected cattle farmers in Kelantan using serological method.

1.2 Rationale of the study

Giardiasis is a worldwide disease affecting both mammals and humans and it has become one of the concerning disease in both developed and developing countries including Malaysia. As the life cycle involves the deposition of infectious cysts via host excreta into the environment, it poses a risk to humans who work on the field and have contact with animals. Occupations such as ruminant livestock farmers including cattle, sheep, pigs and horse have been subjected to the risk of getting the infection. It has been reported that livestock faeces pose a significant threat to the quality of water sources. Contamination may occur directly by faecal deposition or through drainage from the steep slopes, heavy rain or flood (Toledo et al., 2017). The use of contaminated water for daily activities could post a threat to the farm workers or dwellers (Di Piazza et al., 2013). In addition, handling of livestock excreta and cleaning up the farm using untreated water as the norm of farmers' routine tasks exposed the ruminant livestock farm workers to the occupational parasitic disease. Until now, there are limited research regarding the prevalence of giardiasis in Malaysia, especially in Kelantan, when in fact, Kelantan is considered as one of the less industrialize region in Malaysia where most of the populations rely on fishing and agriculture (Lim, Ahmad, & Smith, 2008). Besides, most of local studies conducted are limited to aborigines' population. As the disease might affect the quality of health of the farm workers, a schedule surveillance is pertinent for inspecting potential under-reporting giardiasis. Therefore, the current study will focus on the prevalence of giardiasis in relation to occupational exposure among cattle farmers in Kelantan.

1.3 Research objectives

1.3.1 General objective

To develop an in-house ELISA for detection of anti-giardia IgG in selected serum samples of Kelantan cattle farmers

1.3.2 Specific objectives

The specific objectives for this study are:

1. To develop an in-house ELISA for detection of anti-*Giardia* IgG antibody
2. To compare the distribution of ELISA OD450nm readings among cattle farmers, Orang Asli and blood donors
3. To determine the association between the anti-*Giardia* IgG and demographic variables among cattle farmers

CHAPTER 2

LITERATURE REVIEW

2.1 *Giardia lamblia*

Giardia was first described by Antonie van Leeuwenhoek in 1681 as he was examining his own stool samples. Later in 1859, this organism was described in more details by Lambl and put the organism in the genus *Cercomonas* and thus named as *Cercomonas intestinalis.* Kunstler was the first person to name the organism as *Giardia* in 1882. Since the scientists started to discover more *Giardia* species, they started to name and classify the species by host of origin and morphology of the species. Throughout the centuries, the organism was given name after a name until *Giardia lamblia* was broadly accepted in the medical and scientific literature (Adam, 2001). To date, there are eight species of *Giardia* that has been identified. They are *Giardia agilis, Giardia ardeae, Giardia psittaci, Giardia muris, Giardia microti Giardia peramelis* and *Giardia cricetidarum,* which infect variety of animals including amphibians, birds, rodents and marsupials, and *Giardia lamblia* which infects broad range of host including humans and animals (Ryan & Zahedi, 2019).

Giardia lamblia genotypes are subdivided into 8 assemblages (A, B, C, D, E, F, G, H). Assemblage A and B infect not only human but also pets and livestock animals. Assemblage C and D infect dogs, cats, coyotes and wolves, while assemblage E infects livestock, assemblage F in cats, whereas assemblage G and H are found in rats and marine animals respectively (Fantinatti, Bello, Fernandes, & Da-Cruz, 2016; Gutiérrez, 2017). Study conducted by (Anuar, Azreen, Salleh, & Moktar, 2014) showed that infection with assemblages A and B were not significantly linked with

gender, while infection with assemblage B was found to be significantly higher in teenagers with age of below 15 years old.

2.1.1 The *Giardia* life cycle

The life cycle of *Giardia lamblia* simply alternates between trophozoites that are able to multiply rapidly and cysts that are environmentally stable. Cyst is the infective stage and infection occurs through ingestion of the cysts, followed by excystation, release of the excyzoite and trophozoites colonization of the small intestine (Svärd, Hagblom, & Palm, 2003). Upon ingestion, cysts pass through the stomach and undergo excystation in the small intestine, where the mature cysts transform into trophozoites. The excystation is triggered by gastric acid, bile and trypsin in the duodenum (Einarsson & Svärd, 2015). Trophozoites colonize the duodenum and jejunum of the small intestinal epithelium and multiply rapidly, causes clinical diseases in human (Klotz & Aebischer, 2015). During this stage, the trophozoites must adapt to various abiotic factors including pH, oxygen tension, the normal bacterial flora, the mucus layer as well as intestinal proteases. Besides, they are exposed to destructive factors such as T cells, mast cells, M cells, dendritic cells, antibodies, and cytokines. The ability of *Giardia* to withstand these adaptive responses is a crucial determinant for them to reproduce and survive (Rópolo & Touz, 2010). *Giardia lamblia* reproduces asexually by binary fusion, however, studies also suggested that they are capable of reproducing sexually (Li et al., 2017). A large number of the multiplying trophozoites are transported to the colon while undergoing encystation along the way as they are exposed to the bile salts and other stimuli as well as elevated pH in the lower part of the small intestine (Einarsson & Svärd, 2015). The cysts are then excreted in the faeces and continued another infectious cycle when ingested by a new host. Since *Giardia*

lamblia cysts able to withstand environmental degradation, they can remain infectious for up to months (Naz, Nawaz, Rasool, & Zahoor, 2018). Cysts infectivity decreased by 11% after 49 days at 4°C in soil, and become non-infective after 7 days at 25°C. Meanwhile, cysts remain infectious for up to 56 days at 0°C to 4°C and 14 days at 20°C to 28°C in tap water. They can stand longer in river water with 84 days of infectivity at 0°C to 4°C and 28 days at 20°C to 28°C (Feng & Xiao, 2011).

2.1.2 Distribution of Giardiasis

Giardia lamblia is prevalence worldwide with an estimated 280 million cases per year. About 200 million of cases in Asia, Africa and Latin America have symptomatic giardiasis (Cacciò & Ryan, 2008). Changes in natural and anthropogenic factors largely contribute to the emergence of *Giardia lamblia* infection (Lee et al., 2017). The warm and humid climate may favour survival of *Giardia* cysts (Ehsan et al., 2015). This parasite has contributed for 40.6% of 315 of the parasitic waterborne outbreaks worldwide (Lee et al., 2017). The prevalence rates for giardiasis in developing countries are 8-30% (Deng et al., 2017). Meanwhile, in developed countries, the prevalence rates are lower, ranges from 2% to 5%. Most of the cases accounted for children under 10 years of age, especially those who are malnourished (Naz et al., 2018). In United Kingdom, 1.3% of reported cases are from asymptomatic infection in children, 1.4% of cases are among general practitioner population and 0.9% coming from general population. In England and Wales, there are between 3000-4000 reported cases every year (Waldram, Vivancos, Hartley, & Lamden, 2017). In developed countries, giardiasis is frequent among travellers coming from developing countries (Gutiérrez, 2017). According to Feng and Xiao (2011), the infection rates in countries such as Germany, United States, Portugal, Belgium and South Korea are low,

ranges from 1.1-4.0%. Meanwhile, Italy, Spain, Saudi Arabia, Australia and New Zealand have a slightly higher infection rates, ranges from 0.4-7.6%. A point prevalence survey conducted among orphans in Thailand showed a prevalence of 20%. The giardiasis prevalence in Jordan is 36% through a study conducted among primary school children (Mohamed, Hassan, Hassan, & Rahman, 2008). Infection rates for countries in Asia (Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Nepal, Philippines, Thailand, Turkey, Saudi Arabia, and Vietnam), North America (Cuba, Mexico, and Nicaragua) and Africa (Northern Africa, West Africa, and South Africa) ranging from 8% to 30%, where most of the studies subjected on children (Feng & Xiao, 2011). In addition, studies have documented high cases of human giardiasis in country such as Italy, the Netherlands, Spain, Brazil and Turkey (Table 2.1).

In Malaysia, giardiasis is prevalence among indigenous communities with 5.5% to 28.3% from overall studies conducted. Higher prevalence rate is observed among the Senoi tribe (12.2%) compared to Proto-Malay tribe (3.5%). However, later studies showed a vice versa result where prevalence of the Proto-Malay (33.3% of 150; 10.8% of 268) is found to be higher than Senoi tribe (10.4% of 211; 9.6% of 551) (Lee et al., 2017).

Table 2.1 Prevalence of *Giardia lamblia* in humans

Origin	Nature of the samples (no. of isolates)	Loci tested	Assemblage A (%)	Assemblage B (%)	A + B (%)
Italy	Sporadic (120)	*ssu-rRNA, bg*	65 (54%)	39 (32.5%)	16 (13.5%)
UK	Nursery outbreak (21)	*tpi*		21 (100%)	
The Netherlands	Population survey (18)	*gdh*	9 (50%)	9 (50%)	
The Netherlands	Sporadic (98)	*ssu-rRNA, gdh*	34 (35%)	64 (%)	
France	Sporadic (25)	*tpi*	9 (36%)	16 (64%)	
Spain	Case control study (108)	*tpi*	43 (39.8%)	61 (56.5%)	4 (3.7%)
Norway	Waterborne outbreak (21)	*bg, gdh*		21 (100%)	
Norway	Sporadic (63)	*bg, gdh, tpi*	3 (5%)	60 (95%)	
Albania	Sporadic (22)	*ssu-rRNA*	10 (45%)	12 (55%)	
Uganda	Sporadic (3)	*ssu-rRNA*	3 (100%)		
Ivory Coast	Soldiers (14)	*tpi*		14 (100%)	
Ethiopia	Sporadic (59)	*bg, gdh*	31 (52%)	13 (22%)	15 (25%)
Peru	Sporadic (25)	*tpi*	6 (24%)	19 (76%)	
Brazil	Sporadic (37)	*gdh*	29 (78%)	8 (22%)	
Brazil	Sporadic (62)	*bg*	62 (100%)		
USA	Sporadic (14)	*ssu-rRNA*	14 (100%)		
USA	Sporadic (2)	*tpi*		2 (100%)	
Mexico	Sporadic, children (9)	*bg*	9 (100%)		
Canada	Waterborne outbreak (6)	*ssu-rRNA*	6 (100%)		
Australia	Sporadic (8)	*ssu-rRNA, gdh*	2 (25%)	6 (75%)	
Australia	Population survey (23)	*ssu-rRNA*	7 (30%)	16 (70%)	
Australia	Sporadic (12)	*ssu-rRNA*		11 (92%)	1 (8%)
Turkey	Sporadic (44)	*tpi*	19 (43%)	25 (57%)	
Bangladesh	Case–control study (267)	*tpi*	20 (7.5%)	231 (86.5%)	16 (6%)
India	Sporadic (10)	*tpi*		10 (100%)	
India	Sporadic (19)	*tpi, EF1-α*	6 (32%)	9 (47%)	4 (21%)
India	Sporadic (12)	*gdh*	5 (42%)	7 (58%)	
Laos	Sporadic (5)	*ORF-C4*		5 (100%)	
China	Sporadic (8)	*ssu-rRNA*	4 (50%)	4 (50%)	
Korea	Sporadic (5)	*ssu-rRNA*	5 (100%)		
			391 (35%)	**671 (60%)**	**56 (5%)**

Source: The table is adopted from Caccio` and Ryan (2008)

2.1.3 Route of transmission of *Giardia lamblia*

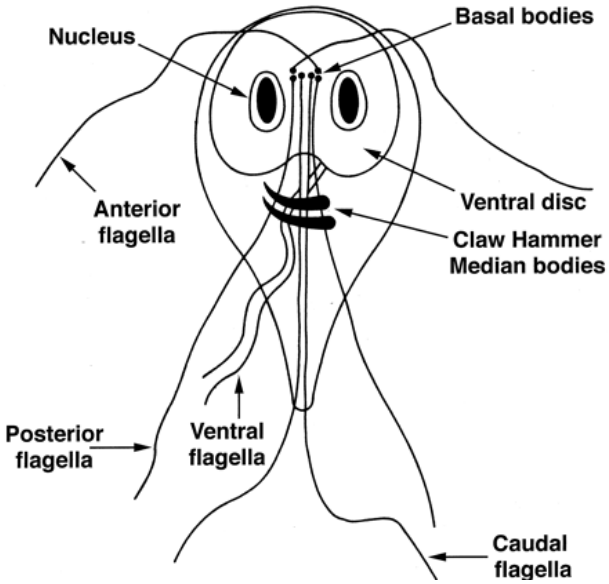
Giardia lamblia transmits via fecal-oral route through direct or indirect ingestion of infectious cysts. Humans are easily infected, indirectly when ingesting food or water contaminated with the cysts. These usually occur through the ingestion of raw food, unboiled water or acquired from unwashed hand (Haider et al., 2013). Besides, infection can spread directly via person-to-person through oral-anal contact (sexual transmission) (Gutiérrez, 2017).

Animal reservoirs crucially contribute to the transmission of different assemblages of *Giardia lamblia*. Stray animals favour the dissemination of the infectious cysts as they wander to places. Meanwhile, infected pet may transmit the cysts within their surrounding such as the pet owner or other domestic mammalian. In addition, infection can easily disseminate in crowded places, including nursery and orphanages (Fantinatti, 2019).

Outbreaks have been associated with drinking of contaminated water and food, and contact with contaminated swimming and wading pools. Study in the UK revealed that giardiasis cases are implicated with swallowing water while swimming, recreational contact with fresh water, drinking tap water and eating green salad. Salad may be contaminated when they are washed with contaminated water or handled by infected person (Espelage, an der Heiden, Stark, & Alpers, 2010). Infection among Orang Asli in Malaysia are mostly due to ingestion of raw vegetables such as tapioca shoots, wild fern shoots and locally planted leaves (Anuar et al., 2014).

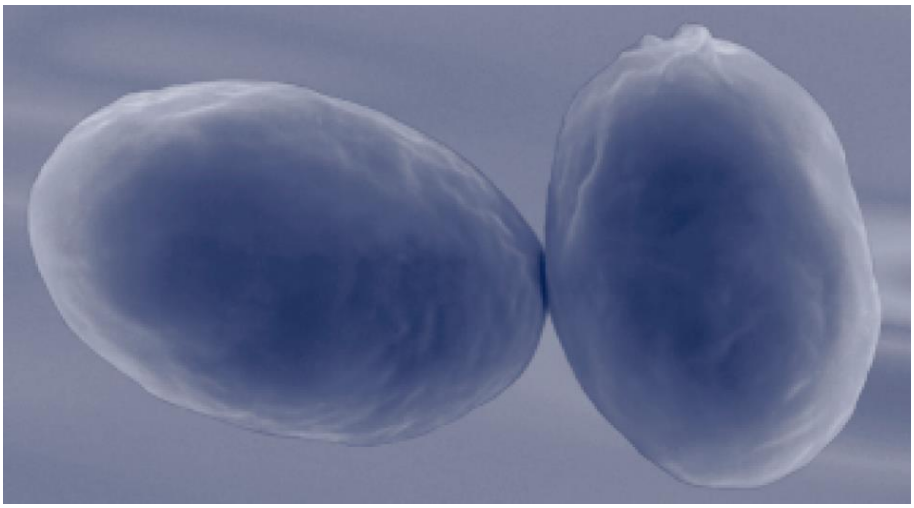
2.1.4 Morphology / characteristics of *Giardia lamblia*

Giardia can exist in two distinct forms the cysts and the trophozoites. The cysts of *Giardia lamblia* are oval in shape, possess four nuclei (quadrinucleated) and a tough hyaline cyst wall that responsible for their survival outside host environment. *Giardia lamblia* cyst measures about 11–14 by 7–10 μm and each cyst can transform into two trophozoites. Actively swimming *Giardia lamblia* trophozoites, like the falling leaves can be seen when viewed under microscope (Gutiérrez, 2017). The trophozoites have a size of about 12 to 15 mm long and 5 to 9 mm wide. The morphology of the trophozoites are considered unique as they look similar to the shape of a pear, possess of two identical nuclei located symmetrically at the anterior part. An adhesive disc at the ventral of the cell surface are responsible for attachment to host intestinal epithelial. Besides, *Giardia* trophozoite also has a claw-shaped median bodies and four pairs of flagella located at the anterior, posterior, caudal and ventral (Figure 2.1). The adhesive disc is crucial for the virulence of *Giardia lamblia* (Adam, 2001; Al Saad & Al Emarah, 2014). Meanwhile, the *Giardia* median body protein (MBP) is an adhesive disc protein which is essential during cell attachment to the host (Woessner & Dawson, 2012). Figure 2.1 and Figure 2.3 respectively show the cyst and trophozoite of *Giardia* under scanning electron micrograph.



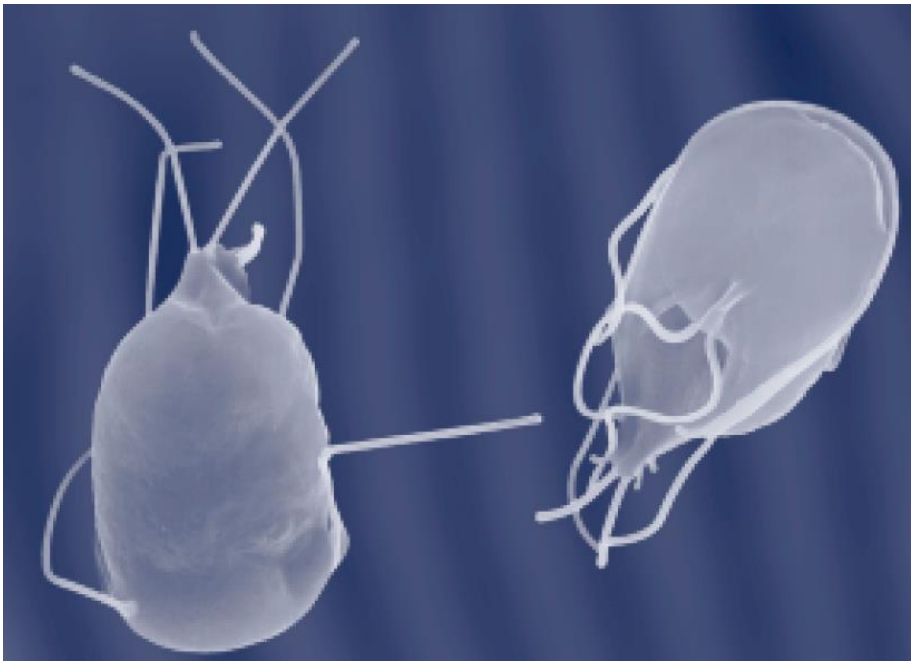
Source: Adopted from (Faubert, 2000)

Figure 2.1 Trophozoite *of Giardia lamblia*



Source:Adopted from Hawrelak (2003)

Figure 2.2 A scanning electron micrograph of *giardia*



Source:Adopted from Hawrelak (2003)

Figure 2.3 *Giardia* trophozoites under the view of a scanning electron microscopy (SEM)

2.2 Clinical manifestation

Human giardiasis shows a wide range of presenting sign, where infected patient may show no symptom or they may present with acute to chronic diarrheal that come along with abdominal pain, nausea, dehydration, vomiting and weight loss (Ferreira et al., 2013). To date, there is no clear explanation on asymptomatic human infection. Assumptions have been made whether the person is infected with non-pathogenic strains or the parasites not completely eliminated from the host, but is maintained at a subclinical level. In most cases, *Giardia* infections are self-limiting, especially among healthy individuals (Espelage et al., 2010). However, some individual may suffer post-infection symptoms such as irritable bowel and chronic fatigue syndrome, arthritis and allergies (Lalle & Hanevik, 2018).

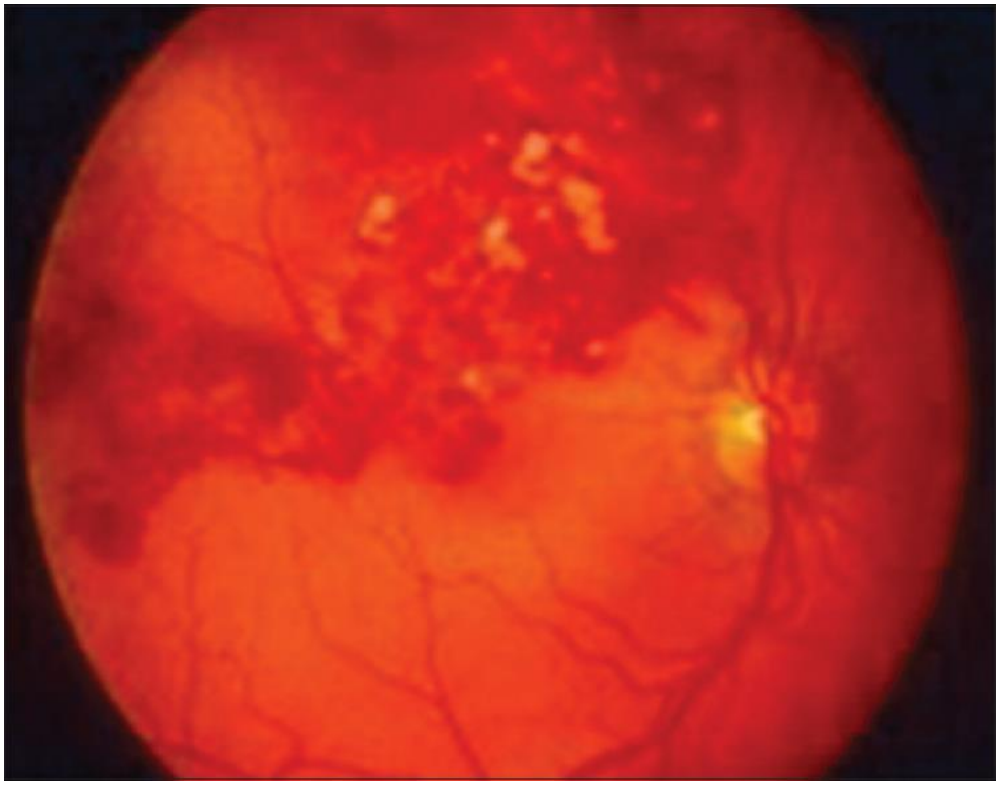
The incubation period for acute giardiasis is 9-15 days. Patient may suffer with intestinal discomfort, anorexia and nausea which subsequently causes prolonged diarrhea. Symptoms such as fever and chills followed by watery, foul-smelling, explosive diarrhoea may last for 3-4 days or persevere for months if left untreated (Haider et al., 2013). Malaise, lassitude, occasional headache as well as malabsorption syndrome have been reported in chronic infection. Symptoms developed differ for each person, depending on the inoculum size, infection period, and individual host and parasite factors. Other than that, symptoms such as urticaria, constipation, cholecystitis, pancreatitis, retinal arteritis and iridocyclitis are observed in chronic giardiasis (Haider et al., 2013; Mohamed et al., 2008).

2.2.1 Long term consequences of giardiasis

Recently, patients infected with *Giardia* are associated with long-term extra-intestinal complications including ocular complications, allergies, muscular complication, and metabolic consequences such as nutritional consequences, failure to thrive, stunting and impaired cognitive function (Halliez & Buret, 2013).

2.2.1 (a) Ocular complications

Ocular complications have been reported to be associated with giardiasis. This include iridocyclitis, choroiditis, and retinal haemorrhages. Recent studies described a "salt and pepper" degeneration associated with the retinal pigmented epithelium in children suffering from giardiasis (Figure 2.4). Damage to the cells of the retina along with release of pigment granules in retinal layer are believed to promote the formation of ocular lesions. However, there is no evidence of direct invasion by the parasite, instead, it may be the result of toxic metabolites produced by the parasites (El-Sayed & Safar, 2015).



Source:Adopted from El-Sayed and Safar (2015)

Figure 2.4 Salt and pepper appearance of the retina in of *Giardia lamblia* infected child

2.2.1 (b) Arthritis

Several case study have reported the association between giardiasis and reactive arthritis or joint pain. However, there is little evidence to support this claim and the magnitude of post-giardiasis arthritis is not known. Until now, there is no large-scale epidemiological studies conducted to assess the association between these two diseases. Recent retrospective cohort study conducted in United State supported the claim of the association between giardiasis and subsequent development of joint pain (Painter, Collier, & Gargano, 2017).

Post-infectious arthritis has a tendency to occur around the joints of the lower limbs especially the knee and ankle. Post-infectious reactive arthritis is listed as a classical spondyloarthropathy which linked with human leukocyte antigen (HLA)-B27, the major histocompatibility complex class I allele present in half of the cases of patients with enteric-infection-related arthritis (Halliez & Buret, 2013).

2.2.1 (c) Muscular complications

Hypokalaemia myopathy is known to be associated with coeliac disease, radiation enteritis and infections. Several cases have been reported in which giardiasis induce hypokalaemia in both immunocompromised and immunocompetent patients. This is evident that *Giardia* infection can cause muscular complications regardless of the immune status of the host. Loss of potassium is associated with the frequency of bouts of diarrhoea per day during the infection (Kanokwanvimol, 2017). Potassium loss causes hypokalaemia which can lead to severe and transient myopathy. Hypokalaemia induced by giardiasis is frequent among elderly, especially among women. However, giardiasis-associated hypokalaemia rarely trigger myopathy and the causes of such complications is still not clear. Suggested mechanism is through the impairment of

nutrient and electrolyte absorption during giardiasis that is thought to play small part in causing hypokalemia and hyponatremia (Halliez & Buret, 2013).

2.3 Pathophysiology of intestinal giardiasis

Over the year, it has been revealed that various factors involved in the pathophysiology of giardiasis which include parasitic, host, dietary, and environmental factors, as well as immunological and non-immunological processes. Acute giardiasis has been associated with increase rate of enterocyte apoptosis. *In vitro* studies revealed a significant upregulation of genes involved in the apoptotic cascade and formation of reactive oxygen species through parasite-host interactions. Another finding discovered the involvement of caspase-3 activation, PARP, cleavage, decreased expression of anti-apoptotic Bcl-2, and upregulation of pro-apoptotic Bax in *Giardia*-induced apoptosis in human epithelial cells. Besides, consumption of host arginine by *Giardia* eliminates the substrate needed for the production of nitric oxide by the enterocytes. Nitric oxide is crucial for the inhibition of giardial growth (Buret, 2008).

Disruption of the intestinal barrier function is also one of the pathophysiological stage involves during acute giardiasis. Some cases have been reported among patients with chronic infections. Both *in vivo* and *in vitro* studies have demonstrated that *Giardia* parasite caused an elevated intestinal permeability during peak of trophozoites colonization. In addition, there are reports that observed an increasing uptake of macromolecules in the jejunum of animal models caused by *Giardia* infection, which also occurs during the peak of trophozoites colonization (Cotton, Beatty, & Buret, 2011). Giardia-mediated alteration of intestinal permeability result from alteration of the tight junctional complex, apoptosis of epithelial cell, and l-arginine starvation. *Giardia* damages the epithelium structure by disrupting the

apical junctional complex (AJC) between enterocytes which composed of tight junctions (TJ), adherent junctions and desmosomes. AJC functions as a selective barrier that protect sub-epithelial compartments from the luminal environment. *Giardia* disrupts the zonula occludens-1 and 2 proteins (ZO-1; ZO-2) which are elements of the TJ. *Giardia* also causes damage to the epithelial occludin and claudin-1, disruption of apical filamentous actin (F-actin) in the cytoskeleton as well as rearrangement of α-actinin which also promotes to the disruption of the AJC. Claudin proteins are crucial element which function as sealing properties of the tight junctions. Disruption of epithelial permeability by epithelial apoptosis implicates the disruption of cellular ZO-1 (Allain, Amat, Motta, Manko, & Buret, 2017).

In addition, giardiasis is known to cause diffuse shortening of brush border microvilli. Diffuse shortening of brush border microvilli leads to malabsorption as mucosal surface area for water, nutrient, mineral, vitamin A and B12 absorption as well as electrolyte transport are reduced. Disruption of microvilli is also linked with decreased activity of brush border digestive enzymes such as disaccharidases, and Na/D-glucose malabsorption. Both diffuse shortening of brush border microvilli and microvillar disaccharidase deficiencies are mediated by activated CD8+ lymphocytes (Allain et al., 2017; Cotton et al., 2011).

2.4 Treatment of giardiasis

The standard treatment used against giardia infection consists of pharmaceutical drugs. Nitroimidazole is considered as the first-line drug in treating giardiasis, with metronidazole as one of the most commonly prescribed. Other drugs drawn from this structural class include tinidazole, secnidazole and ornidazole (Carter, Nabarro, Hedley, & Chiodini, 2018). Metronidazole (commercially known as Flagyl) not only included in the 2015 WHO Essential Medicines List9 but also become the only anti-giardial drug advised by Public Health England (PHE). Recently, tinidazole has been accepted by UK and USA health authorities (Ordóñez-Mena, McCarthy, & Fanshawe, 2018). Metronidazole and tinidazole have a successful treatment rate of 80-90% (Abraham et al., 2019). Mechanism of action of metronidazole utilizes the anaerobic metabolic pathways in the *Giardia*. Metronidazole becomes activated when they are reduced to nitroso radicals. Reduced metronidazole then binds to DNA macromolecules, free thiols or protein cysteines causes DNA damage as they loss their helical structure, damage to the template function, strand breakage, arrest of cell cycle progression and oxidative stress which lead to trophozoites death (Gardner & Hill, 2001; Lalle & Hanevik, 2018). Table 2.2 shows the mechanism of action of drugs used to treat giardiasis.

Table 2.2 Mechanism of action of drugs used to treat *Giardia* infection

Drug	Mechanism of action
Nitroimidazoles	• PFOR produces active metabolite. • Induces double-strand break. • Binds to thiol-containing molecules. • Causes oxidative stress.
Albendazole	• Impairs glucose uptake. • Inhibits cytoskeleton polymerisation. • Induces DNA damage.
Furazolidone	• Reduces producing toxic nitro radicals. • Damages important cellular components, including DNA and functional organelles. • Reduces cysts' ability to differentiate.
Nitazoxanide	• Inhibition of anaerobic energy metabolism pathway. • Interference of NR1. • Compromises cell integrity.
Mepacrine	• Results in intercalation with DNA. • Decreases oxygen consumption. • Reduces cyst viability and excystation
Chloroquine	• Reduces *Giardia's* ability to attach.

NR1/2, nitroreductase 1/2; PFOR, pyruvate ferredoxin oxidoreductase.

Source: Adopted from Carter et al., (2018)

Albendazole (benzimidazole) is a well described alternative giardiasis treatment, known to paralyze the parasite by producing the reactive species, particularly in the nuclei causes oxidative stress and DNA damage. In addition, albendazole has an efficacy rate of such similar to the 5-nitroimidazoles family. Other alternative agents include nitazoxanide, paromomycin, quinacrine, furazolidone and mebendazole. A study revealed that nitazoxanide has 78% of parasitologic cure rate when used as first-line treatment in children. Nevertheless, data on its use for refractory cases are still limited (Abraham et al., 2019; Carter et al., 2018). Despite their effectiveness, these drugs have been reported to manifest side effects such as nausea, fatigue and malaise. Metronidazole is potentially carcinogenic and is not suitable to be prescribed for pregnant patient, especially in their first trimester. Alternatively, furazolidone can be given for this group of patients as this drug is the least teratogenic among the other options (Vesy & Peterson, 1999).

Recent findings suggested albendazole-metronidazole combination therapy is more effective in treating refractory giardiasis compared to albendazole alone, suggesting a synergistic effect. Even so, regardless of the agents, most of giardial therapy does not achieve 100% of cure rates. Recently, the number of nitroimidazole-refractory cases reported has been increasing especially among returning travellers from Asia. Such infection has been associated with drug resistance, suboptimal drug concentrations, reinfection, immunocompromised state and immunoglobulin A deficiency. Reports have been made that some patients exhibit metronidazole-resistance against *Giardia* infection causes failure in the treatment. Resistance to metronidazole has been suggested to be associated with impaired mucosal attachment causes reduced parasite fitness. Meanwhile, a combination of adapting energy supply,

albendazole metabolism reduction and increased cysteine and antioxidant enzymes which increased tolerance to oxidative stress may be the principle for albendazole resistance (Carter et al., 2018). Table 2.3 shows the drugs and their adverse effects, pharmacokinetics and interactions of drugs used to treat giardiasis. Considering these limitations, it is important to continue the research for new effective anti-giardial drug candidates.