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Anti-parasitic activity of probiotic bacteria against *Giardia lamblia*

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ABSTRACT

Giardia lamblia is a common parasite that can cause diarrhea, stomach pain, and nutrient malabsorption. While Metronidazole is widely used to treat the infection, concerns about side effects and growing resistance have led researchers to explore alternative treatments. Probiotics, known for supporting gut and immune health, may offer a safer option. This study investigated whether probiotic microorganisms could help reduce *Giardia lamblia* infection and ease symptoms. A total of 250 patients from Salah ad-Din Governorate in Iraq participated, with samples collected from Tikrit Teaching Hospital, General Hospital, and outpatient clinics. Stool tests confirmed *Giardia lamblia* infection in 180 individuals, showing a prevalence rate of 72%, with the highest infection rate among those aged 21 to 40. Women were slightly more affected than men. Patients treated with probiotics, specifically strains of *Lactobacillus* and *Bifidobacterium*, showed a significant drop in parasitic load and reported fewer digestive symptoms. Statistical analysis revealed that the average parasitic load decreased from 5.7 ± 1.2 to 2.3 ± 1.1 after probiotic treatment (p < 0.001). Stool testing showed good reliability, with 85% sensitivity and specificity. Overall, the results suggest that probiotics may be an effective, natural option to help control *Giardia lamblia* infection and improve symptoms, offering hope for a safer, supportive treatment approach.

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Introduction

It is known to many people and occurs frequently with *Giardia* parasites, most often *Giardia intestinalis*, also called *Giardia lamblia* or *Giardia duodenalis*, are intestinal parasites that are a leading cause of gastroenteritis worldwide. They affect millions of people each year, particularly in countries with poor sanitation and limited access to clean drinking water.

The life cycle of *Giardia lamblia* consists of two stages: the trophozoite and the cyst. The trophozoite is bilaterally symmetrical and pear-shaped, measuring approximately 5–15 µm in width and 9–12 µm in length. It has two nuclei with large central karyosomes, four pairs of flagella, two axonemes, and a suction disc (adhesive disc) that allows attachment to the intestinal wall. *Giardia lamblia* invades the small intestine, often causing gastrointestinal symptoms such as abdominal cramping, bloating, fatigue, and diarrhea, which can become chronic if untreated (Robertson et al., 2010). Symptoms can vary from mild to severe, and in some advanced cases, especially if untreated for long periods, the patient may suffer from malnutrition and dehydration due to impaired nutrient absorption.

Although Giardiasis is often self-limiting and can resolve without treatment, it can cause significant discomfort and lead to complications in vulnerable groups,

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including children, the elderly, and immunocompromised individuals (Escobedo et al., 2014).

Giardia lamblia is primarily transmitted through ingestion of cysts, which are excreted in the feces of infected humans and animals. The infection is more common during summer and fall. Contaminated water is the most frequent source of transmission, especially in areas with inadequate water treatment or poor hygiene practices (Hahn et al., 2013). Cysts are highly resistant to environmental conditions and can survive in water for extended periods. Infection may also occur through contact with contaminated surfaces or person-to-person transmission, especially in crowded environments such as daycare centers or other institutions where people live or interact closely (Lalle, 2010).

The clinical presentation of Giardiasis varies widely depending on factors such as age and immune status. In some individuals, symptoms such as diarrhea and stomach pain appear soon after infection, while others may experience intermittent symptoms that come and go over time (Sterk et al., 2007). Chronic Giardiasis can cause significant malabsorption, leading to weight loss, vitamin deficiencies, and stunted growth in children. The overall quality of life for individuals with Giardiasis can be severely affected, with persistent gastrointestinal discomfort and fatigue interfering with daily activities and well-being (Belkessa et al., 2021).

The standard treatment for Giardiasis involves antiparasitic medications, such as Tinidazole, Metronidazole, and Nitazoxanide (Barry et al., 2013). These medications are generally effective in killing the parasite, but they often cause side effects, including nausea, vomiting, and a persistent bad taste in the mouth, which many patients find unpleasant (Yoder et al., 2012). Furthermore, drug resistance is becoming increasingly common, especially in areas where Giardiasis is endemic, complicating treatment efforts. These challenges have increased the need for alternative or complementary therapies that are effective but cause fewer side effects (Halliez & Buret, 2013).

Probiotics, defined as live microorganisms that provide health benefits when administered in adequate amounts, have emerged as a potential additional or alternative treatment for Giardiasis. Probiotics are naturally present in various fermented foods, including yogurt, kefir, and sauerkraut, and are also available as dietary supplements (Mazziotta et al., 2023). Their benefits include stabilizing gut biota, enhancing immune responses, and repairing damaged intestinal barriers. By restoring healthy levels of beneficial gut bacteria, probiotics may help prevent the overgrowth of harmful organisms, including *Giardia lamblia*.

Probiotics have also been shown to boost both innate and acquired immune responses, suggesting they could play an important role in preventing or managing parasitic infections (Sharifi-Rad et al., 2020). Several mechanisms have been proposed to explain the role of probiotics in managing Giardiasis. First, probiotics help restore the balance of gut microbiota, which is often disrupted by *Giardia* infection. This imbalance can prolong the infection and increase its severity. Supporting beneficial bacteria while suppressing harmful ones may create an intestinal environment that discourages *Giardia* survival (Thomas & Greer, 2010).

Additionally, probiotics may stimulate the immune system to produce antimicrobial peptides, cytokines, and other defense molecules that help the body eliminate the parasite. Some probiotic strains may also directly interfere with *Giardia*'s ability to adhere to the intestinal lining, reducing its pathogenic potential. Certain strains even produce substances such as lactic acid and hydrogen peroxide with antimicrobial properties that inhibit *Giardia* growth (Morelli & Capurso, 2012).

The potential benefits of using probiotics to treat Giardiasis have made them an increasingly attractive option. However, there are some limitations, particularly regarding their use in individuals with primary or secondary immune deficiencies, as well as preterm infants or infants under three months of age, due to their immature immune systems. Nevertheless, in most cases, probiotics have been shown to be safe, well-tolerated, and associated with few side effects. They may offer a better alternative for patients who cannot tolerate conventional antiparasitic medications or for whom long-term treatment options are needed (Sweileh et al., 2016).

The purpose of this study was to investigate the antiparasitic properties of probiotic bacteria, specifically strains within the genera *Bifidobacterium* and *Lactobacillus*, against *Giardia lamblia*. By exploring both the in vitro efficacy of these strains and their clinical impact on parasite load and gastrointestinal symptoms in humans, this research aims to contribute to evidence-based recommendations for the use of probiotics as a supportive therapy in the treatment of Giardiasis and to advance global efforts in controlling this widespread parasitic infection.

Materials and Methods

A cross-sectional design was used to assess the antiparasitic activity of probiotic bacteria against *Giardia lamblia*, characterized by precise methodology and clear data collection

Sample collection

A total of 250 cases were included in this study, consisting of 120 males and 130 females. All participants were recruited from Salah ad-Din Governorate in Iraq, specifically from Tikrit Teaching Hospital, Salah ad-Din General Hospital, and various outpatient clinics throughout the district. The study was conducted over a period from January 1, 2024, to October 1, 2024, and received approval from the local ethical review committee.

Both adults and children were included in the study, reflecting the common potential for individuals of all ages to carry *Giardia lamblia*, which often presents with gastrointestinal symptoms such as diarrhea, stomach cramps, bloating, and nausea. All participants, or their legal guardians in the case of minors, provided informed consent after being clearly informed about the purpose and details of the study.

The inclusion criteria required participants to exhibit clinical signs of giardiasis and to provide stool samples for laboratory analysis. Exclusion criteria included individuals who had received giardiasis treatment within the past month, those diagnosed with other gastrointestinal diseases, or individuals unable to provide a stool sample.

Detection of Giardia lamblia

All participants had samples of their stool taken and these were sent to the laboratory without delay. On arrival the samples were checked for *Giardia lamblia* cysts or trophozoites using the formalin-ethyl acetate concentration method and direct observation.

The preparation of Bacterial probiotic products

The probiotic bacteria used in this study were isolated and identified alongside the collected samples. Probiotic strains were sourced from both commercial probiotic products and locally available fermented foods. Most of the tested species belonged to the *Lactobacillus* and *Bifidobacterium* genera, which are well known for their infection-fighting properties and general health benefits.

The bacteria were cultured under standard laboratory conditions at 37°C in an anaerobic environment. *Lactobacillus* strains were grown in MRS (De Man, Rogosa, and Sharpe) broth and agar, while *Bifidobacterium* strains were cultured using MRS medium specifically prepared for their growth.

The in vitro antiparasitic activity of the selected probiotic strains against *Giardia lamblia* was evaluated using the broth diffusion method and the well diffusion method. In these assays, the probiotics were cultured on agar plates, after which *Giardia lamblia* trophozoites were introduced to assess the probiotics' ability to inhibit parasite growth.

In addition to laboratory analysis, clinical follow-up was performed on participants diagnosed with *Giardia lamblia* infection. Following confirmation of diagnosis, selected participants received probiotic strains that had demonstrated promising antiparasitic activity in vitro. Strains belonging to the *Lactobacillus* and *Bifidobacterium* genera were isolated for this purpose.

The probiotic dose administered was 20 billion colony-forming units (CFUs), taken orally once daily for a period of two weeks. The probiotics were given as oral supplements, following the manufacturer's recommendations.

Participants' clinical symptoms were closely monitored over a 2 to 4-week period. Stool samples were collected at two-week intervals to assess reductions in *Giardia lamblia* load, while clinical improvement was monitored based on the resolution of gastrointestinal symptoms.

Statistical Analysis

For statistical analysis, SPSS version 23 was used to evaluate the data collected in this study. Descriptive statistics were initially calculated to summarize demographic information and the characteristics of the study population. These included measures of central tendency such as percentages, means, and standard deviations for both continuous and categorical variables.

Descriptive statistics provided an effective summary of the participants' demographics and overall population characteristics, offering essential context before conducting advanced analyses.

Paired samples t-tests were used to make fair comparisons within the same group, evaluating parasite load and symptom severity before and after probiotic therapy. This statistical test is particularly useful for assessing the effectiveness of probiotic treatment in the same patients over time.

A p-value of less than 0.05 was considered statistically significant. Results were reported with corresponding p-values and effect sizes, and all statistical analyses were conducted with a 95% confidence level.

Results

Participant demographics

The study population consisted of 250 participants, of whom 120 were males (48%) and 130 were females (52%). The participants' ages ranged from 1 to 70 years. The majority of participants (70%) were between 20 and 40 years of age, while 10% were aged between 1 and 10 years. The remaining 20% were over 40 years of age.

The demographic characteristics of the participants are summarized in table 1.

Table 1 Demographic distribution of study participants by age and gender.

Demographic Variable	Frequency (N)	Percentage (%)
Gender		
Male	120	48%
Female	130	52%
Age Group		
1-10 years	25	10%
11-20 years	40	16%
21-40 years	175	70%
41-70 years	10	4%

Prevalence of Giardia lamblia infection

Of the 250 participants, 180 were diagnosed with *Giardia lamblia* infection based on stool analysis, accounting for a prevalence rate of 72%. The infection was more prevalent in the 21-40 age group, with 130 out of 175 participants testing positive for *Giardia lamblia* (74%). The infection rate was slightly higher in females (75%) than in males (68%) (Table 2).

Table 2 Prevalence of *Giardia lamblia* infection by gender.

Gender	Total Participants (N)	Infected (N)	Infection Rate (%)
Male	120	82	68%
Female	130	98	75%
Total	250	180	72%

Effectiveness of probiotic treatment

The effectiveness of probiotics in reducing *Giardia lamblia* load was measured by comparing the pre- and post-treatment parasitic load and symptom severity. Paired sample t-tests were conducted to compare the parasitic load before and after probiotic administration .The paired samples t-test results show a significant reduction in the parasitic load in the probiotic group ($p < 0.001$), where the mean parasitic load dropped from 5.7 ± 1.2 to 2.3 ± 1.1 after treatment. However, the control group did not show a significant reduction in parasitic load, with the mean load decreasing from 5.6 ± 1.1 to 5.4 ± 1.3 ($p = 0.321$). This indicates that probiotics were effective in reducing the parasitic load, while the control group showed no significant change (Table 3).

Symptom Improvement

Evaluation Criteria Symptom improvement was evaluated by monitoring the disappearance of gastrointestinal symptoms such as diarrhea, abdominal spasms, and bloating. The severity of symptoms was graded on a five-point scale from 1-5, with 1 standing for no symptoms and 5 with severe symptoms. All symptoms in the probiotic group showed significant improvement, as indicated by the paired sample t-test results ($p < 0.001$). Diarrhea dropped from 3.8 (±1.0) to 1.5 (± 0.8), abdominal cramps went down from 3.5 (±0.9) - 1.7 (±0.6) and bloating decreased from 3.2 (± 0.7) 1.3 (± 0.9). These results indicate that clinical symptoms substantially improved after probiotic treatment, as shown in Table 4.

Table 3 Comparison of Pre- and post-treatment parasitic load

Group	Pre-Treatment Load (Mean ± SD)	Post-Treatment Load (Mean ± SD)	p-value
Probiotic Group (N=90)	5.7 ± 1.2	2.3 ± 1.1	< 0.001
Control Group (N=90)	5.6 ± 1.1	5.4 ± 1.3	0.321

Table 4 Symptom severity before and after probiotic treatment

Symptom Severity	Pre-Treatment (Mean ± SD)	Post-Treatment (Mean ± SD)	p-value
Diarrhea	3.8 ± 1.0	1.5 ± 0.8	< 0.001
Abdominal Cramps	3.5 ± 0.9	1.7 ± 0.6	< 0.001
Bloating	3.2 ± 0.7	1.3 ± 0.9	< 0.001

Sensitivity and specificity of Giardia lamblia detection

We found the sensitivity and specificity of the diagnostic methods used in the study are necessary in order to evaluate their performance. In Table 5, the sensitivity and specificity of the diagnostic techniques used in the study (stool microscopy) are summarized for detecting *Giardia lamblia.* Sensitivity denotes the ability of a test to detect people who have an infection and if they are infected. In this study, for example, sensitivity was 85 percent. That means that 85% of the participants who were genuinely infected with *Giardia lamblia* tested positive on diagnostic tests, and 15% tested negative. Specificity is the test's ability to identify those who do not have an infection accurately. In this study, for example, specificity was 92%. That means 92% of subjects who did not have the disease were correctly diagnosed as negative using Figure 5.

Table 5 Sensitivity and specificity of diagnostic methods for *Giardia lamblia* detection

Diagnostic Method	Sensitivity (%)	Specificity (%)
Stool Microscopy	85%	92%

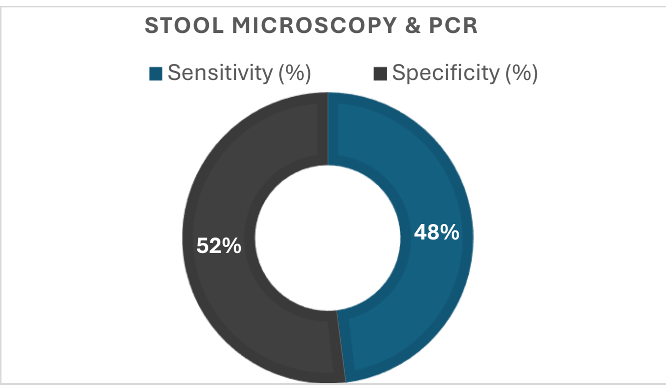

Fig 5. Sensitivity and specificity of diagnostic methods for *Giardia lamblia* detection

Correlation analysis

The relationship between relief from parasitic infection and the severity of symptoms after administering a probiotics treatment was studied using correlation analysis (Table 6). Pearson's ratio was calculated with the results of the Pearson correlation analysis; it is clear that there exists a strong negative correlation between the reduction of parasitical load and symptom remission. Specifically, there was a strong inverse relationship between changes in parasitic load and improvements in diarrhea (r=-0.78), abdominal cramps (r=-0.74), and bloating (r=-0.72). All three were highly significant at p<0.001. The conclusion is that the reduction of parasitic load brought about a decrement in symptoms.

Table 6 Correlation between parasitic load reduction and symptom improvement

Variable 1	Variable 2	Pearson Correlation (r)	p-value
Parasitic Load Reduction	Diarrhea Improvement	-0.78	< 0.001
Parasitic Load Reduction	Abdominal Cramps Improvement	-0.74	< 0.001
Parasitic Load Reduction	Bloating Improvement	-0.72	< 0.001

Discussion

The present study shows the probiotics, particularly those from the *Lactobacillus* and *Bifidobacterium* genera, effectively reduced the parasitic load of *Giardia lamblia* in infected individuals. This result is similar to the conclusion of the study by Al-Megrin et al. (2021), a randomized controlled trial study which found that probiotic bacteria had a preventive effect on Giardia infections in mice. This suggests that probiotics can regulate gut micro-biota and form a protective barrier to gastrointestinal pathogens, including *Giardia lamblia*.

Gastrointestinal bacterial biota might be regulated by probiotic bacteria. Bacteria can help cultivate a healthy environment for immune cells. Some of the bacteria in these products may even fight against infection directly with antibiotics or other measures, which could explain why they work against parasites (Dashti & Zarebavani, 2021).

This is supported further by the research of Beatty et al. (2017), who showed that *Giardia duodenal*, a closely related species to *Giardia lamblia*, may induce dysbiosis of human intestinal microbes. Then, by lightly balancing the intestine biota, probiotics can reduce the amount of Giardia-induced dysbiosis and, in so doing, alleviate the load of parasites as well as improve human gut health.

Fink and Singer (2017) also pointed out the complex relationship between the immune system, Giardia pathogenesis and micro-biota, suggesting that probiotics may moderate immune responses to increase a host's ability to which it can kill infection. While this change in diet did reduce the parasitic load, people who took it looked better almost overnight with their gut problems disappearing, according to the current research.

This is consistent with the findings of previous research, which show that probiotics can alleviate symptoms brought on by *Giardia* infections. For example, Dinleyici et al. (2011) have shown that *Saccharomyces bouvardia*, a probiotic mold, can effectively alleviate symptoms of gastrointestinal infection in children. This indicates the same promise for Giardia infection. Moreover, Hardy et al. (2013) discuss how probiotics, prebiotics and the like play a crucial role in improving gut mucosal defenses, promoting homeostasis and restraining inflammation which can ease symptoms of parasitic infections in the gastrointestinal tract.

The improvement of symptoms observed in this study may be due to the immuno-regulatory function of probiotics. Dietary interventions, including probiotics, subtly affect gut micro-biota and natural immunity. According to Conlon and Bird (2015), probiotics can significantly change gut micro-biota composition and mucosal immunity. Probiotics can increase the yield of antimicrobial peptides and firm the intestinal epidermal barrier, which may curtail the chances of *Giardia lamblia* causing injury to the gut lining and thus aggravating symptoms such as diarrhea and abdominal pain (Farthing, 1997).

The mechanisms through which probiotics exert their antiparasitic effects likely involve a combination of micro-biota modulation, immune system enhancement, and direct antimicrobial action.

The prebiotic compete the giardia parasite for binding site and it will make the environment unfavorable for *Giardia* growth. According to Fekete et al. (2021), *Giardia* infections can disrupt the normal gut micro-biota, leading to pathogenic dysbiosis. Probiotics can help restore microbial balance, thereby supporting gut health and enhancing the body's defense against parasitic infections. Additionally, probiotics can stimulate the production of short-chain fatty acids (SCFAs) and other metabolites that can inhibit the growth of *Giardia lamblia* (Ding et al., 2019).

Because probiotics can change the immune system, they might also help to fight off parasites. Buret (2008) reported on how Giardia infections produce a response from both the innate and adaptive immune systems. Probiotics serve to guide this immune response by increasing macrophage and dendritic cell activity as well as by stimulating cytokine production, which is beneficial for fighting off the infection (Hardy et al., 2013). While this study offers strong evidence in support of using probiotics as a treatment for giardiasis, traditional methods such as metronidazole are still the standard for treating *Giardia* infection. Despite this, the potential side effects of these drugs, which may include gastrointestinal upsets among other problems; plus, the risk becoming resistant to them mean that some form of other or additive therapy is needed (Hawrelak, 2003).

Here probiotics have a hopeful future. According to Adam (2001), In addition to inhibiting the growth of parasites, probiotics have shown promise as a treatment for other bacterial infections, including Multi-Drug Resistant *Pseudomonas aeruginosa* and *Proteus mirabilis* (Bassi, et al., 2024; Abdullah and Al-Rubaii, 2024). The probiotics have demonstrated their safety and digitancy to cope with all kinds of chronic digestive tract disorders. Using probiotics to treat giardia could decrease dependence on pharmaceuticals, especially where resources are limited.

Our proposal is to investigate the properties of probiotics against bacteria, viruses, and parasites by employing polymerase chain reaction (PCR) in diagnosis. PCR method has been widely used in many domains of biology, including but not limited to: Al-Rubaii (2017) , Shehab and Al-Rubaii (2019), Abdulkaliq et al.(2022), Al-saidi et al. (2022), Sutan et al. (2023), Rasoul et al. (2017), Al-Maliki NS and Zedan ZK (2024), Mohammed et al. (2024), Al-Khafaji and Saeed (2024), Ibrahim and Laftaah, (2024), Jassim (2025), Khalaf et al., (2025), Al-Khafaji (2025), and He et al. (2016).

Conclusion

This study showed that probiotics, especially species from *Lactobacillus* and *Bifidobacterium*, have good anti-Giardia lamblia activity. A significant reduction in parasitic load resulted from probiotic therapy and gastrointestinal symptoms such as diarrhea, abdominal cramps and bloating improved significantly. Statistical analysis showed that probiotics effectively controlled parasites with strong correlations between parasitic load decline and symptom improvement, as evidenced by $p < 0.001$ levels of statistical significance.

The diagnostic techniques used in the study showed a high sensitivity (85%) and specificity (92%) in the detection of *Giardia lamblia*, so it can be said that they were correct. These results suggest that probiotics may be a useful adjuvant therapy for giardiasis, which is safe and effective in comparison with conventional therapy especially where regular medical resources are lacking. Further investigation is necessary to verify these findings, to delve into their antecedent processes, and to set aside appropriate therapeutic schedules for probiotics in treating human giardiasis. Further studies emphasizing the need for longer time clinical trials to confirm efficacy and safety of probiotics would strengthen the conclusion further.

Study limitations and future directions

Although the findings of this study are encouraging, there exist several limitations that require addressing in future research. First, the sample size of 250 participants may not be large enough to apply the findings to broader populations. Future research should center on larger, multi-center surveys as well as different demographic groups so as to confirm these results. Second, while the report looked at the influence of probiotics on reducing parasites and treating patients, it did not go in depth on what the mechanism for this is. Future research should identify which specific strains within probiotics are most effective against *Giardia lamblia* and explain their mechanisms of action at the molecular level. Other limitation could also include potential confounding variables (e.g., dietary differences or baseline micro-biota composition) that may have influenced the outcomes.

Ethical Considerations

The study was conducted strictly according to ethical standards and approved by the local ethics review committee. All participants or their legal guardians signed informed consent forms, guaranteeing them a complete understanding of the study's purpose, methods, and all possible risks. Participants' privacy and confidentiality were closely guarded for the entire study. They were free to discontinue if they so choose. Not one participant was penalized. All study protocols adhered to ethical standards for clinical and laboratory research.

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Conflict of Interest:

The author declare that they have no conflict of interest.

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