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REVIEW

Diagnosis and Treatment of *Giardia lamblia* Article Review

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ABSTRACT

Giardia lamblia is a microscopic parasite belonging to the genus Giardia, renowned for its complex antigenic mechanisms. This parasite predominantly inhabits the small intestine, leading to a range of symptoms, including diarrhea, abdominal pain, and discomfort, affecting both humans and various animals such as dogs, cats, cattle, and sheep. The transmission of Giardia lamblia occurs through contaminated food, surface water, and fecal matter. The parasite spreads primarily through the fecal-oral route when hands contaminated with infected feces is exposed to the mouth, or when the infected cysts are ingested directly or indirectly.

Giardia lamblia goes through two primary life stages after entering the host: the vegetative trophozoite phase and the cyst phase. The parasite takes 9 to 15 days to begin incubating before symptoms appear. Giardiasis is usually diagnosed by stool analysis, employing direct smear techniques with wet or saline preparations. A duodenal biopsy may be necessary in certain rare cases after a positive serological test since duodenal aspiration has been shown to identify up to 80% more cases than stool analysis, making it essential for successful treatment and prevention plans.

Numerous diagnostic techniques are available to determine whether *Giardia lamblia* is present. To find cysts or trophozoites in stool samples, concentration techniques and direct fixation methods are used. Cysts are typically visible on wet preparation slides, although the trophozoite stage is more likely to be visible on permanent mounts. Furthermore, the parasites and their antigens are found using sophisticated diagnostic methods such enzyme immunoassay and immunofluorescence, respectively.

There are various potent antibiotics used in the treatment of giardiasis. Known by its brand name Flagyl, metronidazole is the most generally prescribed antibiotic. On the other hand, adverse reactions like nausea and a metallic aftertaste are reported. Tinidazole, another potent antibiotic that is taken in a single dose, with adverse effects that are comparable to those of metronidazole. In order to avoid negative responses, patients receiving therapy with metronidazole or tinidazole must abstain from alcohol.

The pathogen *Giardia lamblia* is the source of giardiasis and poses a significant hazard to human and animal health. The spread of giardiasis and its effects can be considerably decreased by being aware of the parasite's life cycle, employing suitable diagnostic techniques, and adhering to recommended treatment regimens.

Keywords: Antibiotic treatment, Cyst stage, Duodenal biopsy, Giardia lamblia, Metronidazole (Flagyl), Serological diagnosis

1. Introduction

The *Giardia lamblia* is a micro parasite of the genus Giardia, known as *Giardia duodenalis*, *Giardia intestinalis*, or *Giardia lamblia*, settles in the small intestine and causes a variety of symptoms, including diarrhea and abdominal pain, particularly in children (Simner, 2017; Buret et al., 2020; Rumsey and Waseem, 2023). This parasite, whose main reservoirs are food, surface water, and excrement, can infect humans as well as animals, including dogs, cats, cattle, and sheep (Bacteria, 2023; Ali and Mohammed, 2010).

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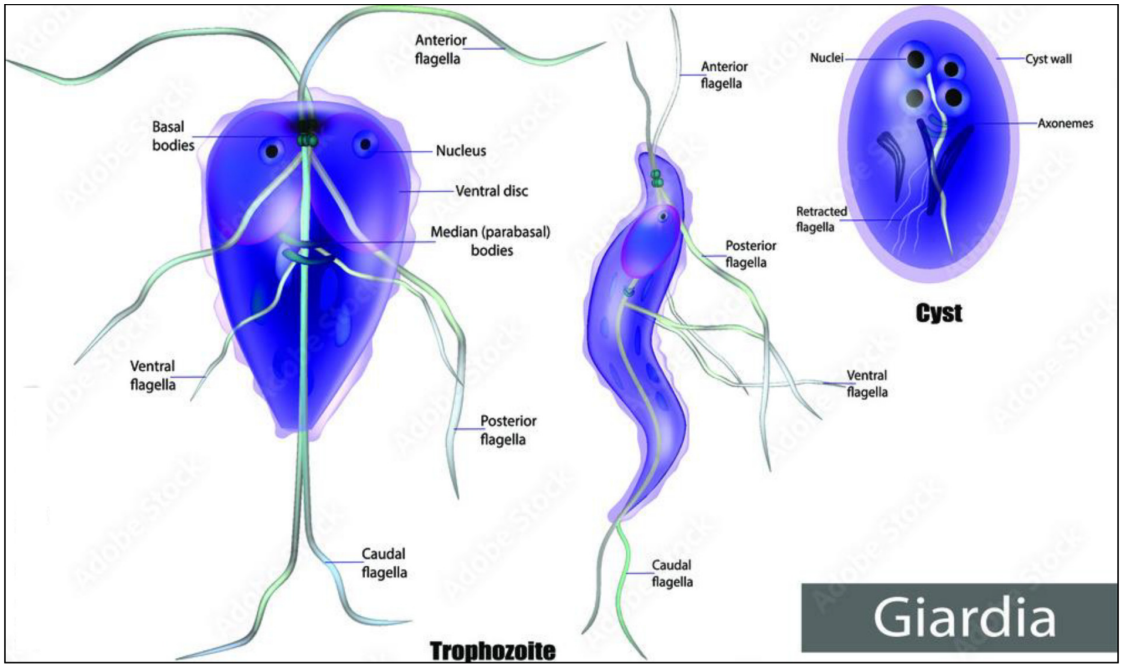

Fig. 1. Cystic and trophozoite stages of *Giardia lamblia*'s life (Giardia "Cyst" images–browse 81 stock photos, vectors, and video, https://n9.cl/mvjuqe).

Giardia lamblia spreads by hands infected with stool, and then oral transmission happens when infected cysts are consumed either directly or indirectly (Bacteria, 2023; Balcioglu et al., 2007). *Giardia lamblia* takes 9 to 15 days to develop after being ingested, during this time its life cycle is made up of the cystic and trophozoite stages (the components of which are depicted in figure 1 below (Corrales, Izurieta and Moe, 2006; Kia et al., 2008).

Contaminated infected cysts are the main sources of infection in the environment; symptoms of the disease can range from none to nausea, vomiting, abdominal pain, and severe watery diarrhea (Hussein and Meerkhan, 2019; John, Petri and Martin, 2006).

Giardia lamblia is a parasite that also affects young adults, causes poor absorption and weight loss, and attaches to the mucosal tissue of the lining of the small intestine, causing weakness or lack of intestinal villi and deficiency of digestive enzymes in severe cases. As a result, the parasite prevents food absorption, resulting in weight loss syndrome (John, Petri and Martin, 2006; Molan and Farage, 1989).

2. Diagnosis and treatment of *Giardia lamblia*

Giardia lamblia can be identified by taking the stool, performing a direct swab (wet or saline), and looking for Giardia. In rare circumstances, a duodenal biopsy is used to diagnose a parasite after a positive serological examination since duodenal aspiration finds 80% more cases than stool.

Giardiasis can be recognized through direct mounts or concentration techniques that look for trophozoites or cysts of *Giardia lamblia* in the stool. Wet mount preparations usually contain cysts, whereas permanent mounts frequently contain trophozoites. It might be necessary to take many samples. Furthermore, trophozoites may be present in both duodenal biopsy samples and duodenal fluid samples (such as Enterotest). Two other detection techniques are immunofluorescence for identifying parasites and enzyme immunoassays for identifying antigens. A direct immunofluorescence assay (DFA), *Giardia lamblia* infections may also be found by combining the stool with fluorescently tagged antibodies and incubating the mixture. The spherical, brilliant green objects that make up Giardia cysts are visible under a fluorescent microscope as shown in Fig. 2 (Giardiasis, https://www.cdc.gov/dpdx/giardiasis/dx.html).

The test of choice for diagnosing giardiasis is microscopy with direct fluorescent antibody testing, as it offers greater sensitivity than non-fluorescent microscopy techniques. There are various tests available to detect Giardia, some of which are more sensitive and specific than others. Additional alternative techniques for detection include of:

- Enzyme immunoassay kits.
- Rapid immunochromatographic cartridge assays.

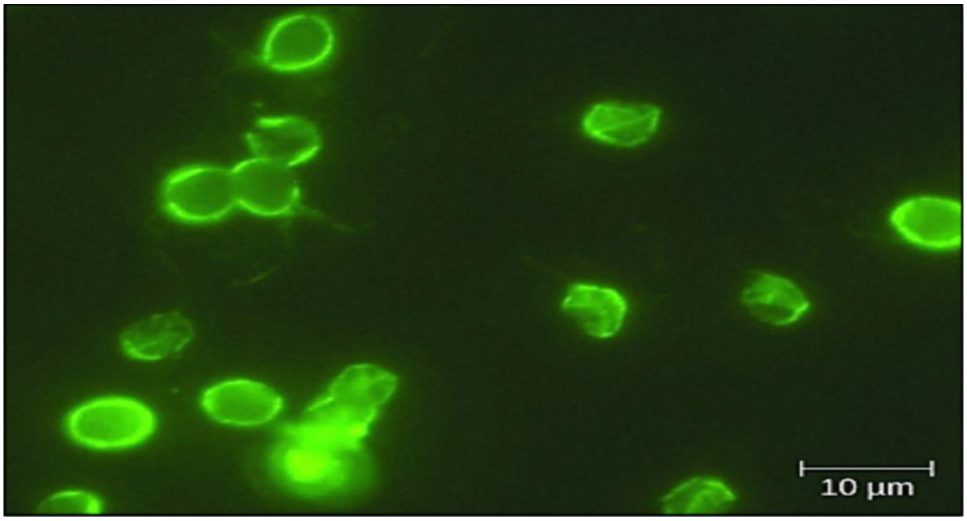

Fig. 2. Giardia cysts appearance (Giardia "Cyst" images–browse 81 stock photos, vectors, and video, https://n9.cl/mvjuqe).

- Trichrome staining used in microscopy.
- Assays using molecules.

To get more accurate test results, the Centers for Disease Control and preventive (CDC), a government agency that supports and promotes health promotion, preventive, and preparedness initiatives, advises taking three stool samples from patients over the course of several days. In the US, commercial products are available for diagnosing giardiasis. The only method available for identifying Giardia strains is molecular testing, such as DNA sequencing. Table 1 compares the diagnostic performance of microscopy and *Giardia lamblia* PCR. PCR analysis revealed that, of the 157 samples, 44 were positive and 113 were negative for *Giardia lamblia* (Cdc.gov, 2021).

Information displayed as percentages (%) and numbers (n). The 95% confidence interval is shown by data in parenthesis. Both negative and positive predictive values (NPV and PPV) are present. The text in bold faces $P < 0.05$.

3. Sensitivity, specificity and predictive values of microscopy

Table 1 displays the validity of the microscopy. When compared to PCR, microscopy showed a sensitivity of 64.4% (48.9–78.1). Therefore, the likelihood of a positive test under microscopy conditions involving *Giardia lamblia* was 0.644. When compared to PCR, microscopy showed an 86.6% (78.9–92.3) specificity. Therefore, the likelihood of a negative test under microscopy conditions where *Girdia lamblia* was absent was 0.866. When microscopy and PCR were compared, the positive predictive value was 65.9% (53.5–76.5). Consequently, 0.659 percent of patients with positive test results had *G. lamblia*. Additionally, the diagnostic test produced a negative predictive value of 85.8% (80.1–90.0%), indicating that in the event that the test is negative, there is a 0.858 chance that the patient does not have *Girdia lamblia*. A PCR and microscopy agreement that was moderate was indicated by a Cohen's kappa score of 0.51. Nonetheless, a substantial difference ($P < 0.001$) between the two tests was found using McNemar's test.

Centrifugal flotation of zinc sulfate, centrifugal sedimentation (with the aid of a commercial fecal concentration tool called the TF-Test kit®), and PCR-based techniques. 277 (48.3%) of the stool samples that were evaluated tested positive for commensal protozoa and/or intestinal parasites. For Giardia infections, centrifugal flotation showed the highest diagnostic sensitivity. The kappa index showed that, for commensal protozoan and helminth infections, respectively, acceptable (72%) and poor (35%)

Table 1. Comparison of polymerase chain reaction and direct stool analysis for *G. lamblia*.

Technique	Samples numbers	Positives samples	Vulnerability (%)	Specificity (%)	PPV (%)	NPV (%)	Cohen's coefficient of Kappa	(P) McNamar Test
Microscopy	157	29	64.4	86.6	65.9	85.8	0.51	<0.001
PCR	157	44	100	100	100	100	—	—

concordance was found, and both coproparasitological procedures closely agreed with the Giardia diagnosis (86%). Concerning Giardia molecular diagnosis, glutamate dehydrogenase, an enzyme involved in amino acid metabolism that is frequently utilized to identify Giardia infections, is encoded by the (glutamate dehydrogenase -gdh) gene. Besides that, the enzyme triosephosphate isomerase (tpi), which is essential for glycolysis, is also employed. From 71 microscopy-positive samples, specific amplification of (gdh) and (tpi) fragments was noted in 68 (95.7%) and 64 (90%) samples, respectively. In microscopy-positive Giardia samples, (gdh) and (tpi) amplification was seen in 95.7% and 90% of cases, respectively. The gdh and tpi gene amplification products were recovered from 8.3% and 35.9% of the 144 microscopy-negative samples. The degree of agreement among these genes was about 40%. According to the current investigation, the centrifuge-floatation-based approach was the most effective way to diagnose Giardia because it was both affordable and accurate (Cdc.gov, 2021).

4. Method

In order to guarantee impartial results while comparing the diagnostic methods, this study used blinding procedures. Here is a thorough explanation of every step in the approach (Monaghan et al., 2021).

4.1. Blind method

The use of a blind approach means that neither the patient's condition nor the anticipated results were known to the people performing the microscopic inspections or the serological testing (such as ELISA and counterimmunoelectrophoresis). By using this method, observer bias is removed and the validity of the compared findings is guaranteed.

4.2. Microscopic examination

- Stool Specimen maintained: To ensure that stool samples remain intact for analysis throughout time, they were maintained.
- Concentrated Specimen: Concentrating the stool samples increased the chance of finding parasites, especially if they were scarce. Concentration methods that assist enhance parasite eggs, cysts, or trophozoites in the sample include sedimentation and flotation.
- Permanently Stained Specimen: The stool might be stained to make parasites easier to see under a microscope. Stains that are frequently used for feces samples include iodine or acid-fast stains for other organisms, as well as trichrome stain, which increases the contrast of parasites like amoebas.

4.3. Enzyme-Linked Immunosorbent Assay (ELISA)

- Trophozoite-Immune Rabbit Serum: Using antibodies from immunized rabbits against trophozoites (the parasite's active feeding form), ELISA was carried out. Antigens found in the trophozoites are particularly bound by the antibodies in the rabbit serum.
- This technique finds antigens unique to parasites in the stool. The extremely specific and sensitive ELISA method enables quantitative or semi-quantitative evaluation of the parasite infection.

4.4. Counterimmunoelectrophoresis (CIE)

- Cyst-Immune Rabbit Serum: For this test, antibodies against the parasite's cyst form—a more resistant, latent stage—were present in rabbit serum.
- Using an electric field to induce an antigen-antibody response, the electrophoretic technique known as CIE enables the identification of antigens in a sample. It's a quick technique for finding immune complexes in diagnostics.
- Under the influence of an electric current, antigens from cysts in the stool sample would travel in the direction of antibodies in the immune serum, generating visible precipitation lines that would signal a positive test result.

From the foregoing, it is evident that the comparison was meant to assess the precision and dependability of immunological tests (ELISA and CIE), which rely on the identification of particular parasite antigens, against classical microscopy, which examines stained stool samples. Higher sensitivity can be obtained using immunological techniques such as ELISA and CIE, especially in cases when parasite concentrations are low or the organisms are challenging to see under a microscope. Nonetheless, because it allows for the direct observation of the parasite's morphology, microscopy is still frequently considered the gold standard.

When these techniques were applied to 118 stool specimens from clinical microbiology laboratories (of which 53 had *G. lamblia*) and 239 toddlers from day care centers (of which 39 contained *Giardia lamblia*), the outcomes were comparable. For counterimmunoelectrophoresis and ELISA, in comparison to microscopy, we observed the following differences:

concordance, 89%; positive predictive value, 86 vs 76%; negative predictive value, 98 compared 97%; sensitivity, 88 versus 94%; and specificity, 97 versus 95%. Microscopy confirmed that the ELISA false-positive rate in toddlers from day care centers was 24% (10 of 42), whereas in healthy adults, it was only 3% (1 of 32) (P less than 0.04). This disparity raises the possibility that ELISA is more sensitive than microscopy, which is the accepted gold standard. It also raises the possibility that the results could be influenced by the study subjects' infection epidemiology.

Microscopy is the most commonly used method for determining the presence of a *Giardia lamblia* infection, despite its lack of sensitivity and long processing time. We developed a real-time PCR method based on the small subunit ribosomal RNA gene of *Giardia lamblia* to uniquely detect its DNA in stool samples. Next, we contrasted the outcomes using antigen detection and microscopy. The *Giardia lamblia* real-time PCR test produced positive results in 102 of the 104 fecal samples known to contain *Giardia lamblia* cysts, including 10 fecal samples where *Giardia lamblia* antigen was found but no cysts were seen following microscopic analysis of concentrated fecal samples. Real-time PCR is as selective and sensitive as antigen detection, and it is more sensitive than microscopy. Furthermore, in two individuals, we were able to identify *Giardia lamblia* earlier than we could have done with any other method over the course of the sickness. When stool samples are examined under a microscope and trophozoites or cysts are found, giardiasis is often diagnosed. However, because the cysts shed periodically, microscopic analysis of a single stool specimen has a limited sensitivity (46%) in this regard. Thus, in order to obtain 94% accuracy in diagnosing positive Giardia patients, a minimum of three fecal samples must be collected and analyzed over the course of three to five days (Corrales, Izurieta and Moe, 2006; Kia et al., 2008). This method's primary flaw is that it requires a lot of time and labor and is dependent on the expertise of a seasoned microscopist. Furthermore, bile pigments may conceal the parasite, making it impossible to see with a wet-mount examination (Hussein and Meerkhan, 2019; John, Petri and Martin, 2006).

Giardia lamblia was found using microscopy, which has a 64.4% sensitivity and an 86.6% specificity. Additionally, this test demonstrated a 64.2% sensitivity and an 83.6% specificity for diagnosing *Entamoeba histolytica*. Under a microscope, it was discovered that *Giardia lamblia* and *Entamoeba histolytica* had Cohen's kappa values of 0.51 and 0.47, respectively (John, Petri and Martin, 2006).

Polymerase chain reaction (PCR) testing, the gold standard, was used to stool samples that were thought to include *Giardia. lamblia* and *Entamoeba histolytica*. The same samples were subsequently subjected to microscopy, and the results of the two tests were compared (Hale and Rowe, 2014).

The gold standard technique for diagnosing giardiasis and amebiasis is PCR (Bacteria, 2023; Abed, Mohammed and Razoqi, 2020). However, it is not realistic to use PCR for routinely diagnosing *Giardia. lamblia* and *Entamoeba histolytica* in situations with low resources. Therefore, the most effective method for diagnosing *Giardia lamblia* and *Entamoeba histolytica* is to combine serologic testing with microscope detection. However, in most circumstances with inadequate resources, to detect the majority of intestinal parasites, microscopy is the sole diagnostic tool available. To guarantee that microscopy is reliable, it is crucial to do ongoing testing and monitoring. Additionally, there are differences in the training, experience, and skill sets of laboratory professionals. As a result, it is essential that they be assessed on an ongoing basis by verifying their reports against the gold standard. Therefore, in a resource-constrained environment in Western Kenya, this study compared the effectiveness of microscopy to PCR, using an early strategy diagnosis and treatment is imperative to prevent and control infection of *Giardia*. The Infectious Disease Society of America's diagnostic criteria state that stool studies are the preferred method for diagnosing Giardia; duodenal aspirate microscopy is the only other recommended diagnostic technique in situations where stool studies produce negative results but suspicion is still high. We report on a patient whose duodenal biopsy material, taken while treating a gastrointestinal bleed, unintentionally showed Giardia. The evidence presented in this study suggests that duodenal biopsy may be a highly sensitive diagnostic technique for Giardia infections, particularly in cases when stool tests produce conflicting results. By directly observing Giardia trophozoites adhered to the intestinal lining, the biopsy improves the precision of the diagnosis. Research indicates that it performs better in identifying low-burden or persistent illnesses than stool tests and the authors (Groudan et al., 2021), highlight how it can be useful in cases that are challenging to diagnose.

Various methods have been proposed to enhance the conventional microscopic diagnosis, such as the intestinal impression smear, intestinal biopsy, duodenal aspirate, and string test. However, these more expensive, intrusive tests offer little assistance in the diagnosis (Molan and Farage, 1989). These days, an immunological diagnosis can be made by identifying

Giardia antigens and anti-Giardia antibodies in stool specimens (Kirchner, 1998).

The most often used and advised antibiotic for giardiasis patients is metronidazole, also known as Flagyl. However, this medication has certain side effects, including nausea and a metallic taste. There is also tinidazole available in a single dose, which has many of the same side effects as metronidazole. Although the liquid version of the antibiotic nitazoxanide makes it easier for kids to take, there are some side effects to be aware of, including nausea, stomach gas, yellowing of the eyes, and bright yellow urine. The US Food and Drug Administration (FDA) has approved tinidazole for the treatment of giardiasis in individuals who are older than three (John, Petri and Martin, 2006; Molan and Farage, 1989).

It is crucial to counsel patients not to drink alcohol when taking metronidazole or tinidazole. The FDA to treat giardiasis in individuals older than a year has licensed Nitazoxanide. A single dosage of 2 g of metronidazole can also be given to nursing mothers, if they take a 12- to 24-hour break from breastfeeding. To yet, there have been no published reports of unfavorable effects on breastfed newborns. Being poorly absorbed in the intestines, pregnant women who have giardiasis may benefit from treatment. Because monoamine oxidase inhibition occurs when you use a drug or food for longer than five days, there may be significant interactions. For more precise information, go to Table 2 below, which provides further pharmacological information. For example, quinacrine is only used in situations of refractory giardiasis (Hale and Rowe, 2014; Cdc.gov, 2021).

5. Discussion

Giardia lamblia is parasite that is thought to be one of the main causes of diarrhea in both children and adults. Giardiasis is a condition marked by diarrhea, vomiting, and weight loss due to watery diarrhea (Simner, 2017; Nematian et al., 2004).

The parasite causes acute diarrhea in human beings, especially in children, and has the potential to infect other creatures. *Giardia lamblia* is a parasite that lives in the small intestine and colonizes the mucous lining of the duodenum along with jejunum. It has two life stages, the bag stage and the double - moving trophozoite stage, and the best way to spread it is by consuming contaminated food and water. The most effective way to prevent getting the parasite is to washing hands before eating (Yilmaz, Akman and Göz, 1999).

Before any negative results are recorded, at least three stool samples must be tested. Stool samples should be concentrated in formalin or another fixative before being used for microscopy (for example, 10 min at 500 g when using the formalin-ethyl acetate concentration procedure), but samples used for ELIZA or rapid cartridge assays shouldn't be concentrated because antigenic components are lost during the process (Cacciò et al., 2003).

Santos and Vittori stated that there were no statistically significant differences in hemoglobin levels among Giardia and control groups, but the percentage of eosinophil showed a very significant difference. The results of several studies revealed a non-significant change in the concentration of

Table 2. Medication used to treat giardiasis.

Drugs (Alphabetically listed)	Dosage
	Adults (not pregnant)
Albendazole	Four hundred milligram orally once a day for 5 days.
Furazolidone	Hundred milligram orally 4 times a day for 7–10 days.
Metronidazole	Two hundred and fifty milligram orally 3 times a day for 5–7 days.
Paromomycin	Five hundred milligram (Thirty milligram/kg/day) orally 3 times a day for 7 days.
Quinacrine	Hundred milligram orally 3 times a day for 5 days.
Tinidazole	Two grams orally once.
	Women (pregnant)
Paromomycin	Five hundred milligram (Thirty milligram/kg/day) orally 3–4 times a day for 7 days.
	Children
Albendazole	Four hundred milligram orally once a day for 5 days.
Furazolidone	Six to eight milligram/kg/day orally divided three to fourtice a day for 7–10 days.
Metronidazole	Fifteen milligram/kg/day orally divided thrice a day for 5 days (Max. 300 mg/day).
Paromomycin	Thirty milligram/kg/day orally thrice a day for 7 days.
Quinacrine	Six milligram/kg/day orally divided thrice a day for 5 days (Max. 300 mg/day).
Tinidazole	Fifty milligram/kg/day orally once (Max. 2 grams).
Refractory cases	(Refractory disease is defined as resistance to multiple drugs with different mechanisms of action owing to the persistence of physical symptoms and high disease activity.)
Metronidazole	Seven hundred and fifty milligram orally 3 times a day for 14 days.
Quinacrine	Hundred milligram orally 3 times a day for 14 days.

hemoglobin level and the volume of packed cells (Abed, Mohammed and Razoqi, 2020).

On bright field microscopy, cysts have an oval form and are typically between 11 and 14 microns in size (with a range of 8 to 19 microns). Cysts have fibers within the cytoplasm, and mature cysts have two nuclei whereas immature cysts have four (Cacciò et al., 2003).

Trophozoites and cysts have to be viewed under a microscope and recognized by the detection of antigens in a stool sample in order to determine the parasite. In this regard, the ELIZA, direct fluorescent antibody, and PCR tests are very helpful since they show. Metronidazole can be used as the first stage of treatment for giardia infection. Patients who do not respond well to treatment are frequently given multiple courses of standard medications; as the course lengthens and doses increase, these medications are frequently metronidazole, furazolidone, and tinidazole, which are widely available but no longer used on a regular basis (Yilmaz, Akman and Göz, 1999; Abid and Alsaadi, 2019).

For rate of infection, according to (Al-Saeed and Issa, 2006) findings increasing because of frequent interaction with domestic animals and their products as well as direct usage of contaminated river water for a number of uses. 13.4% of infections had trophozoite stages, compared to 79.3% with cyst stages. Cyst stages made up more of the patient's stool than trophozoite stages did. They discovered that giardia infection increased gastrointestinal symptoms after treating 100 patients with diverse parasite infections, including 9 patients who had the giardia parasite (Al-Aboody et al., 2020).

Utilizing a direct smear for the investigation of intestinal parasites, the microscopic examinations revealed variable results, with (43.4%) representing positive findings and (56.6%) representing negative results. This high incidence may be caused by sewage line problems, poor water quality, malfunctioning sewage systems, and insufficient quantities of chlorine, which is utilized for eliminating bacteria in public water sources but cannot eliminate Giardia cysts (Elwan, 2021).

The study revealed that 8.1% of samples tested positive for *Giardia lamblia* through microscopic examination. Male patients exhibited a slightly higher infection rate at 8.2%, compared to 7.0% in females. Geographically, rural areas had a higher infection prevalence at 11.8%, while urban areas had a lower rate of 7.2%. In terms of age, children aged 1–10 years had the highest infection rate at 10.0%, whereas the lowest rate of 4.5% was observed in adults aged 21–30 years (Elwan, 2021; Kanaem, 2017).

As for prevention, chlorination, filtration, and purifying or filtering drinking water are good strategies for removing the parasite since children who have a *Giardia lamblia* infection can spread cysts easily if poor sanitation is utilized. Many causes of Giardia can be avoided by hand cleaning thoroughly before eating, before preparing food, and after utilizing the bathroom, using boiling water or iodine in endemic areas, washing potentially contaminated produce, and washing vegetables and fruits. Giardia-infected children should avoid public pools and untreated water sources such as rivers, lakes, and streams. The Giardia will be eliminated and killed by first boiling the water (Nematian et al., 2004; Yilmaz, Akman and Göz, 1999; Cacciò et al., 2003).

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