The diagnosis and clinical importance of Giardiasis

by Dr T. Mank

Globally *Giardia lamblia* is one of the most important non-viral causes of human diarrhoea, with infections occurring not only in developing countries but also in the developed world. The ingestion of cysts does not usually result in clinical illness, but *Giardia* infection can produce a broad spectrum of gastrointestinal symptoms which can persist for long periods if left untreated. This article discusses the biology and epidemiology of the parasite, and considers the various techniques that can be used for its diagnosis. Microscopic examination of stool samples is still the mainstay for routine diagnosis despite the fact that progress has been made in developing and validating non-morphologically based diagnostic tests and the proven utility of EIAs.

*Giardia* is a ubiquitous enteric protozoan parasite that affects humans, domestic animals and wildlife throughout the world. The parasite is a flagellate, which was discovered by the inventor of the microscope, Antoni van Leeuwenhoek. In a letter written to Hooke at the British Royal Society of Parasitology in 1681 he accurately described the motile trophozoite of *G. lamblia*, which he observed in a sample of his own stool as "very prettily moving animacules, some rather larger, others somewhat smaller than a blood corpuscle and all of one and the same structure ...." "their bodies were somewhat longer than broad, and their belly, which was flatlike, furnished with sundry little paws...." The descriptive information of this historic letter was not widely distributed and it was Vilem Lambi who comprehensively described the trophozoites, which he found in the stools of paediatric patients in Prague in 1859. His published observations were accompanied by beautiful drawings of the organism based on his many microscopic observations.

The life cycle

The life cycle of *Giardia* is well-known and comprises two developmental stages; the trophozoite and the cyst [Figure 1]. The most common location of the former stage is in the crypts within the duodenum of the host (e.g. human) where trophozoites live closely applied to the mucosa. There, binary fission repeatedly takes place, the eventual result of which is the establishment of the protozoan, often in enormous numbers. The binucleate trophozoite is usually described as being teardrop-shaped with the posterior end being pointed; it measures 10-20 µm [Figures 2 and 3]. The anterior portion of the ventral surface of the organism is modified to form a sucking disc, which serves to attach the organism to the mucosa. The transformation of trophozoites into cysts takes place in the host intestinal tract, when the trophozoites, together with the faecal mass, move down through the colon. The cysts, which are shed into the environment by the host, may be either round or oval, and they contain four nuclei [Figure 4]. The cyst represents the resting stage of the organism; its rigid outer wall protects the parasite against changes in environmental temperature, dehydration, and such disinfectants as chlorine, all of which would destroy the trophozoite. The cyst is also the infective stage; the cycle continues when a suitable host ingests the cyst. Stomach acidity and other factors trigger the excystation process, which usually takes places in the new host’s small intestine.

Although various criteria, including host specificity, differing body dimensions, variations in structure, and molecular tools have all been used to differentiate species of *Giardia*, there is still considerable debate over the appropriate classification and nomenclature regarding this group of organisms. However, five species of *Giardia* are currently recognised on the basis of parasite morphology and host occurrence. These are *G. agilis* (amphibians), *G. ardeae* (birds), *G. duodenalis* (most mammals including humans), *G. microti* (voles and muskrats) and *G. muris* (rodents) [Figure 5].

Most species of *Giardia* are host-adapted, with the exception of *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*) which seems to have a much broader host range infecting many mammalian species. Despite disagreement concerning the names "duodenalis", "intestinalis", and "lamblia" all three continue to be used to describe this organism although the term *Giardia lamblia* is predominantly used in human medicine.

Clinical features in human infections

The natural course of giardiasis is often very mild; in most cases, the ingestion of cysts will not result in any clinical illness. On the other hand, *Giardia* infection can
molecular techniques to understand. Host factors, such as immune status, nutritional status and age, as well as differences in virulence and pathogenicity of strains are recognised as important factors determining the severity of infection. The recent application of molecular techniques to *Giardia lamblia* isolates has revealed high levels of genetic diversity within this species. Currently, there are six recognised variants or assemblages (assemblage A, B, C, D, E and F), each having a varying degree of host specificity. Several studies on the correlation between clinical presentation and genotypes have been performed. However due to the conflicting results reported in the literature, it is still not completely clear what the relation is between symptoms and infection with different genotypes, in particular those belonging to assemblages A and B, which are so far the only genotypes known to cause human disease.

In the majority of healthy individuals the infection is self-limiting, a proportion (estimated at 30-50%) of infected patients, however, will go on to chronic giardiasis, characterised by steatorrhoea accompanied by a classic malabsorption syndrome with substantial weight loss, general debility and fatigue. Chronic infection in early childhood is associated with poor cognitive function and failure to thrive.

The factors determining the variability in clinical outcome in giardiasis are poorly understood. Host factors, such as immune status, nutritional status and age, as well as differences in virulence and pathogenicity of *Giardia* strains are recognised as important factors determining the severity of infection. The recent application of molecular techniques to *Giardia lamblia* isolates has revealed high levels of genetic diversity within this species. Currently, there are six recognised variants or assemblages (assemblage A, B, C, D, E and F), each having a varying degree of host specificity. Several studies on the correlation between clinical presentation and genotypes have been performed. However due to the conflicting results reported in the literature, it is still not completely clear what the relation is between symptoms and infection with different genotypes, in particular those belonging to assemblages A and B, which are so far the only genotypes known to cause human disease.

Unfortunately in contrast to the case of infections with *Entamoeba*, where it was possible to define two distinct genotypes that are now named *E. histolytica* and *E. dispar*, applying such a dichotomy is not (yet) possible with *Giardia* infections. Worldwide co-operation, including the exchanging of the various genotypes and standardisation of methods, will eventually reveal some answers to the remaining questions. By genotyping the different strains it will become possible to study the variety of associated symptoms, although host factors, e.g. host immune response to infection, will also have to be taken into account.

**Diagnosis in human infections**

Progress has been made in developing and validating non-morphologically based diagnostic tests for intestinal parasite antigens. Immunofluorescence microscopy (IF), enzyme linked immunosorbent assays (ELISA), parasite DNA polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), and real time PCR (RT-PCR) have all been utilised for *Giardia* diagnosis. However, microscopy is still the cornerstone and gold standard for detecting intestinal parasites in stool samples in both clinical and veterinary diagnostic parasitology laboratories.

Traditionally fresh or preserved stool samples are microscopically examined directly or with (permanent) staining, with or without concentration. Unfortunately, the sensitivity of this conventional ovum-and-parasite (O&P) examination on a single stool sample for *G. lamblia* is less than optimal. It was recently determined by Cartwright to be only 74% [1]. The poor sensitivity of a single parasitological stool examination for diagnosing giardiasis is mainly due to the variable excretion or low-level shedding of the parasite in both symptomatic and asymptomatic patients. Furthermore, symptoms can occur before intact parasites are detected in the stool, hence repeated examinations are necessary until morphological forms are seen, as is well described and recommended in many parasitology textbooks. To overcome sampling issues, the Triple-Faeces-Test (TFT) was recently developed in the Netherlands as an all round test for the laboratory diagnosis of intestinal parasites. The advantages of both fixatives, permanent staining methods and multiple sampling are combined in this test [2].

Serological approaches to the diagnosis of giardiasis have been developed and have been proven to be most useful in epidemiological surveys [8-10], PCR assays for specific gena/genotypes of intestinal parasites are useful for surveys, but not for the clinical diagnostic laboratory. Stools must be screened for a variety of pathogens and the cost of different PCR assays would be too high. RT-PCR may have a role in diagnostic and reference laboratories, as several targets could be combined in one multiplex RT-PCR assay, allowing the simultaneous detection of multiple infections such as *E. histolytica*, *G. lamblia* and *Cryptosporidium* species and genotypes infectious to humans.

With the use of the new approaches in routine parasitological stool examinations, a substantial increase in sensitivity and specificity in the laboratory diagnosis of intestinal protozoan infections can be achieved. Nevertheless it is still extremely important to familiarise physicians with the clinical relevance and epidemiology of these infections.

Physicians should include a parasitological stool examination in their diagnostic work-up of patients with persistent (lasting longer than 1 week) or intermittent diarrhoea. This is indicated because of the frequency of protozoal diarrhoea in general practice, and the negligible diagnostic value of symptoms and other anamnestic data from patients infected with *Giardia lamblia* or other intestinal protozoa, as well as the fact that most intestinal protozoal infections can be treated.

**Epidemiology**

*Giardia lamblia* is recognised as a major cause of diarrheal illness in humans and livestock. Worldwide it is one of the most important non-viral infectious agents causing diarrhoeal illness in humans [11-12]. The parasite is not restricted to people living in the developing countries, where sanitation is frequently non-existent and people are forced to drink unfiltered surface water, but also occurs among people living in developed, market economies where public hygiene is good and water supplies are purified and piped.

The infection is endemic in all regions of the world, and also occurs in sporadic epidemic bursts. Exact figures on incidence and prevalence depend of the population examined. In the Netherlands, the prevalence of the infection varies between 2% and 14%, being high in patients who consult their general practitioner with persistent diarrhoea and low (2%) in asymptomatic subjects [13-15]. Survival in fresh water has enabled *Giardia* to achieve the reputation of being the most common cause of epidemic waterborne diarrheal disease. The first record-
ed waterborne outbreak of giardiasis involved travelers to St. Petersburg, Russia. Since then many epidemics have been well characterised in both North America and Europe, and they have usually been associated with inadequate water treatment. High rates of infection have also been reported for hikers and campers in the USA. Because some of these areas were remote from human habitation, infected wild animals, especially beavers, are suspected of being a host or reservoir for *Giardia lamblia* [16-17].

Direct person-to-person spread by faecal-oral transmission is another major mechanism by which the disease is transmitted. The organism tends to be found more frequently in children or in groups that live in close quarters. Outbreaks of giardiasis are well recognised in day care centres, residential institutions and schools. Many infections are asymptomatic but spread of cysts can occur to other members of the family or to other residents. Transmission of the parasite also occurs during sexual activity, in particular as a result of intimate oro-anal contact.

The contribution of foodborne transmission of giardiasis has not been well characterised. However, there have been a number of reports where food has clearly been identified as the source in several outbreaks of giardiasis. In most cases an infected food handler has been implicated, presumably transmitting cysts to freshly prepared food [18].

**Treatment**

Nitro-imidazoles (metronidazole and tinidazole) and albendazole are the drugs of choice for giardiasis, but lack of treatment compliance and side effects can result in treatment failures. For treatment of special patient groups e.g., pregnant and immunocompromised patients, alternative drugs, such as paromomycin, and/or prolonged or combination therapy may sometimes be necessary.

**References**

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