DEVELOPMENT OF AN IN-HOUSE PCR FOR THE DETECTION OF *Giardia lamblia* IN CAT AND DOG STOOL SAMPLES

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by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

~	About
%	Percentage
^{0}C	Degree Celsius
µg	Microgram
µL	Microliter
dH_2O	Distilled water
ELISA	Enzyme linked immunosorbent assay
et al.	*et alii* – 'and others'
x *g*	Gravity
g	Gram
Ig	Immunoglobulin
IHA	Indirect hemagglutination assay
L	Litre
h	Hour
min	Minute
mL	Milliliter
mM	Milimolar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PBS	Phosphate Buffered Saline
r*Eh*SREHP	Recombinant *Entamoeba histolytica* Serine Rich Protein
s	Second
rpm	Revolutions per minute

PEMBANGUNAN PCR DALAMAN UNTUK PENGESANAN *Giardia lamblia* DALAM SAMPEL TINJAL KUCING DAN ANJING

ABSTRAK

Giardiasis ialah penyakit yang disebabkan oleh parasit protozoa yang tersebar melalui laluan dubur dan -mulut, dan membawa risiko penularan penyakit zoonotik. Haiwan peliharaan di rumah, seperti kucing dan anjing, boleh menjadi pembawa parasit ini dan, menyebabkan risiko penularan kepada kumpulan rentan seperti kanak-kanak dan warga emas. Walaupun jangkitan *Giardia* kronik dan intermiten jarang mengakibatkan kematian, namun ia boleh menyebabkan kekurangan nutrisi dan pertumbuhan terbantut pada kanak-kanak dan warga emas. Menjadualkan pemeriksaan berkala tinja kucing dan anjing adalah penting untuk mengurangkan risiko giardiasis zoonotik. Pemeriksaan tinja menggunakan mikroskop adalah kaedah yang kukuh dan kos efektif untuk mengesan parasit ini; Bagaimanapun, kepekaannya yang rendah boleh menghasilkan keputusan negatif yang palsu. Sebaliknya, PCR mempunyai kepekaan yang lebih tinggi dan boleh mengurangkan keputusan negatif palsu yang disebabkan oleh pelepasan berkala parasit dalam tinja. Oleh itu, kajian ini bertujuan untuk membangunkan asai PCR dalaman untuk mengesan DNA *Giardia* dalam sampel tinja kucing dan anjing. Trofozoit *Giardia* telah dikultur secara asenik dan DNA *Giardia* telah diekstrak untuk digunakan sebagai templat ujian. PCR dalaman telah dibangunkan menggunakan primer Gia2029 dan Gia2150c yang mensasarkan amplikon 250 bp. Selain itu, PCR kawalan dalaman (IC-PCR) telah dibangunkan menggunakan primer T7-promoter dan T7-terminator yang mensasarkan gen *Entamoeba histolytica* SREHP (800 bp) dalam plasmid pET14b-SREHP. Suhu

penyepuhlindapan telah dioptimumkan kepada 58°C untuk kedua-dua PCR *Giardia* dan IC-PCR. Had pengesanan analisis adalah kira-kira 20 pg DNA genomik. Dalam saringan awal *Giardia* menggunakan mikroskop terhadap sampel tinja kucing (n = 7) dan anjing (n = 3) adalah negatif, namun amplikon tidak khusus ditemui dalam sampel tinja kucing (6/7), tetapi tidak dalam sampel anjing (0/3). Semua spesimen tinja menunjukkan amplikon PCR positif untuk gen kawalan dalaman seterusnya, menyingkirkan gangguan oleh agen perencat PCR. Kesimpulannya, kaedah PCR berdasarkan primer Gia2029 dan Gia2150c adalah sesuai untuk sampel anjing, manakala penilaian lanjut menggunakan penjujukan DNA diperlukan untuk sampel kucing.

DEVELOPMENT OF AN IN-HOUSE PCR FOR THE DETECTION OF *Giardia lamblia* IN CAT AND DOG STOOL SAMPLES

ABSTRACT

Giardiasis is disease caused by a parasitic protozoan that can be transmitted through faecal-oral routes and carries a zoonotic transmission risk. Household pets, such as cats and dogs, can serve as carriers of this parasite, posing a transmission risk to vulnerable groups like children and the elderly. Although chronic and intermittent *Giardia* infections rarely lead to fatal outcomes, they can result in malnutrition or stunted growth in children and the elderly. Scheduling regular screenings of cat and dog stools is crucial to minimize the zoonotic risk of giardiasis. Stool microscopy is a robust and cost-effective method for detecting the parasite; however, its low sensitivity can lead to false negatives. On the other hand, PCR has higher sensitivity and can reduce false negatives due to the intermittent release of the parasite in stool. Thus, our study aimed to develop an in-house PCR method for detecting *Giardia* DNA in dog and cat stool samples. We established an axenic *Giardia* trophozoite culture and extracted *Giardia* DNA from these cells for use as an assay template. An in-house PCR was developed using Gia2029 and Gia2150c primers targeting a 250 bp amplicon. Additionally, we set up an internal control PCR (IC-PCR) using T7-promoter and T7-terminator primers targeting the Entamoeba histolytica SREHP gene (800 bp) in the pET14b-SREHP plasmid. The optimized annealing temperature for both *Giardia*-PCR and IC-PCR was 58 °C. The analytical limit of detection was approximately 20 pg of genomic DNA, equivalent to around 1000 trophozoites. In our preliminary PCR screening of *Giardia* in environmental stool samples from cats (n = 7) and dogs (n =

3) that tested negative by microscopy, we found unspecific amplicons in cat stool samples (6/7), but none in dog samples (0/3). All stool specimens showed positive PCR amplicons for the internal control gene, ruling out interference by PCR inhibitors. In conclusion, the PCR method based on Gia2029 and Gia2150c primers appears suitable for dog samples, while further evaluation via sequencing is required for cat samples.

CHAPTER 1

INTRODUCTION

1.1 Background

Giardia lamblia is a type of single-celled organism that lives in the upper small intestine of humans and other vertebrates. It is also known as *Giardia intestinalis or Giardia duodenalis*. This parasite is responsible for causing giardiasis, which can manifest as asymptomatic or with acute and chronic symptoms such as diarrhoea and malabsorption (Carranza & Lujan, 2010). It is estimated that around 280 million people are infected with *G. lamblia* every year worldwide, in human and animals. Most cases are found in developing countries, where poverty and poor hygiene can result in a childhood prevalence rate of up to 30%. However, even in developed countries, *G. lamblia* can be a significant cause of waterborne illness, particularly in areas where water treatment and sanitation systems are inadequate (Carter et al., 2018). The World Health Organization (WHO) stated that globally, around 200 million people are infected with this disease, primarily children aged between 2 and 12 years (Vivancos et al., 2018). In 2016, there were approximately 1.6 million reported cases of deaths caused by diarrhoea. Out of these cases, around 90% of the deaths occurred in South Asia and sub-Saharan Africa. It is important to note that diarrhoea is responsible for one in nine child deaths, resulting in 2,195 deaths per day, which adds up to a total of 801,000 child deaths annually. This figure is higher than the combined cases of malaria, measles, and AIDS. The Centre for Disease Control and Prevention (CDC) (2015) reported that malaria, measles, and AIDS have caused many illnesses, and roughly 250 million reported cases of diarrhoea are associated with *G. lamblia*, a parasitic disease. Diarrhoea is the second leading cause of morbidity and mortality

after pneumonia among children, underscoring the importance of controlling giardiasis to reduce diarrhoea-related deaths. Clean water and sanitation are the two primary strategies for addressing giardiasis, which is prevalent and severely impacts children, health, and socioeconomics in disadvantaged communities. Therefore, giardiasis was included in the WHO Neglected Diseases Initiative in 2004. Furthermore, giardiasis is of significant clinical and economic importance in livestock and pet animals, necessitating an integrated approach to comprehensively control the disease under the One Health framework (Roshidi et al., 2021).

The pathogenesis of giardiasis may involve the death of enterocytes, along with rearrangement of the cell's cytoskeleton, caused by toxic substances released by the trophozoites. This leads to increased permeability of the epithelial layer and disruption of tight junctional proteins. The toxins produced by trophozoites, along with T-cell activation, cause a reduction in the length of microvilli in the small intestine's brush border and decreased activity of brush border enzymes, particularly lipase, some proteases, and the disaccharidases lactase and maltase. Malabsorption caused by giardiasis is linked with increased numbers of intraepithelial lymphocytes and a decreased ratio of villi to crypts. This malabsorptive diarrhoea can lead to reduced weight gain. The presence of mucus in the diarrhoea may be due to the decreased activity of lipase and increased mucin production by goblet cells. Giardiasis can also decrease food transit time in the gut and increase gut contractility, which could explain the abdominal cramps frequently observed in giardiasis (Thigeel, 2016).

The risk factors associated with *G. lamblia* infection include water contamination, poor sanitation, travel and tourism, animal contact, person-to-person transmission, immunocompromised individuals, foodborne transmission, and

socioeconomic factors. Consumption of untreated or inadequately treated water from contaminated sources, such as rivers or wells, increases the risk of infection. Inadequate sanitation practices and poor personal hygiene contribute to transmission. Traveling to regions with a high prevalence of *G. lamblia* exposes individuals to contaminated water and food. Direct or indirect contact with animals, including pets or livestock, can lead to transmission. Close contact with infected individuals in settings with poor hygiene or crowded environments facilitates the spread. Immunocompromised individuals are more susceptible to infection. Consumption of contaminated food, especially raw or undercooked fruits, vegetables, or seafood, can be a source of transmission. Socioeconomic conditions, such as poverty, limited access to clean water, sanitation facilities, and overcrowded living conditions, increase the risk. Understanding these risk factors is vital for developing targeted interventions and preventive strategies to reduce the transmission and impact of *Giardia lamblia* infection (Leung et al., 2019).

1.2 Problem statement

G. lamblia is a common protozoan parasite that causes gastrointestinal infections in humans. The current diagnostic methods for detecting *G. lamblia* in stool samples, such as microscopy is suffering from low assay sensitivity and unable to be scale up. To scale up output of microscopy examination, more well-trained microscopists are required (Garrett, 2006). The sensitivity of parasite detection is often limited by the inability to culture the stools as the practice in bacterial and viral analysis. Intermittent shed of the parasite cysts in stool samples also increase the challenge of accurate diagnosis as it could lead to misdiagnosis. PCR, on the other hand, could amplified the DNA of live or dead parasite, could aid in detection stool samples with low parasite

count. Therefore, there is a need to develop an in-house PCR assay. By developing an in-house PCR assay, it would be possible to optimize the detection process and improve the efficiency and accuracy of diagnosis. Additionally, the development of an in-house PCR assays a cost-effective alternative to commercial kits, making it more accessible for laboratories with limited resources. This PCR assay can also aid in investigating potential zoonotic transmission of *Giardia* in humans and mammals such as cats and dogs, in addition to its diagnostic purpose (Dixon, 2021).

1.3 Objectives

1.3.1 General objective

This study aimed to develop an in-house PCR for the detection of *G. lamblia* in cat and dog stool samples.

1.3.2 Specific objectives

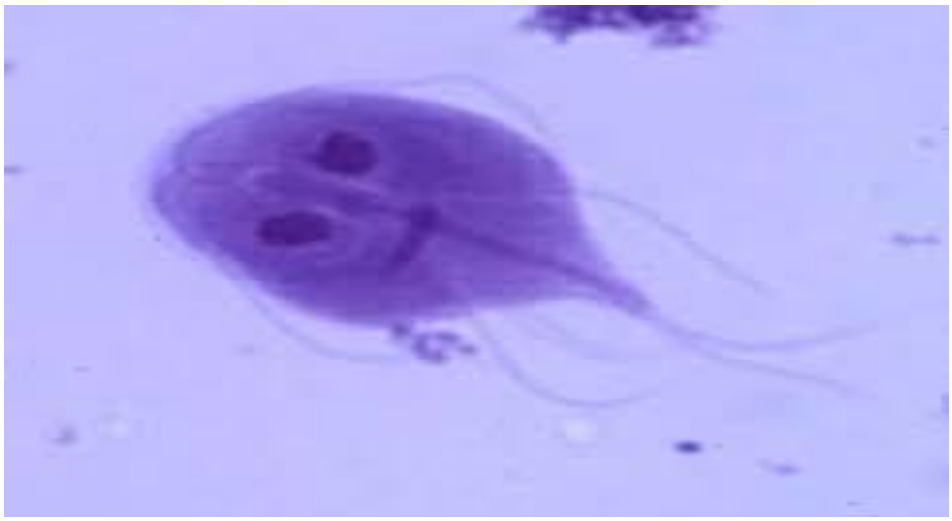
1. To establish the *Giardia* trophozoite culture
2. To develop an in-house *Giardia*-PCR and internal control PCR
3. To determine the detection limit of an in-house *Giardia*-PCR
4. To screen cat and dog stool samples with a developed in-house *Gardia*-PCR

CHAPTER 2

LITERATURE REVIEW

2.1 *Giardia lamblia*

Giardia lamblia is a single-celled parasite that has flagella and lives in the small intestine of its host. When this parasite infects the host, it can cause giardiasis, a gastrointestinal illness that is a significant cause of illness and death around the world (Roshidi et al., 2021). Antony Van Leeuwenhoek originally discovered *Giardia* in 1681, describing the parasites as "animalcules a-moving very prettily" in a sample of his own stool. He noted their body shape, flat belly with small paws, and slow movement. A stained image of a single *Giardia* parasite matching his description is shown in Figure 1. Later, in 1882 and 1883, Kunstler identified a similar organism in tadpoles and named it Giardia, thus establishing the genus name for these parasites (Chang et al., 2023).



Source (Anderson, 2020)

Figure 2.1 Unicellular *Giardia lamblia* trophozoites.

Giardia belongs to the Domain Eukaryota, Kingdom Excavata, Superphylum Metamonada, Phylum Fornicata, Order Diplomonadida, Family Hexamitidae, and Genus Giardia. With this classification, there are over 40 different types of *Giardia* that can be described. Some of the distinct species of *Giardia* include *G. muris from mice, G. agilis* from amphibians, and a third group that infects various warm-blooded animals. This third group contains at least four species, including *G. ardeae and G. psittaci from birds, G. microti* from muskrats and voles, and *G. duodenalis* (also known as *G. intestinalis and G. lamblia*), a species complex that infects a wide range of mammals, including people and domestic animals (Adam, 2021).

G. lamblia can be classified into eight different assemblages or genotypes, labelled as assemblages A-H. These assemblages can infect a broad range of mammals, including humans (Atan Edinur et al., 2023). Assemblages A and B are known to infect both humans and other mammals, while the other assemblages (C, D, E, F, G, and H) are not known to cause infection in humans. Assemblages C and D are known to infect domestic animals like dogs, while assemblage E mainly infects hoofed animals, domestic ruminants, and pigs. Assemblage F infects cats, while assemblage G primarily infects rodents like mice, and assemblage H infects seals (Chang et al., 2023). table 2.1 shows the established *Giardia* species and *G. intestinalis* assemblages.

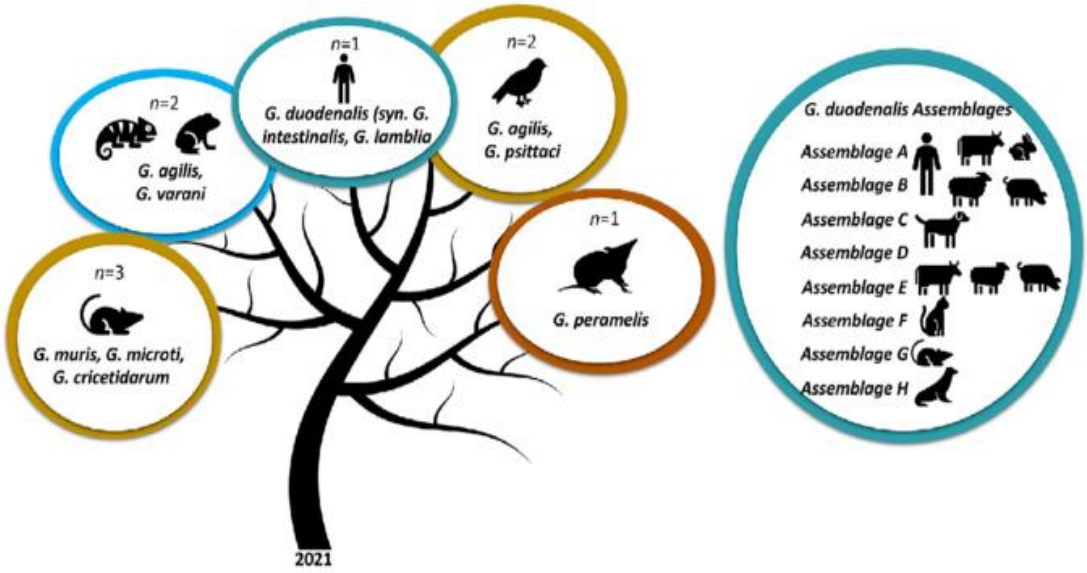
Humans can be infected with *Giardia* assemblages A-I to A-IV and B, while dogs are primarily infected with C and D, and cats with F. Assemblage A-I has been found in both humans and animals, A-II in humans, and A-III and IV exclusively in animals, whereas assemblage B can infect both humans and animals. Rats have G and seals have H, making *Giardia* less likely to be zoonotic. Over 40 species of birds, amphibians, and mammals serve as hosts for Giardia. The most common mammals

infected with *Giardia* include dogs, cats, rabbits, cattle, sheep, horses, swine, and humans. Animals are believed to be the source for human outbreaks, as they can shed assemblages A and B which can infect humans. Additionally, animals can become infected by humans via sewage and wastes entering water sources, making them a possible zoonotic host (Chang et al., 2023). Figure 2.2 shows *Giardia* species and *G. lamblia* assemblage

Table 2.1 *Giardia* species and assemblages.

Species	Genotype	Proposed species name	Host
G. intestinalis	Assemblage A	*G. duodenalis*	Humans and other mammals
	Assemblage B	*G. enterica*	Humans and other mammals
	Assemblage C,D	*G. canis*	Domestic and wild canids
	Assemblage E	*G. bovis*	Hoofed animals
	Assemblage F	*G. cati*	Cats
	Assemblage G	*G. simondi*	Rodents
	Assemblage H		Sea mammals
G. muris			Rodents
G. agilis			Amphibians
G. microti			Rodents
G. ardeae			Herons
G. psittaci			Parakeets

Source: Liu (2019)



Source: Feng and Xiao (2011)

Figure 2.2 *Giardia* species and *G. lamblia* assemblage

2.2 Epidemiology of *Giardia*

G. lamblia, a single-celled organism, is found worldwide and has a cosmopolitan distribution. Studies have shown that this parasite is a common cause of parasitic infections in children, particularly in areas with poor sanitation and low economic status. Approximately 280 million people in Asia, Latin America, and Africa are infected with *G. lamblia* annually (Seher et al., 2019). In low-income countries, giardiasis has a high prevalence, ranging from 20% to 40%, with young children under 5 years of age being at the greatest risk. In high-income countries, the prevalence is lower, ranging from 2% to 7%, and is mostly associated with travellers returning from highly endemic areas (Roshidi et al., 2021).

Giardiasis has a high prevalence in western Nepal, with a reported occurrence of 73.4%, which is largely attributed to inadequate sanitation. In the United States, approximately 2.5 million cases of giardiasis are reported annually since 2002. Poor

access to clean water is a common cause of diarrhoea among travellers to regions of Africa, Asia, and Latin America (Fida et al., 2023).

A surveillance survey conducted in 2011 and 2012 found that giardiasis affected 16,868 cases per 100,000 population in 2011 and 5.8 in 2012. It was found to be the leading cause of diarrhoea in children aged 1-9 years, possibly due to increased exposure to recreational water, poor hygiene, and contact with infected children in childcare centres. Males, particularly adults, were more likely to be affected, possibly due to their engagement in outdoor activities, occupation, and homosexual practices (Painter et al., 2015).

Giardiasis may be underreported in Malaysia, and it is most prevalent among aboriginal communities, with a prevalence rate of 29.2%. The number of cases diagnosed in West Malaysia is higher than in East Malaysia. Giardiasis has been observed to be most prevalent among aboriginal communities in several states of Malaysia, including Pahang, Negeri Sembilan, and Kedah, with the highest rate reported in Pahang at 15.9%. In contrast, the prevalence was found to be relatively low in Malacca at 4.6%. In West Malaysia, the occurrence of *Giardia* infection was higher in children under 12 years of age in aboriginal communities. This is due to several factors such as inadequate sanitary and healthcare facilities, lack of clean water supply, poor hygiene practices, insufficient health education, and poor housing conditions. (Choy et al., 2014). Figure 2.3 shows the distribution of giardiasis in Malaysia.

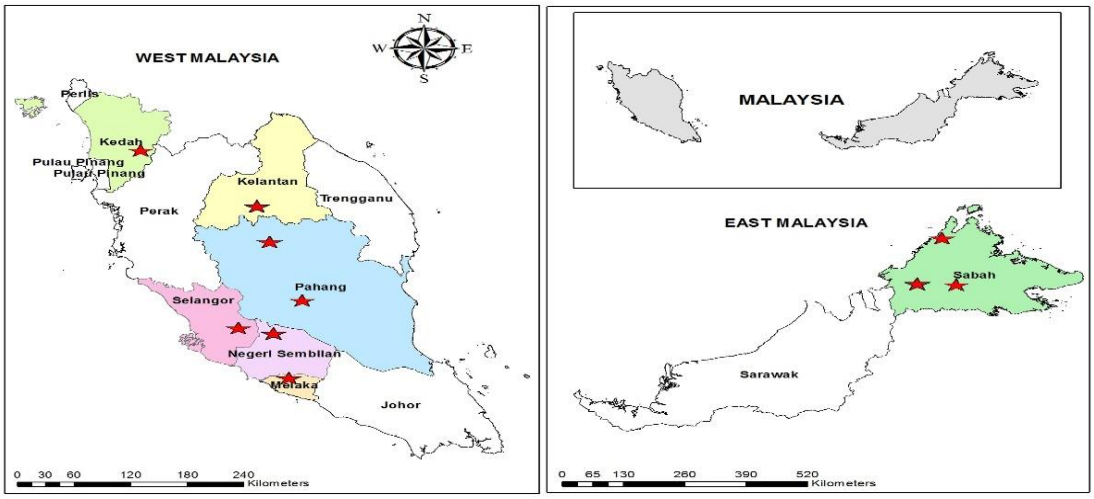


Figure 2.3 Occurrence of giardiasis in Malaysia.

Source: A geographical map showing the location of district (stars) and states (coloured) involved. Reported by Choy et al., (2014)

2.3 Life cycle of *Giardia* sp.

The *Giardia* parasite has a simple life cycle consisting of two main metabolic states: the trophozoite and cyst. Trophozoites are motile and actively replicate, while cysts are non-motile and metabolically dormant. It also includes two intermediate stages: the excyzoite and encyzoite. The excyzoite stage is when the parasite rapidly exits its protective cyst wall and transforms into four replicating trophozoites. The encyzoite stage is when the trophozoite slowly rebuilds its protective coating and returns to a dormant state. Infection with *Giardia* occurs when a susceptible host ingests viable cysts through contaminated water, food, or materials. Within hours, the cysts open in the upper part of the intestine, releasing two newly formed trophozoites that infect the intestinal cells. The parasite multiplies on the lining of the small intestine, causing diarrhoea that can interfere with nutrient absorption. The trophozoites move to the end of the intestine and form new cysts that are excreted in faeces. The number of cysts released varies, and they can survive in the environment, allowing the parasite to infect other susceptible hosts (Thigeel, 2016).

Giardia trophozoites possess four pairs of flagella and are characterized by their motility. They exhibit a distinctive twisting motion, but they are mainly found attached to the epithelial cells of the small intestine. The *Giardia* trophozoites attach to the intestinal epithelium using an adhesive disk located on the ventral side of the parasite. They obtain nutrients from the intestinal lumen by either pinocytosis or contact digestion. Trophozoites multiply by binary fission in the small intestine and don't have any intracellular stages. This leads to the prepatent period, which lasts for about 3-10 days. After this period, *Giardia* undergoes cyst shedding, which can last for several days to weeks and is intermittent in the chronic phase. Cysts shed by *Giardia* can survive for several weeks in the small and large intestine environment, while the trophozoite form cannot (Vivancos et al., 2018).

Giardia can form cysts as an alternative to replicating the trophozoite. During encystment, the parasite changes its shape, separates from the intestinal epithelium, and secretes a cyst wall. To induce encystment, trophozoites are deprived of bile at pH 7, followed by exposure to high bile concentrations at pH 7.8. The production of cyst wall proteins and large secretory vesicles in the parasite cytoplasm occur after encystment induction. During cyst maturation, the *Giardia* parasite undergoes one round of nuclear division without cytokinesis, resulting in two or four nuclei. The flagella and adhesive disk are lost during maturation, while the axonemes and median bodies persist. The mature cysts are oval-shaped and can measure 8-12 µm in length and 5-6 µm wide. These cysts are passed in faeces and can survive for up to three months under appropriate temperature and moisture conditions. The mature cysts are infective to the next possible host if ingested, completing the life cycle. Figure 2.4 shows the summary of *G. lamblia* life cycle (Dixon, 2021).

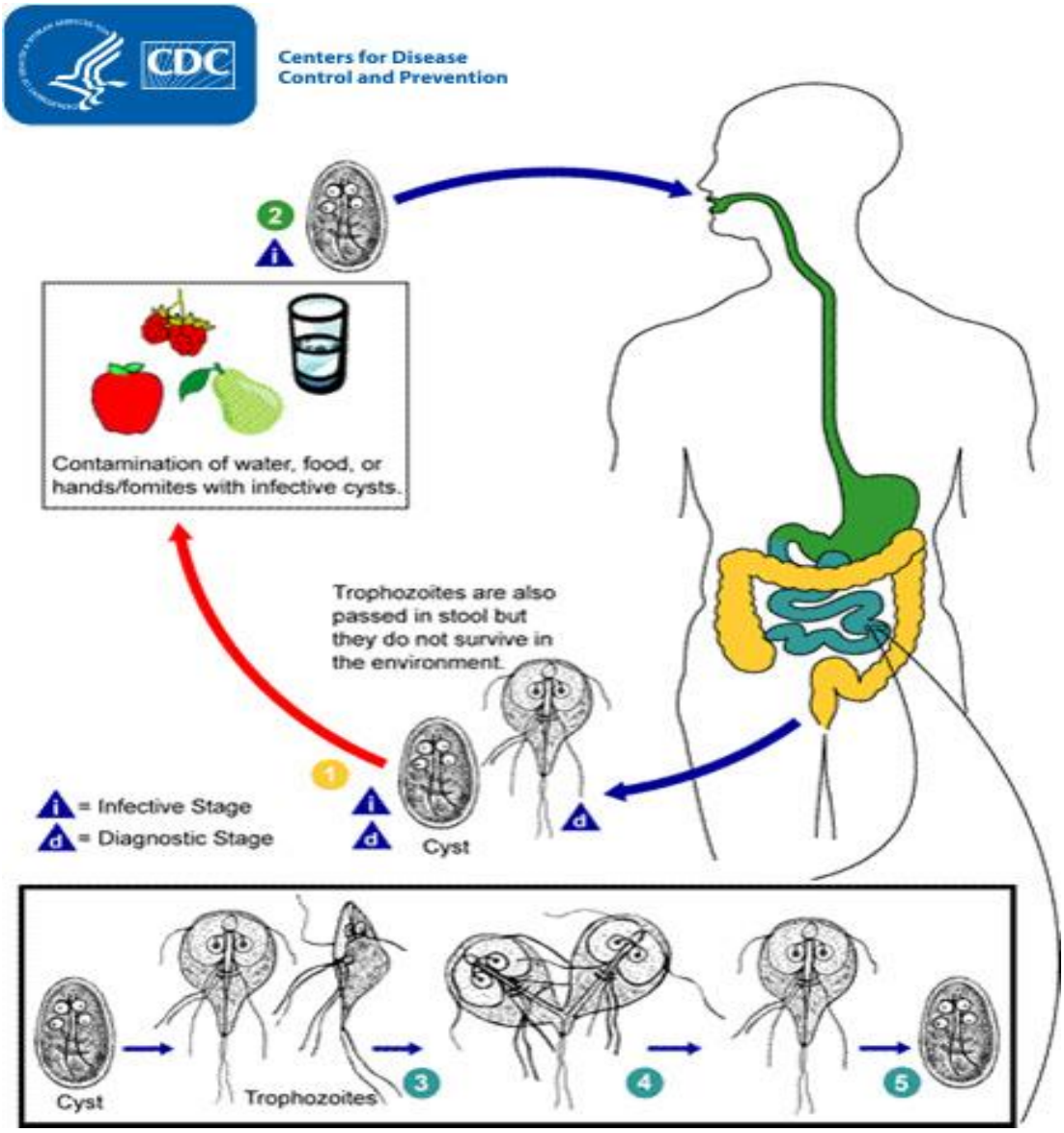


Figure 2.4 Life cycle of *Giardia lamblia*

2.4 Protozoan morphology

The life cycle of *G. lamblia* includes two different stages, which are the trophozoite and cyst stages.

2.5 Cyst

The *G. lamblia* is an oval-shaped organism that measures around 12×6 microns in size. Its granular cytoplasm contains the remnants of flagella, and it has two pairs of nuclei

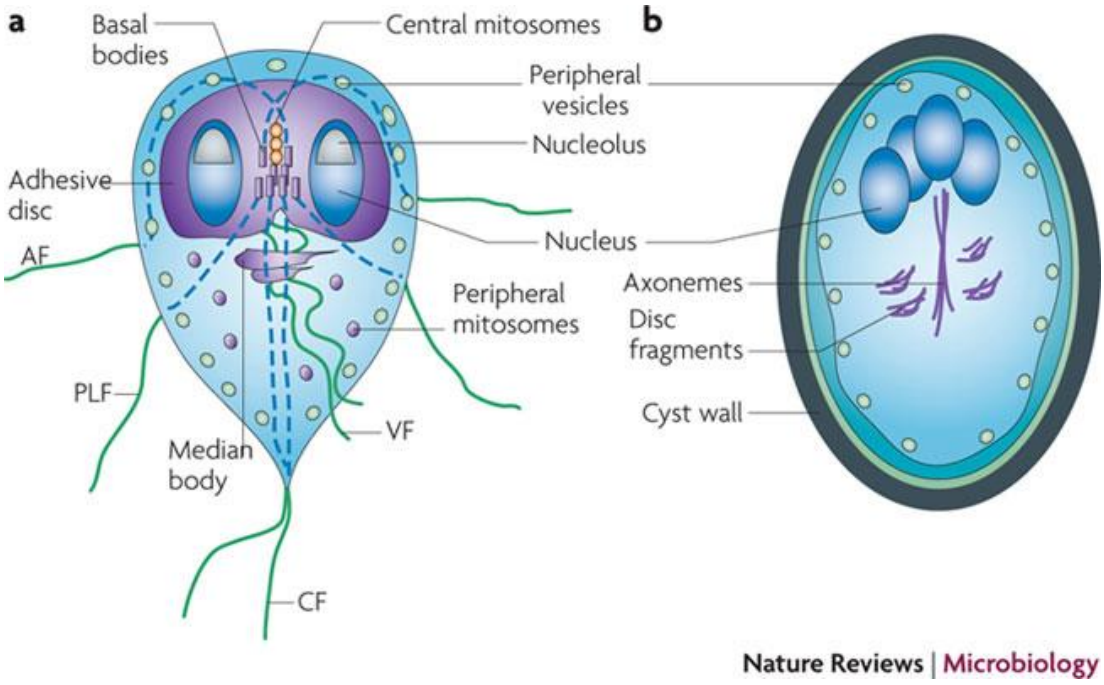
that can either cluster at one end or occur in pairs at opposite ends. The organism's cyst has a clear space between its cytoplasm and protective cell wall. It can adapt to the external environment and can survive for weeks or months (AL-kahfaji & Alsaadi, 2019).

The *Giardia* parasite is protected by a strong cyst wall made up of a meshwork of sugar and protein at a 3:2 ratio, with the carbohydrate component consisting of a homopolymer of β (1-3)-N-acetyl-d-galactosamine. The remaining 40% of the cyst wall is made up of cyst wall proteins (CWPs) which strongly interact with the carbohydrates. Among the identified CWPs are three leucine-rich repeat-containing proteins (CWP1-3) and a high-cysteine non-variant cyst protein (HCNHp). These oval-shaped cysts, which make up most of the parasite's life outside of the host, are responsible for transmitting the disease from one host to another. *Giardia* cysts can survive for extended periods of time in cool and moist conditions in the environment. However, they are vulnerable to desiccation and direct sunlight and are destroyed more quickly in hot and dry conditions. Studies have shown that cysts can survive in tap or lake water for up to two months at 0-8 °C, in tap water for 2 weeks at 20-28 °C, and in lake water for 1 month at 17-20 °C. In river water, cysts remained viable for almost 3 months at 0-4 °C and 1 month at 20-28 °C, while in seawater they survived for more than 2 months at 4°C. In soil held at 4°C, almost 90% of cysts were still viable after 49 days, but infectivity was lost within 7 days at 25 °C. Cysts were also found to survive for one week in solid cattle manure at 4 °C, and for as long as 18 days in human faeces (Fever, 2005).

2.5.1 Trophozoite

Giardia is a pear-shaped organism, measuring around 12-15 microns in length and 5-9 microns in maximum width. It has two sucking disks on the underside, which it uses to attach to the mucosal surface of the small intestine. The organism is bilaterally symmetrical and has two nuclei positioned symmetrically with an oval shape. The nuclei display differences in nuclear pore number and distribution during division. The organism also possesses two axostyles in the middle of the body and a rod-like structure known as the median body running across the axostyles. The trophozoite has eight flagella, with a pair located at the front, two pairs in the middle, and a pair at the back, which it uses to move around (AL-kahfaji & Alsaadi, 2019).

Giardia trophozoites have an adhesive disc on their ventral side, composed of microtubules and microribbons. It contains proteins such as *giardins, α and β-tubulin*, SALP-1, and aurora kinase. The adhesive disc is used for attachment to the host epithelium and avoiding elimination due to intestinal peristalsis. The attachment is strong and can result in an imprint in the epithelium. The suction created by the ventrolateral flange that surrounds the ventral disc is an important factor in parasite attachment. Attachment is also achieved by surface adhesive lectins. Altering the growth substrate to a spiky surface can decrease parasitemia in vitro, indicating that steric hindrance can play a role in preventing attachment (AL-kahfaji & Alsaadi, 2019).



Source: AL-kahfaji and Alsaadi (2019)

Figure 2.5 Trophozoite and cyst stages of *G. lamblia*.

2.6 Giardiasis in human

It is believed that the infective dose of *G. lamblia* for humans who display symptoms is relatively low, with an estimated range of 10 to 100. When infected, humans intermittently shed *Giardia* cysts, with up to 2 x 10^6 per gram of faeces. Giardiasis can cause mild to severe illness, with common symptoms including abdominal cramps, diarrhoea, bloating, flatulence, nausea, and weight loss. The illness typically lasts for 1-2 weeks but can persist for up to seven weeks in some cases. Malnourished children are at risk of developing chronic giardiasis. *Giardia lamblia* is typically found in the upper portion of the small intestine, and this may be influenced by factors such as the virulence of the infective strains or the number of cysts ingested, as well as host factors such as age and immune system status. Human giardiasis can be categorized as either acute or chronic. The acute phase is typically short and involves symptoms such as abdominal cramps, flatulence, and watery diarrhoea that becomes bulky with an

unpleasant Odor. The chronic phase is characterized by malaise, weight loss, and malabsorption, with diarrhoea that is frequent, small in volume, and pale or yellow in color (Thigeel, 2016).

2.7 Giardiasis in animal

The two assemblages of *G. lamblia* that infect dogs are C and D, while assemblage F is responsible for infecting cats, although assemblages A and B can infect both dogs and cats. The prevalence of *G. lamblia* in companion animals varies depending on the region, diagnostic method, and general health of the animal. In the USA, the estimated prevalence of *G. lamblia* in kennel/shelter dogs was 39.0%, and in household dogs, it was 34.0%, both diagnosed through microscopic examination. However, prevalence estimates for clinically affected dogs were 15.6% using immunochromatography and 4.0% using microscopic examination. The prevalence of *Giardia* in cats, diagnosed using microscopic examination, was relatively low at 0.58% compared to dogs. Young animals tend to shed more cysts than adult animals, and this is due to the slow development of their adaptive immune system. This may make young animals more likely to transmit the disease directly to other susceptible animals and contribute to environmental contamination with *Giardia* cysts (Thigeel, 2016).

2.8 Zoonotic consideration of *G. lamblia*

Giardia lamblia is considered a zoonotic agent by the World Health Organization because of waterborne outbreaks in humans caused by infected beavers. This means that the disease can be transmitted from animals to humans, and reverse zoonosis (infection from humans to animals) can also occur. Among the eight assemblages of *G. lamblia*, assemblages A and B can infect both humans and animals, with assemblage

A having a greater zoonotic risk than assemblage B. Assemblage A has four subgroups, with AII being the most common subgroup found in humans, while AIII and AIV are commonly isolated from animals. The subgroup AI is the only subgroup with confirmed zoonotic potential, as it is isolated from both humans and animals. Assemblage B has four subgroups, with BIII and BIV commonly isolated from humans, while BI and BII are commonly isolated from animals. Some studies have reported the zoonotic potential for assemblage A only. Direct transmission between humans can also maintain both assemblages, and they can also infect companion animals, livestock, and wildlife (Thigeel, 2016).

The zoonotic transmission of *Giardia* from dogs or cats to humans is still not fully understood, and there is limited data available on the frequency of such transmission. Although *Giardia* isolates in assemblage A have a higher zoonotic risk than those in assemblage B, several studies have reported the zoonotic potential for assemblage B as well. However, there is a considerable genetic variation between and within the *Giardia* genotypes, and some patients infected with assemblage B can also develop persistent diarrhoea. Studies conducted in different parts of the world have shown that dogs or cats can be infected with host-adapted and/or zoonotic *Giardia* assemblages, but further research is needed to understand the extent of zoonotic transmission from companion animals to humans (Thigeel, 2016).

2.9 Route of transmission of *G. lamblia*

Giardia lamblia is a parasite that spreads through the faecal-oral route, either directly or indirectly. Indirect infection is common and occurs when contaminated food or water is consumed. Poor hygiene practices, such as not washing hands properly, can

contribute to the spread of the parasite (Ivanov, 2010). Person-to-person transmission is also possible through oral-anal contact. In institutional settings such as day care centres and nursing homes, proper hygiene practices are crucial in preventing the spread of Giardia. A possible outbreak among humans may result from a failure in water treatment facilities or poor sanitary conditions. Gender doesn't seem to have a significant effect on *Giardia* rates, but the number of cases is notably higher for individuals aged 15 to 54 years old (Masrin et al., 2019).

Animal reservoirs play a crucial role in the transmission of various assemblages of *Giardia lamblia.* Stray animals are particularly significant in spreading infectious cysts as they wander around different places. Infected pets can also contribute to the spread of cysts within their immediate surroundings, such as their owners or other domestic mammals. Moreover, transmission can easily occur in crowded places, including nurseries and orphanages (Fantinatti, 2019).

Outbreaks of giardiasis have been linked to a variety of sources such as consuming contaminated water and food and encountering contaminated swimming and wading pools. In the UK, research has shown that cases of giardiasis are associated with activities such as swallowing water while swimming, recreational contact with fresh water, drinking tap water, and eating green salad. The contamination of salad may occur when it is washed with contaminated water or handled by an infected person (Espelage et al., 2010). In Malaysia, the Orang Asli population is mainly infected through the consumption of uncooked vegetables like tapioca shoots, wild fern shoots, and locally grown leaves (Anuar et al., 2014).

2.10 Immune response of giardiasis

2.10.1 Innate immunity

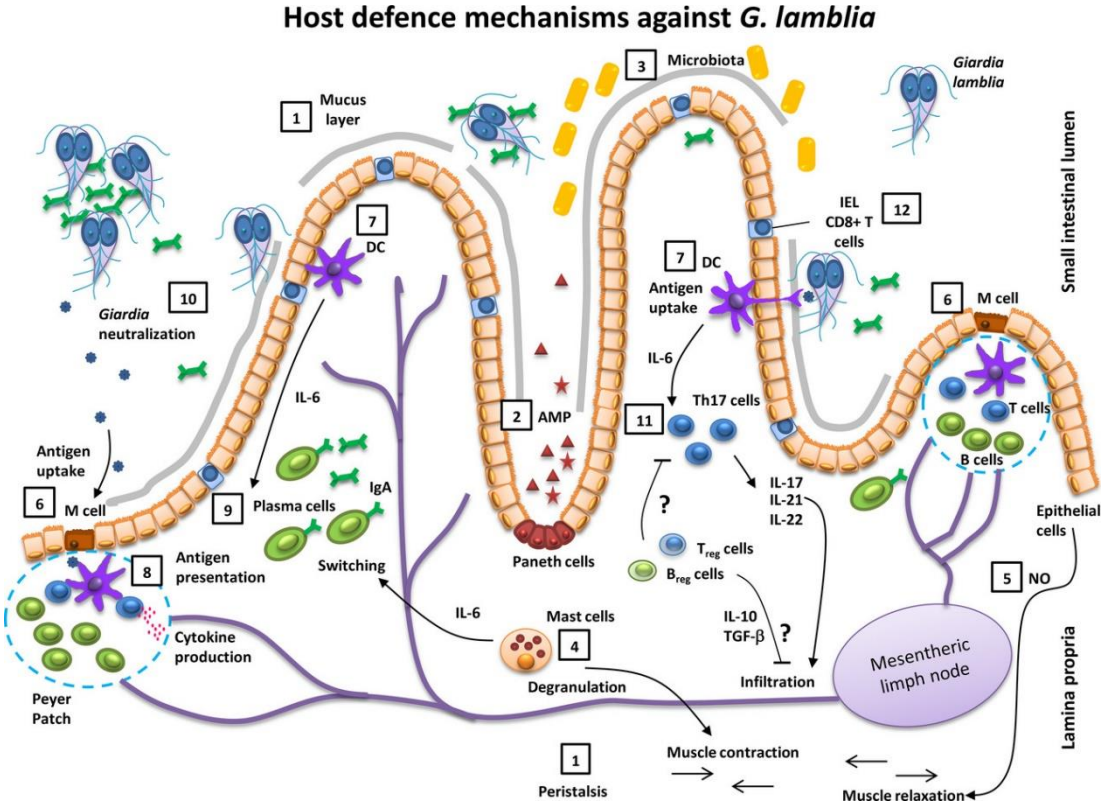
The host's innate immune response is the primary defence against *G. lamblia* infection and is responsible for recognizing the organism. When trophozoites meet the epithelial cells lining the small intestine, they stimulate the production of chemokines and cytokines, which regulate innate immunity and help eliminate the parasite. Dendritic cells are also able to recognize and respond to *G. lamblia* trophozoites. Beneficial microbes in the small intestine lumen can increase resistance to parasites. Studies have shown that human milk contains lipase enzyme, which can destroy and kill *Giardia* trophozoites. Nitric oxide has also been found to play a crucial role in inhibiting trophozoite differentiation into cysts and stopping the replication process (Adam, 2021).

2.10.2 Acquired immunity.

Giardiasis triggers an immune response against the parasite, resulting in the production of antibodies. The presence of specific antibodies and complement inhibits the replication of the parasite. Immunoglobulin A (IgA) is a crucial factor in controlling and clearing the parasite and is found in maternal milk and intestinal secretions. Mast cells also play an important role in detaching the parasite from the mucosal surface by increasing smooth muscle contraction and intestinal motility. CD4 (+) T cells are essential in the immune response against Giardia, as they activate and stimulate the differentiation of B cells to produce antibodies specific to the parasite. The immune response of the host is crucial for the elimination of *Giardia* from the intestine and provides protection against future infections. Both the innate and adaptive immune systems work together to protect the host. Antimicrobial peptides produced by the

epithelial paneth cells have *anti-giardial* properties and can kill the trophozoites. Reactive oxygen species and nitric oxide can also inhibit the excystation process and are toxic to the *Giardia* trophozoite. The presence of mast cells in the host contributes to the survival and activation of B-cells, which differentiate into plasma cells and produce IgA antibodies. *Giardia* infection also leads to the maturation of dendritic cells, resulting in increased expression of cytokines and surface molecules, which activate the adaptive immune mechanisms (Adam, 2021).

After *Giardia* infection, the adaptive immunity response is activated within two weeks, with CD4+ T-cells detecting and recognizing the presence of antigens and becoming helper cells, while CD8+ T-cells become cytotoxic. These T-cell-mediated immune responses are crucial in clearing the infection, as a decrease in CD4+ T-cells can lead to chronic giardiasis. CD4+ T-cells are important in the activation, maturation, and differentiation of B-cells into plasma cells that produce immunoglobulins such as IL-17, which then move to the infection sites and kill the parasites. However, the activation of CD8+ T-cells may cause damage to the host's intestine. The antimicrobial peptides produced by epithelial paneth cells and mast cells also contribute to parasite clearance, while the activation of dendritic cells induces the adaptive immune mechanisms. Figure 2.5 illustrates the host's Défense mechanisms against *Giardia* infection (Adam, 2021).



Source: Lopez‐Romero et al. (2015)

Figure 2.6 Host Défense Mechanism against *Giardia*

2.11 Clinical manifestation

The symptoms of a *Giardia* infection can vary depending on factors such as the severity of the infection, the type of host, and the duration of the parasite's presence. The *Giardia* parasite infects the small intestine and can cause an increase in intestinal permeability, leading to a shortening of microvilli in the small intestine. This can result in decreased absorption of water, electrolytes, and nutrients, leading to malabsorptive diarrhoea and lower weight gain. Acute signs of *Giardia* infection can include flatulence, abdominal pain, nausea, loss of appetite, and explosive, foul-smelling diarrhoea. Stools associated with *Giardia* infection are often described as loose, bulky, frothy, and/or greasy, and may lack blood or mucus Other gastrointestinal symptoms may include bloating, anorexia, cramps, sulfuric belching, and, in rare cases,

Hematochezia (the presence of blood in stools). Chronic symptoms of *Giardia* infection may include intermittent diarrhoea, weight loss, poor growth, and poor appetite. Some individuals may overcome acute symptoms but remain asymptomatic carriers, while others may have occasional acute symptoms (Dixon, 2021).

Acute giardiasis, which is commonly observed in travellers, is characterized mainly by prolonged diarrhoea. The incubation period for this infection typically ranges from 9 to 15 days. The acute phase of the disease often starts with a sense of discomfort in the intestinal region, followed by loss of appetite and nausea. This may be accompanied by mild fever and chills, and is then followed by explosive, foul-smelling, watery diarrhea, abdominal pain, flatulence, and belching. These symptoms usually last for around 3 to 4 days and may resemble other types of travellers' diarrhoea. If left untreated, the symptoms may persist for several months (Adam, 2021).

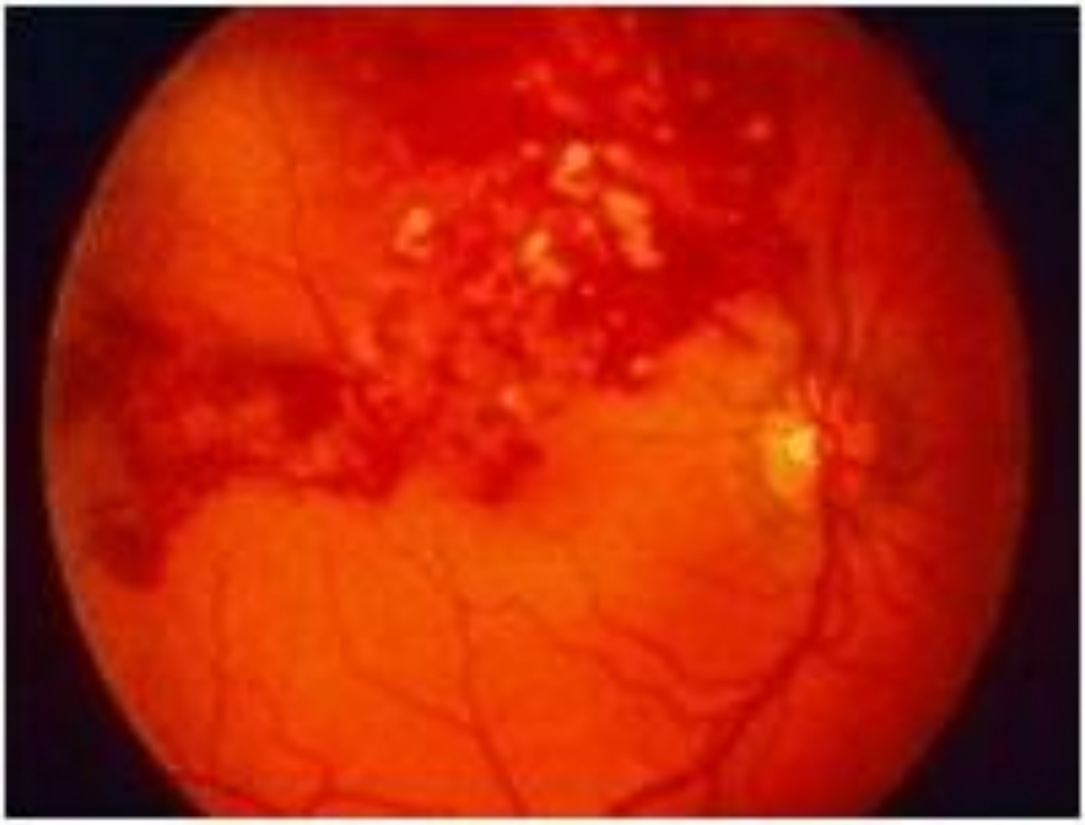
2.11.1 Long term consequences of giardiasis

Recent studies have linked *Giardia* infection in patients to a range of extra-intestinal complications that persist in the long term. These complications may affect different parts of the body such as the eyes, muscles, and metabolism. Examples of these complications include allergies, muscular problems, and nutritional consequences such as stunting, failure to thrive, and impaired cognitive function (Adam, 2021).

2.11.2 Ocular complications

Giardiasis has been linked to ocular complications such as iridocyclitis, choroiditis, and retinal hemorrhages. A recent study revealed that children with giardiasis showed

a "salt and pepper" degeneration in the retinal pigmented epithelium (as shown in Figure 2.7). This degeneration is thought to occur due to damage to the retina cells and the release of pigment granules in the retinal layer. There is no direct evidence of invasion by the parasite in the ocular tissues, but it is believed that toxic metabolites produced by the parasites may be responsible for these ocular lesions (El-Sayed & Safar, 2015).



Source: El-Sayed and Safar (2015)

Figure 2.7 Typical salt and p2epper appearance of the retina in a child infected with *Giardia lamblia*.

2.12 Arthritis in giardiasis

According to some case studies, there seems to be a link between giardiasis and reactive arthritis or joint pain. However, there is limited evidence to support this claim and the extent of post-giardiasis arthritis is still uncertain. To date, no large-scale epidemiological studies have been conducted to investigate the relationship between these two conditions. However, a recent retrospective cohort study conducted in the United States has provided some evidence to support the claim of an association between giardiasis and the subsequent development of joint pain (Dixon, 2021).

After an infection, reactive arthritis typically affects the joints of the lower limbs, particularly the knee and ankle. Reactive arthritis is classified as a type of spondyloarthropathy and is associated with human leukocyte antigen (HLA)-B27, a major histocompatibility complex class I allele that is present in about 50% of patients with enteric-infection-related arthritis (Halliez & Buret, 2013).

2.13 Muscular complications

The condition known as hypokalaemia myopathy has been linked with coeliac disease, radiation enteritis, and infections. There have been several reported cases of hypokalaemia occurring in patients with giardiasis, both in those with weakened immune systems and those with normal immune function. It appears that giardiasis can cause muscle-related issues regardless of the host's immune status. The loss of potassium is associated with the frequency of diarrhoea episodes during the infection. The loss of potassium due to giardiasis can result in hypokalemia, which can cause transient and severe myopathy. This condition is common among elderly people, particularly women. However, while giardiasis-related hypokalemia is frequent, it