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GIARDIA/CRYPTO (S SINGER, SECTION EDITOR)

Can *Giardia lamblia* Assemblages Drive the Clinical Outcome of Giardiasis?

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Abstract

Purpose of Review To carry out a bibliographic survey from June 2001 until March 2021 on the possible association between *Giardia lamblia* genotypes and clinical manifestations of giardiasis.

Recent Findings *G. lamblia* infection leads to a broad spectrum of clinical conditions, ranging from asymptomatic to acute or chronic symptomatic forms. There are several open questions regarding the direct damage of the parasite and the host response to giardiasis pathogenesis. The wide genetic variability of *G. lamblia* prompts speculation on the possible relationship between its assemblages and the clinical manifestations.

Summary Studies in human giardiasis focus on the association between individual symptoms and infection by assemblages A, B, or even E. This review points out that this topic is still little explored and the results are inconclusive and contradictory.

Keywords *Giardia lamblia* · Clinical giardiasis · Assemblage

Introduction

Giardiasis is an intestinal pathology caused by a flagellated protozoan called *Giardia*. *G. lamblia* (syn. *G. duodenalis*, *G. intestinalis*) is a species with a high affinity for mammalian hosts, which gives it a high (anthropo) zoonotic potential. This affinity, combined with its cystic wall resistance, guarantees cyst integrity in the environment and makes *G. lamblia* an organism that is easily dispersed and associated with outbreaks due to the consumption of contaminated water and food.

G. lamblia infection can present a broad spectrum of clinical conditions, ranging from asymptomatic to acute or chronic symptomatic forms. Asymptomatic infection is the most common form in children and adults. However, the intervening factors in this parasite-host relationship, which allow the persistence of the infection without the occurrence of clinical expression, are not yet defined [1]. Individuals without symptoms have considerable epidemiological relevance, especially in the direct transmission of *G. lamblia*, since they do not seek medical follow-up and adequate treatment.

In locations with precarious economic and sanitary conditions, high *G. lamblia* infection rates are expected because its transmission is closely related to inadequate water supply and sewage treatment. In endemic areas, the frequency of infection is inversely proportional to increasing age. Interestingly, in these regions, when present, the manifestation of symptoms occurs in children in early childhood. This phenomenon is possibly related to high exposure to the parasite throughout life, since individuals who have second and third episodes of infection have lower levels of fecal lactoferrin (a non-invasive marker of inflammatory bowel diseases), suggesting some protection against giardiasis severity [2].

In areas with high socioeconomic status, asymptomatic cases are also reported [3, 4]. However, the relative frequency of symptom manifestation in cases of adult infection is higher [5]. In these areas, the occurrence of giardiasis is normally related to occasional cases, at times imported (known as "traveler's disease"), or from outbreaks via an indirect transmission (food and mainly contaminated water) [6–9].

This article is part of the Topical Collection on *Giardia/Crypto*

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When symptomatic, diarrhea is the main clinical manifestation. Other alterations can also be observed: abdominal pain, nutritional malabsorption, weight loss, nausea, flatulence, headache, and fetid stools with or without the presence of fat. However, the clinical characteristics of the infection can vary widely, and it is not clear whether this phenomenon is related to the host, the parasite, or even to the host-parasite-environment interaction.

In adults, the main consequence of infection is the nutritional deficit that regresses once treated. However, in children, the infection can compromise physical and cognitive development, especially in cases of prolonged exposure to infection (chronic cases, repeated episodes of reinfection, or therapeutic failure) regardless of the manifestation of symptoms [10–14].

The absence of symptoms and the wide variability of clinical conditions and consequences of the infection raised the hypothesis that *Giardia* could act as a commensal microorganism or a pathogen [15, 16]. However, this theory has been deconstructed because asymptomatic infections can still harm the host [12, 14].

Clinical Symptoms and Signs, and Association with *Giardia lamblia* Assemblage

The *G. lamblia* species is phylogenetically subdivided into assemblages named in alphabetical order from A to H. Assemblages A and B are the most commonly reported in humans [17–19]. Recently, assemblage E has also been increasingly reported as infecting humans in several countries [20–22]. On the other hand, assemblages C, D, and F were only observed in specific cases in China and Slovakia [8, 9], Germany [7], and Ethiopia [6], respectively. The other assemblages have not yet been reported.

In addition to genetic differences, much has been speculated about whether these assemblages could show differences in dispersibility and transmission potential, drug resistance, pathogenesis, and clinical manifestation.

Studies were attempting to determine whether a *G. lamblia* phenotypic profile that drives clinical giardiasis focuses on an association between symptoms and assemblages A and B. Although assemblage E has been reported in humans, the clinical characteristics surrounding this infection are not yet known. In humans, assemblages A, B, and E are associated with damage to the intestinal mucosa, but there was no relationship between the immune response profile and the infecting assemblage [23, 24]. In mice, assemblages A and B reduce glial cells in the small intestine [25].

It has been seen that trophozoites of axenic cultivation of assemblage A have greater cell growth and are less susceptible to nitric oxide action than assemblages B and E [26, 27]. In Brazil, high rates of parasitic persistence by assemblage A are reported in children after treatment with nitroimidazole in an area of sympatric circulation of the three assemblages (A, B, and E) [28]. In Spain, almost 20% of patients had persistent infection with assemblages A and B after using nitroimidazole [29]. The susceptibility to in vitro drugs of the assemblages varies, and assemblage A is shown to be the least susceptible to the action of nitroimidazoles [26]. However, in a gerbil experimental model, there are no differences in susceptibility to different drugs [26].

In general, assemblages A and B are associated with reports of asymptomatic and symptomatic cases of *Giardia* infection in humans [30]. Despite the wide variety of symptoms of giardiasis, genotyping studies correlate genetic characteristics mainly with the occurrence of diarrhea, but other symptoms such as abdominal pain, fever, nausea, and vomiting are occasionally reported. Part of the studies classifies individuals as symptomatic without specific symptoms.

Studies that attempt to connect a specific *G. lamblia* assemblage to a specific giardiasis clinical picture are still scarce in humans. When carrying out an exploratory and descriptive search on the online electronic database Medical Literature Analysis and Retrieval System (Medline) in March 2021, using the MeSH terms "Giardia," "assemblage," and "symptom," 259 published manuscripts were found since the first publication in June 2001. However, the vast majority are not successful in associating assemblages with symptoms ($n=69$) or refer to non-human hosts ($n=121$). Furthermore, some studies establish asymptomatic ($n=2$) or symptomatic (or even with specific symptomatology) groups as description criteria ($n=13$), not enabling correlations with the presence or absence of symptoms.

Studies that correlate specific symptoms, as well as disease severity, differ in their findings. Some authors have reported that assemblage B is more associated with clinical manifestations and may be associated with anthroponotic transmission, especially in children [3, 4, 6, 31–40]. Others find the exact same association when assemblage A was evaluated [5, 41–50]. However, in the vast majority of studies, a low number of clinical samples were analyzed, reducing or making it almost impossible to guarantee the statistical power of the association.

Although infection by both assemblages manifested a broad spectrum of symptoms, studies in India and Saudi Arabia successfully found a statistical association between clinical characteristics and *G. lamblia* assemblages. In India, the infection by assemblage B was more associated with malnutrition and weight loss [51]. In Saudi Arabia, patients infected with assemblage B were symptomatic, despite low number of samples ($n=40$) [33].

Several studies conducted in different countries do not observe any difference between clinical giardiasis and assemblages A or B [2, 31, 34, 35, 50, 52–61]. Although assemblage B was more common in Egypt than assemblage

A, associations between the assemblages and epidemiological information were not detected, except for assemblage B being more frequent in younger children [35]. When residents of a province in Iran and Algeria were evaluated, no significant difference was observed between demographic factors, such as age, sex, and clinical manifestation of those infected by assemblages A and B of *G. lamblia* [50, 62]. In Cuba, although there is no difference between the clinical picture and the assemblages, assemblage B seems to be more prone to inducing clinical symptoms [54].

In addition to the division into assemblages, some authors evaluate the characteristics of the subassemblages. Although both assemblages (A and B) cause disease in Iran, the AII infections were more often associated with abdominal pain, nausea, and vomiting [44]. However, in Brazil, the clinical symptoms of few analyzed samples ($n=5$) showed that assemblage A was related only to AI, whereas cases of infection by AII were asymptomatic [63]. Similarly, in Iraq and Saudi Arabia, assemblage B was observed in symptomatic individuals while cases of infection by subassemblages AI and AII were asymptomatic or presented only mild symptoms and without diarrhea [33].

In the city of Damascus, Syria, the subassemblage AII was correlated with weight loss in adults [46]. Iranian researchers observed a similar event, in which the AII assemblage was significantly associated with abdominal pain, nausea, and vomiting. Despite this, the infection by all assemblages presents similar clinical giardiasis [44]. In Egypt, assemblage B was the most common in symptomatic infections. The AI subassemblage was less common in children with gastrointestinal symptoms, and the AII subassemblage was reported only in asymptomatic individuals [64].

In Spain, giardiasis affects individuals of all ages, especially children. After genotyping 97 *G. lamblia* isolates obtained during 7 years, investigators observed that assemblage B was more common than assemblage A in children under 12 years of age. In contrast, both assemblages have a similar distribution in adults [4]. Assemblage B was already reported as the most prevalent in patients with clinical giardiasis in these Spanish isolates [3, 4]. However, the association between assemblage A and symptomatic infections, and between assemblage B and asymptomatic infections was also observed in the past [65]. The apparent controversies observed in these studies could be justified by geographic particularities or temporal differences between these studies.

Clinical presentations can be used to identify the occurrence of giardiasis, and assemblage characterization can be used to identify transmission routes. *G. lamblia* is a common parasite in children with gastrointestinal symptoms in Egypt, and the predominance of assemblage B suggests that they share common sources of infection [39]. Both the identification of assemblages B and A points to the possibility of anthroponotic and zoonotic transmission [32, 36, 62, 66, 67].

Other potential routes of transmission associated with the high parasitic load of cysts eliminated in the feces and with their ability to resist in the environment increase the pressure of infection in contaminated areas. In Cuba, children that excrete more cysts show more symptomatic manifestations regardless of the assemblage [54]. In Brazil, children infected with assemblage B release more cysts than those infected with assemblage A [2]. Synergistically, individuals infected by assemblages A and B excrete even more cysts than children infected by a single assemblage [2]. Similarly, although assemblage AII has been associated with a higher frequency of diarrhea episodes in South India when patients are infected by both assemblages (A and B), the diarrheal picture was more intense [68].

Personal Observations

Our research group has been working in a Brazilian low-income endemic area for *G. lamblia* infection, where public health is threatened by social and health vulnerability. Over the years of study, the epidemiological scenario of *G. lamblia* assemblage distribution has been altered. Initially we identified only assemblages A and E [20]. Since then, the assemblage B was introduced [28]. Now, assemblages A, B, and E are found with sympatric circulation and detected in humans. Of utmost importance, during the year of checking the circulation of assemblage B, the number of episodes of diarrhea and abdominal pain increased considerably. Although assemblages A, B, and E are found infecting symptomatic and asymptomatic children, an experimental design that allowed us to establish any clinical-genotypic profile association was not yet drawn. Several factors could interfere and distort a correlation (contribute to misinterpreting possible correlations) between assemblage and *Giardia* infection clinical presentation, such as:

1) The clinical picture of giardiasis is similar to other infections. Therefore, research on other etiological agents of diarrhea must be carried out to exclude the manifestation of symptoms associated with co-infection by another parasite.
2) The common practice of taking antibiotics for treating bacterial infections may interfere with the intestinal microbiota and, consequently, alter the stool consistency, a common clinical symptom presented by the preschoolers we studied.
3) Preschoolers are introducing solid foods into their nutritional menu, which can change stool consistency.

4) Individuals with precarious economic conditions may have nutritional deficiencies.

Conclusion

The remarkable divergence between findings points out that it is not yet possible to assertively establish the correlation between the assemblage profile of *G. lamblia* and a symptom or set of symptoms. Thus, it is not possible to rule out that the clinical pictures are related to factors associated with the host. This would help justify the higher frequency of symptom manifestation in children and adults in non-endemic areas since they have a weakly stimulated immune system for the parasite.

It is worth mentioning that the high frequency of *G. lamblia* infection is commonly seen in places with poor basic sanitation and socioeconomic conditions. Thus, studies that aim to establish the association between infectious assemblage and symptom manifestation need to ensure that the clinical condition observed is a consequence of the exclusive infection by *G. lamblia* and is not associated with other pathogens, nutritional conditions, or dietary changes. Using a susceptible experimental model, mirroring human giardiasis' clinical aspects can control and overwhelm these limitations.

In the environmental context, we must consider the hypothesis that in endemic areas for a given assemblage, the introduction of a new assemblage profile can predispose people to more severe symptoms. Moreover, infection by two different assemblages could produce a synergistic increase in gastrointestinal tissue damage.

Other factors of the parasite (such as multiplication rate, expressed variable surface proteins, resistance to pharmaceutical products, and the ability to invade the immune response) and its interaction with host factors can also contribute to the pathophysiology observed for clinical giardiasis. It is still worth mentioning that factors such as the infective burden as well as the parasitic load can help to justify the differences in virulence and pathogenesis of assemblages A and B observed between the studies.

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Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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