

GIARDIASIS

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INTRODUCTION

Giardiasis is the infection of the small intestine with the flagellate protozoa *Giardia lamblia*. The parasite normally lives in the duodenum and upper jejunum and may be associated with gastrointestinal symptoms particularly diarrhoea.¹⁻³

HISTORICAL NOTES

Giardia lamblia is amongst the first of the protozoan parasites to be seen under the microscope. Antonj van Leeuwenhoek described an organism that was probably *Giardia lamblia* in his own diarrhoeal stools in 1681.⁴ It was reported as *Cercomonas intestinalis* by Vilem Fedorovic Lambl in 1859. The genus name *Giardia* was given by Kunstler in 1882. In 1888, Blanchard called it *Lamblia* as a synonym of *Giardia*. Unfortunately, so far there has been no agreement on the taxonomy and nomenclature of this protozoa and a state of disagreement persists until today. Americans call the human parasite *Giardia lamblia*,⁵ West Europeans refer it to as *Giardia intestinalis*, whereas the east Europeans call it *Lamblia intestinalis*. Other names found in the literature are *Giardia duodenalis* and *Giardia enterica*.⁶ In this article, the parasite is referred to as *Giardia lamblia*.

Taxonomically, the genus *Giardia* is placed in the Phylum *Sarcomastigophora* Honigberg and Balamuth, 1963; subphylum *Mastigophora* Dising 1866; Class *Zoomastigophoracida* Wenyon, 1926 and Family *Hexamitidae* Kent 1880.⁶

GEOGRAPHICAL DISTRIBUTION

Giardia lamblia has a world wide distribution. It is more common in the tropics and subtropics than in the temperate countries. In eighty-six surveys of 134,966 people throughout the world summarised by Belding,⁷ its prevalence was 2.4-67.5% with a mean of 10.4%. The most frequently identified intestinal parasite in public health laboratories in the United States is *Giardia lamblia*.⁸ It has been reported widely from many parts of the United States and in some areas, it is endemic.⁹⁻¹² It was

found in 7.4% of 35,299 persons in 24 surveys in the United States.⁷ It has been widely reported from Europe^{13, 14} and from tour groups returning from the Soviet Union.¹⁵⁻¹⁷ *Giardia lamblia* infection is endemic in Australia.^{18, 19}

In Southeast Asia it is endemic and has a wide distribution especially among children.^{1-3, 20-24} A review of literature on giardiasis in Malaysia revealed prevalence rates varying between 4.8% to 25.0%.²⁵⁻²⁹ In all endemic areas, it is more common in children than in adults.²¹

MORPHOLOGY

Giardia lamblia has two stages in its life-cycle; the feeding form or trophozoite and the propagative form or the cyst.

The trophozoite is pear-shaped with a broad rounded anterior end and a tapering posterior end. The trophozoite measures about 9 to 18 μm long, 5 to 8 μm wide and 2 to 4 μm thick. The dorsal surface is convex while the ventral surface is concave. The organism is bilaterally symmetrical with a large sucking or adhesive disc on the ventral side. It occupies most of the anterior region of the ventral surface. There are two large anterior vesicular nuclei. Four pairs of flagella are present. The first pair of flagella emerges anteriorly between the cell edge and dorsal surface, the second or lateral pair posterio-laterally from a groove between the margin and the dorsal surface, the third or the ventral pair from a groove posterior to the adhesive disc and the fourth pair caudally from the tapered end. The flagella which runs backwards through the middle of the body have been referred to as the median rods or axonemes. Two median bodies composed of bundles of microtubules arranged irregularly in the shape of the claws of a claw-hammer are present. These disappear in the course of division and it has been suggested to have something to do with the formation of a new adhesive disc. The shape of the median body is used to divide giardias into groups.³⁰

The trophozoites usually encyst before they leave the jejunum. It is not known whether the factors that initiate the encystation are intrinsic or en-

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environmental like changes in the intestinal pH. Most probably, it is a combination of both.

The cysts are ovoid to ellipsoidal and slightly asymmetric, measuring about 9 μm to 12 μm by 7 μm to 8 μm . The cysts from the same host often show variations in size and shape. The cysts have 4 nuclei when mature and a variable number of fibrillar remnants of the organelles of the trophozoite. The cyst wall is smooth without any characteristic markings. Cysts from different hosts appear to be morphologically indistinguishable.³¹

The fine structure

Studies on fine structure of various organelles of *Giardia lamblia* trophozoites obtained from human biopsies³² show that the sucking disc occupies the ventral portion of the entire anterior region. The disc has a characteristic striation due to alternating light and dense lines or ridges. The ridges are concentric within the centres of the lobes and are longitudinally arranged at the margins. At the midline anterior to the nuclei is an area devoid of striation termed the "naked area". The sucking disc is composed of perpendicular ribbons attached to microtubules aligned along the ventral plasma membrane. The central disc is considered to be the organelle of attachment.³³ When the parasite is in motion, all the flagella seem to contribute to the motion but when the parasite becomes attached, only the ventral flagella beat with vigorous synchronous rhythm while the others are quiescent. The synchronised beating produces a strong fluid flow that creates an area of lowered pressure in the region of the ventral disc resulting in the adhesive force necessary to hold the parasite against the mucosa. This is further supported by the fact that when the ventral flagellar motion stops; the parasites become detached from the substrate.

The median body of the *Giardia* trophozoite is described³⁴ as a group of straight or slightly curved double membranes which when cut in cross-section show circular appearances indicating them to be microtubules. There is no connection between the median bodies and other organelles of the parasite. It was concluded that the median bodies were possibly energy producing lipoprotein reserves.^{32, 34-36}

Each flagellum originates within the cytoplasm as an axoneme which consists of 9 rows of double microtubules encircling two central microtubules. All of these pass through the cytoplasm and protrude out of the main body of the trophozoite covered by a membrane to form a flagellum.

A series of circular to oval vacuoles situated near the dorsal surface of the trophozoite have been

seen. They are arranged in a single row between the plasma membrane and the granular cytoplasm. These vacuoles have been thought to be involved in the encystation process.¹ The cytoplasm contains large granules, which are probably glycogen and smaller granules which appear like individual ribosomes.³⁷ No Golgi bodies are seen.³⁸

The ultrastructure of the *Giardia lamblia* cyst was described by Sheffield and Bjorvatn.³⁹ The cyst wall is 0.3 μm in thickness and is composed of thin fibrous elements interspersed with fine particles. The central mass of the parasite appears to be separate from the thin layer adjacent to the cyst wall.

The life cycle is well known. As stated earlier, mechanisms that trigger encystation are still unknown. For excystation *in vitro*, a low pH is needed.⁴⁰ Within a few minutes of ingestion of viable cysts, excystation occurs and two trophozoites are liberated from each cyst. Each of these then multiplies by successive longitudinal binary fission to large numbers and colonise the duodenum and upper jejunum. When conditions in the gut are unfavourable or when the intrinsic stimulus is triggered, encystation occurs. During encystation, a resistant cyst wall is secreted by the parasite and the nuclei of the cyst divide once into a 4-nucleated cyst.

Enormous numbers of cysts may be found in the stool. The 4-nucleated cysts may need a period of maturation before excystation occurs *in vitro*.⁴⁰ Under suitable conditions, cysts will survive in the external environment for several weeks. Cysts have been shown to survive up to 77 days in tap water at 8°C.⁴¹

If the patient has diarrhoea, motile trophozoites, sometimes in large numbers, may be liberated in the watery stool. These are not infective and disintegrate within a short time.

TRANSMISSION

Transmission occurs by the faecal-oral route with viable cysts. Cysts are ingested in water,^{42,43} food contaminated by cysts or are transmitted by flies, food handlers⁴⁴ or possibly during intimate oro-anal contact among homosexuals.^{45,46} In recent years, several water-borne epidemics have been reported in the USA, some of which have been traced to municipal water supplies.^{11,43,47,48} Some of these were due to contamination of the water supply by sewage.^{43,49} In developed countries, the most common mode of transmission is through untreated drinking water.⁴²

Experiments with human volunteers^{41,50} have shown that the infecting dose can be as small as 10 cysts although infection is more certain with 100

cysts or more. The prepatent period is between 6 and 15 days and the incubation period is between 7 and 21 days.

CLINICAL FEATURES

A great majority of persons harbouring *Giardia lamblia* are asymptomatic. Symptomatic giardiasis can be acute or chronic. Children are more susceptible than adults. The usual time between infection and onset of symptoms in travellers is 15 days.^{9,10,15} The acute stage of infection is manifested by a sudden onset of explosive watery and often foul smelling diarrhoea. There is an increased frequency of stools (5-20 times a day). Symptomatic giardiasis may be characterised by intestinal malabsorption,⁵¹⁻⁵³ steatorrhoea,^{54,55} increased flatulence and abdominal distention. These patients often have mid-epigastric or sometimes generalised abdominal pain with mild weight loss.^{55,56} Less frequently, there may be vomiting, chills, low grade fever, headache, belching and generalised weakness. The acute stage may last from a few days to 2-3 months; rarely it persists longer. The most common chronic complaints are periodic episodes of mushy foul stools alternating with constipation.^{57,58}

PATHOLOGY

Giardia lamblia trophozoites are found next to the mucosa in the upper part of the small intestine. They attach themselves to the convex surface of the epithelial cells of the intestine with the aid of their sucking discs. The powerful sucking discs of the parasites can cause considerable disturbance of intestinal function by mechanical irritation to the mucosa and are also capable of affecting the fuzzy coat of the microvillus.^{59, 60} The physical attachment of parasites to the mucosal surface of the villi produces morphological alteration in the microvillus border and leads to impairment of normal intestinal function. A number of different mechanisms have been postulated to explain pathogenicity.^{35,56,61} Competition for essential nutrients, mechanical blockage and functional impairment of the mucosa^{54,60} are well documented, but tissue invasion is not proven. The mucosa in patients with giardiasis is normal or may show partial villous atrophy.^{59,62,63} *Giardia lamblia* can cause acute diarrhoea and is a potential cause of malabsorption.^{48,52,64} Mechanical interference with absorption particularly of fat from the intestine by a layer of parasites adhering to its wall like a carpet, may lead to malabsorption of vit. B₁₂, d-xylose, disaccharides, cyanocobalamin, fat and fat-soluble vitamins.^{1,2,9,10,48,52,56,65,66} Large amounts of unabsorbed fat in the stools causes persistent, recurrent diarrhoea often with large

amounts of yellowish mucus. These symptoms resemble those of coeliac disease or chronic gall bladder disease. The malabsorption is more pronounced for carbohydrate than for fat and diminished levels of thiamine, folic acid and cobalamin have been reported.^{52,67} Malabsorption of vitamin A has also been reported.⁶⁷ Lactase production is seriously depressed.⁶⁸ The presence of anaerobic bacteria may contribute to the steatorrhoea.^{67,70}

Patients with hypo- or agammaglobulinaemia who become infected with *Giardia* are likely to have malabsorption and abdominal symptoms.⁷¹ In immunologically normal patients,⁷² circulating antibodies (IgG) against *Giardia* is present. Although reduced levels of secretory IgA, IgM and IgG are reported in patients with giardiasis,^{73,74} this was not confirmed by others.⁷⁵⁻⁷⁷

DIAGNOSIS

In a great majority of infections, a diagnosis can be made on stool examination by finding cysts or trophozoites. In diarrhoeic stools, only trophozoites may be present. *Giardia* cysts are usually shed intermittently⁵⁰ and therefore a single stool examination may be unrewarding. To diagnose light infection with *Giardia*, it is usual to examine two direct faecal smears from a single stool sample; one suspended in physiologic saline and the other in 1% iodine in potassium iodide. In addition, 2 faecal smears that are permanently stained with iron haematoxylin may be made. In light infections, it may be necessary to use a formal-ether concentration method.⁷⁸ Duodenal contents and biopsy specimens may then be essential for diagnosis. Motile trophozoites are often readily obtained in fresh duodenal fluid. The organism may also be readily seen in Giemsa stained smears prepared from aspirated fluid or from a fragment of tissue or mucus contained within the biopsy capsule.⁷⁸⁻⁸⁰ Duodenal washings can also be passed through a Millipore filter and the organism seen on the filter after appropriate staining. Mucosal impression smears are easier and quicker to evaluate than biopsy sections and can be diagnostic.⁸⁰ It is difficult to detect the parasites during routine histology of biopsy material.^{59,81}

It is possible to grow trophozoites from jejunal aspirates in axenic cultures.⁸²⁻⁸⁴ A strain of *Giardia lamblia* was shown to grow very luxuriously in Diamond's TPS-I Medium.⁸³ The generation time was found to be 12.2 hours at 37°C. Serologic tests will facilitate epidemiologic and immunologic investigation of the disease. An indirect immunofluorescence test⁷² has been described but has its limitations. The difficulty in detecting specific antibodies may be due to the poor immunogenic

activity of *Giardia*.⁸⁵ Preliminary results using axenically cultivated *G. lamblia* as antigen^{83,86} suggest the feasibility of an immunofluorescence antibody test for the detection of anti-*G. lamblia* antibodies. Recently, there have been studies showing that indirect immunofluorescence for *G. lamblia* antibodies is specific, reproducible and may be useful in epidemiologic and immunologic studies of giardiasis.⁸³

EPIDEMIOLOGY

In developed countries, giardiasis, in addition to being endemic, is also reported to occur in epidemic forms. In the last decade or so, several outbreaks of giardiasis have been reported from the USA^{9,42,43} and Europe.^{13,14} Most of these outbreaks have been due to fecal contamination of drinking water.^{9,49} An outbreak of giardiasis in Washington, USA has been thought to be associated with infected beavers in streams supplying the drinking water.⁸⁷ A survey of wild and free ranging domestic animals and man for *Giardia* conducted in Colorado, USA showed that 33 species of vertebrates were positive for *Giardia*. Six species of mammals were reported to harbour *Giardia*: beaver (*Caster canadensis*); coyote (*Canis latrans*); cattle (*Bos taurus*); domestic cat (*Felis domesticus*); dog (*Canis familiaris*) and man (*Homo sapiens*). Laboratory, domestic and wild animals fed with *Giardia* from man became and remained positive for *Giardia* from 1 day to 3 months.^{42,43} Some outbreaks have been reported in campers who were exposed to mountain streams or pond water.⁸⁷ Giardiasis is a common infection among travellers who visit highly endemic areas. Over the past 13 years, several University students and other Malaysians who visited India for the first time contracted the disease (unpublished data). Similar infections in visitors from Britain to India and Nepal have also been reported.⁸⁸ American visitors to Leningrad, Russia, have been infected^{15,16} from contaminated tap water. Epidemics of giardiasis have not been so far reported from Malaysia or neighbouring countries.

In epidemic outbreaks, people of all ages are infected and morbidity is often high unlike communities where giardiasis is endemic. Transmission in endemic areas, on the other hand, is normally continuous and infection rates are low in adults, the disease being confined mainly to young children between 6 and 10 years of age.⁸⁹ This presumably is due to development of protective immunity in adults in endemic areas.⁸⁸ Serum antibodies have been demonstrated in symptomatic patients.⁷² In endemic communities, infectivity rates may be higher in irrigation and sewage workers. Direct

transmission has been shown to occur in homosexual males.⁴⁶

Giardiasis normally has a limited duration with few clinical symptoms which resolve spontaneously in 4 to 6 weeks.⁵⁰ In a small number of patients it remains as a persistent, chronic infection. Chronic and severe cases are probably due to humoral or cellular immune deficiencies in the host⁷¹ although some individuals with normal immune responses may have persistent giardiasis.³⁸ In chronic infections, excretion of cysts tends to be intermittent and erratic^W and up to 900 x 10⁶ cysts may be passed daily.⁹¹

The effect of giardiasis on the community is influenced by host factors. In developing countries where the parasite is endemic, most infections occur in children, many of whom are malnourished. Epidemics in developed countries do not cause mortality, although they can produce considerable morbidity. The morbidity rate in populations not previously exposed may reach up to 20 per cent or more. The cysts are resistant to concentrations of chlorine usually used for treatment of piped water, especially at low temperatures. This is an important factor in water-borne epidemics of giardiasis. Lack of sanitary facilities and inadequate sewage and water treatment systems are also important factors in the epidemic outbreaks of giardiasis in developed countries.

ZOONOTIC ASPECTS

In nature, many animals including mice, rats, hamsters and other animals are infected with *Giardia* species. In a recent survey of wild and domestic animals in the USA, cattle, dog, domestic cat, beaver and coyote were found to be positive with *Giardia* infection.⁸⁷ Two species of *Giardia* which infect mammals, namely *G. muris* in the mouse, rat and hamsters and *G. duodenalis* (*G. lamblia*) in all other mammals including man were compared and found to have no structural differences.³⁰ On the other hand, *Giardia* have been believed to be host specific⁹² and different names have been given to it from each host.

In experiments⁸⁷ for cross-species transmission with *Giardia* cysts from humans, animals like laboratory rats, gerbils, guinea pigs, beavers, dogs, racoons, bighorn, mouflon and prong horn sheep became infected. These animals were positive for infection within 3 months and cysts were first found in the faeces from 6-34 days after exposure. Hamsters, domestic rabbits, laboratory mice, black bears and domestic sheep, on the other hand, did not become infected.

Giardia cysts which are morphologically identical to *Giardia lamblia* of man, and which were infec-

tive to and cause disease in SPF beagle puppies, have been obtained from beavers. Cysts from human sources have produced *Giardia* infections in puppies.⁹³

Except for the outbreaks (mentioned earlier) where beavers were suspected as the source of human infection, the role played by animals in transmission of giardiasis to man is largely unknown. Infected persons seem to be the most important source of infection to man. Zoonotic aspects of giardiasis in Malaysia are not known.

CONTROL AND PREVENTION

In tropical regions, particularly in developing countries, giardiasis is a problem. The treatment of the parasites would effectively cut off the vicious cycle of parasitic infections. Drugs like Fasigyin, Metranidazole, Tinidazole, Ornidazole and Furazolidase have proved to be quite effective against *Giardia lamblia*. In conjunction with treatment, the most practical methods of control and prevention are providing opportunities for health education and motivating the people involved in a variety of public health measures, including hygiene, sanitation and nutrition, through the setting up of a parasite control programme. The improvement of environmental sanitation includes the supply of safe drinking water through proper and safe methods of disinfecting water by filtration and chlorination. The normal amount of chlorine (10 ppm) in drinking water is not strong enough to kill the cyst of *Giardia*. They are killed after exposure to 0.5% chlorinated water for 2-3 days. Recently, it has been shown that a 1-3% solution of chlorine killed only 10% of *Giardia lamblia* in 30 minutes to 24 hours. A 10% solution killed 30-40% in 1-6 hours. Many studies have shown that cysts of *E. histolytica* and *Giardia* are highly resistant to chlorine. In countries or places where no water filtration or chlorination is available, people should be educated to drink water only after boiling. The provision of latrines and their location is important. It is imperative that latrines should be built away from water supplies so that water is not contaminated with faeces. Proper disposal of faeces, improvement in water supply and mass treatment of infected persons will help to control and prevent giardiasis.

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