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Giardia: a pathogen or commensal for children in high prevalence settings?

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Abstract

Purpose of review—*Giardia* is a common intestinal parasite worldwide, and infection can be associated with clear and sometimes persistent symptomatology. However, in children in high prevalence settings, it is not associated with or is perhaps even protective against acute diarrhea, and the association with long-term outcomes has been difficult to discern.

Recent findings—Recent studies have made progress in helping us disentangle this apparent paradox. First, prospective, well-characterized cohort studies have added to the data on the association between *Giardia* and diarrhea in these settings and have further characterized associations between *Giardia* infection and nutrition, gut function, and growth. Second, animal models have further characterized the host response to *Giardia* and helped elucidate mechanisms by which *Giardia* could impair child development. Finally, new work has shed light on the heterogeneity of human *Giardia* strains, which may both explain discrepant findings in the literature and help guide higher-resolution analyses of this pathogen in the future.

Summary—The true clinical impact of endemic pediatric giardiasis remains unclear, but recent prospective studies have confirmed a high prevalence of persistent, subclinical *Giardia* infections and associated growth shortfalls. Integrating how nutritional, microbial, metabolic, and pathogen-strain variables influence these outcomes could sharpen delineations between pathogenic and potentially beneficial attributes of this enigmatic parasite.

Keywords

Giardia; diarrhea; child growth; child development; environmental enteropathy

INTRODUCTION: WHENCE THE BLACK BOX?

Giardia lamblia (also known as *G. intestinalis/duodenalis*) is one of the most common intestinal parasitic infections in both children and adults worldwide. Giardiasis has a clear

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Conflicts of interest

There are no conflicts of interest.

case definition characterized by malabsorptive diarrhea, abdominal bloating/cramping, and weight loss [1] that is reproducible, if strain-dependent, in human volunteers [2] and resolves with treatment. Infection can lead to intestinal inflammation [3], systemic cellular immunity [4], and lingering symptoms, even for several years [5]. *Giardia* has also been implicated as a cause of diarrhea in travelers from non-endemic to endemic countries [6], with the greatest risk amongst backpackers in Asia [7]. Children living in limited-resource settings, many of whom are undernourished, harbor a particularly large burden of *Giardia* infection.

However, recent assessments of pathogen-specific diarrhea in such settings find no *Giardia*-attributable burden of diarrhea in this population [8, 9*]. Indeed, the finding in several studies that *Giardia* infection is associated with a decreased likelihood of acute diarrhea [6, 10, 11] has fostered speculation that such colonization is not only inconsequential, but even protective in these children. Thus, despite its high prevalence in this vulnerable population, *Giardia*, unlike other enteropathogens (e.g., *Campylobacter* or *Cryptosporidium*) is completely absent from global burden of disease estimates [12]. Here we review recent work that addresses this epidemiological conundrum that seemingly categorizes *Giardia* simultaneously as both harmful pathogen and intestinal commensal.

DOES *GIARDIA* CAUSE ACUTE DIARRHEA IN HIGH-PREVALENCE SETTINGS?

The recently-completed Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) allows for the most comprehensive analysis of *Giardia* infection in these settings to date. Over the period of November 2009 to February 2014, *Giardia* was detected in two-thirds of 1,741 children completing the study through 2 years of age, with wide variation in median time to first detection among the eight study sites [13]. *Giardia* was detected in 37 to 95% of children at each site within the first two years of life and persistent infection was common. However, when adjusting for *Giardia* detection in non-diarrheal stools and the presence of co-pathogens, *Giardia* was not significantly associated with diarrhea, regardless of site or child age [9*]. In other work, *Giardia* was not an independent risk factor for diarrhea in children presenting to clinics after travel to sub-Saharan Africa, Latin America, and South Asia, even though it was among the most frequently detected pathogens in 10.1% of stools [14]. The strikingly high prevalence of non-diarrheal *Giardia* infections [6, 8, 9*, 15, 16**] is such that *Giardia* detection has frequently been negatively associated with diarrhea [6, 8, 17, 18]. Several factors could preferentially decrease the sensitivity of *Giardia* detection preferentially in diarrheal stools. First, metronidazole is commonly used as empiric therapy for diarrhea in these settings. Thus, diarrhea could be a marker of metronidazole exposure, which could reduce *Giardia* detection in diarrheal stools. Second, detection artifact due to a decreased sensitivity of microscopy-based detection in liquid stools might partially explain this phenomenon [18]. However, studies using antigen-based diagnostics that are presumably more resistant to such a dilutional effect have corroborated these findings [8], and quantitative nucleic-acid based diagnostics have also suggested that higher quantities of *Giardia* are associated with even greater reductions in diarrhea risk [17], an inversion of

Koch's molecular postulates and the opposite of what is seen with pathogens that are clearly diarrhea associated [17,19,20]. This suggestion of a protective effect of *Giardia* against diarrhea has primarily come from case-control studies, however prospective studies have also found evidence of such [10, 11]. These findings are not universal, and *Giardia* was not seen to be protective against diarrhea in a recent birth cohort study in Bangladesh [16**].

If real, what could explain this protective effect? The list of potential explanations is long, and data are few. Early *Giardia* infection may be an environmental marker of exposure to multiple enteropathogens. Thus, children with *Giardia* infection at baseline in longitudinal studies may be more likely to have already developed immunity to these pathogens and be less likely to have subsequent clinical diarrhea due to enteric infections. Second, *Giardia* may induce a specific micronutrient deficiency (e.g., iron) that is protective against diarrhea [21]. Intriguingly, in one prospective study, the protective effect of *Giardia* infection on acute diarrhea was not discerned in children who were randomized to receive micronutrient supplementation [11]. Thirdly, *Giardia* may more broadly modulate immune responses to other pathogens [22] or even directly bind enterotoxins [23]. Finally, unidentified environmental or host factors such as breastfeeding practices, genetics, or intestinal microbiota could differentially influence *Giardia* manifestations in young children. Further work is needed to understand the specificity and potential mechanisms driving this intriguing association.

IS *GIARDIA* INFECTION ASSOCIATED WITH POOR GROWTH?

Child growth, rather than incident diarrhea, might be a more relevant outcome for estimating the burden of endemic pediatric giardiasis. Historically, associations between *Giardia* and impairments in child growth have varied by study, population, and site, leaving unanswered questions regarding causal relationships between the parasite and child developmental sequelae. In MAL-ED, no consistent association was seen between a high burden of *Giardia* infection during the first two years of life and height attainment over that interval [24]. However, there was a strong association between persistent *Giardia* infection within the first six months of life and height attainment at 2 years (Rogawski ET, Bartelt LA, Platts-Mills JA, unpublished data). Similarly, in a single site birth cohort in Bangladesh where *Giardia* infection was nearly universal, with an average of 3.5 *Giardia* positive monthly surveillance stools per child in the first year of life, an impact on growth attainment at two years of age was seen in children with a first *Giardia* detection in the first six months of life [16**]. Since the majority of first *Giardia* exposures occur after one year of age, other factors such as total pathogen burden had a greater overall effect on childhood growth at two years of age [24]. These findings could suggest an early, critical window of childhood vulnerability which is seldom captured in a focused manner. However, it is also possible that the chronic consequences of *Giardia* persistence into the second year of life were not yet evident at the completion of follow-up. Additionally, ongoing exposures to deworming agents with anti-giardial activity such as mebendazole for soil-transmitted helminths which overlap with *Giardia* prevalence [25, 26] in older children could also interrupt *Giardia* persistence and abrogate the impact of *Giardia* on growth in older children.

WHAT IS THE PATHWAY BETWEEN *GIARDIA* INFECTION AND POOR GROWTH?

The current working model of enteropathogen-associated growth shortfalls in children links nutrient deficiencies with microbial-driven intestinal inflammation, gut dysfunction, and increased intestinal permeability, termed environmental enteropathy (EE)[27, 28**]. Chronic *Giardia* infection has been associated with altered intestinal architecture and chronic lymphocytic inflammation in humans and some experimental models [4, 29, 30]. In MAL-ED, *Giardia* infection was associated with an increased lactulose:mannitol (L:M) ratio, indicative of increased gut permeability, but fecal myeloperoxidase (MPO), a marker of neutrophil inflammation, was paradoxically lower in children with *Giardia,* and *Giardia* was not associated with increased fecal neopterin as a marker of T-cell activation or the acute phase reactant α-1-acid glycoprotein (Rogawski ET, Bartelt LA, Platts-Mills JA, unpublished data). Thus, while impaired gut function represents a putative pathway for poor growth during giardiasis, the mechanisms appear to be independent of both diarrhea and inflammation.

Recent laboratory work supports these findings and suggests new lines of investigation for understanding the association between *Giardia* and poor growth. First, both parasite-induced and host cellular- or cytokine-mediated alterations in tight junctions could participate in increased gut permeability during giardiasis. For example, direct contact between intestinal epithelial cell monolayers and some *Giardia* strains lead to degradation of cytoskeletal villin protein as well as the tight-junction protein zonula occludens 1 (ZO-1) [31*]. In an experimental model, *Giardia* degraded epithelial cell tight junction proteins occludin and claudin-4 [32*] and increased para-cellular translocation of intestinal bacteria. Inflammation, gut hypersensitivity, and evidence of ongoing bacterial translocation, were present well after parasite clearance, suggesting that *Giardia*-induced alterations in epithelial barrier may explain chronic intestinal sequelae following *Giardia* exposures [5, 33]. Finally, host immune responses during experimental *Giardia* infections also contribute to epithelial cell dysfunction and are entirely sufficient to cause microvillus alterations in the absence of the parasite [34].

Second, the absence of an association between *Giardia* and markers of inflammation in MAL-ED are consistent with the frequent lack of acute inflammation on histopathology during *Giardia* infection, decreased fecal neutrophil chemotactic markers such as IL-8 during more prolonged *Giardia* infections [35], and an association between *Giardia* and lower systemic C-reactive protein levels [11]. Indeed, *Giardia* contains an impressive repertoire of immunoevasive products [36*] that are upregulated upon sensing secreted host factors even prior to parasite attachment [37] and could have implications for modulating host responses to co-pathogens or resident microbiota. For example, cathepsin B-producing *Giardia* strains are capable of cleaving IL-8 and attenuating neutrophil chemotaxis to inflammatory stimuli [38, 39]. Interestingly, inhibition of cathepsin B prevents *Giardia*-induced epithelial cell villin protein degradation, potentially linking a single virulence factor that could lead to divergent gut permeability and inflammation findings [31*]. Translating

similar outcomes into children will require further elucidation of *Giardia* strain differences since this effect was restricted to specific laboratory isolates.

Third, both the finding that persistent *Giardia* infections have the greatest influence on childhood growth and the clear association between *Giardia* and persistent diarrhea [6] suggest that a subset of children fail to make an appropriate immune response. While host factors associated with clinical recurrent giardiasis have generally focused on secretory IgA, recall assays in travelers with giardiasis have identified IL-17A-producing $CD4^+$ T-cells as the predominate memory cell phenotype [40*] in those who cleared parasites more rapidly, consistent with recent murine models invoking the role of Th17 cells in *Giardia* clearance [41]. The specific relevance of the Th17 axis during *Giardia* infection has not been elucidated, but since IL17 has been shown to enhance intestinal epithelial barrier function [42], determining whether children with persistent *Giardia* infection have impairments in IL17-mediated immunity and consequently barrier dysfunction even in the absence of increased inflammation may reveal additional mechanisms driving increased intestinal permeability in this population.

Finally, while growth is an important outcome for prospective studies of early child development, growth shortfalls remain a non-specific outcome for EE. Ongoing work into better non-invasive measures of EE may help also help resolve the relationship between *Giardia* and childhood growth via EE [26, 43, 44]. Recently identified regulators of β-oxidation and energy flux via nicotinamide metabolism may represent novel metabolic pathways driving childhood malnutrition [45*]. Children with *Giardia* infection excrete greater amounts of urinary lipid markers of oxidative damage and erythrocyte catalase, suggestive of chronic systemic oxidation [46*]. Experimental models are actively pursuing how persistent *Giardia* infection dysregulates gut microbial-host co-metabolic adaptations to the nutrient-deprivation condition. Ascertaining whether such changes are due to direct effects of *Giardia* or alternatively whether either microbial interactions or the immune modulation associated with *Giardia* infection influence the metabonome will be important to resolve.

GIARDIA BY ANY OTHER NAME…

G. lamblia is conventionally categorized into eight genotypically distinct assemblages, each with a restricted host range, with the majority of human *Giardia* infections due to assemblage A or B. Unlike the characteristics that distinguish the pathogenicity of some other enteric infections, no single marker ascribes virulence potential to clinical *Giardia* isolates. Despite clear differences in experimental models and in human volunteers, there remains no consistent delineation between assemblage infection type and symptoms [47]. An assemblage A human isolate strain (Human *Giardia* Invader, HGINV) was recently identified with mucosal invasive potential, but weight loss in an animal model was no greater than infection with a non-invasive and generally less virulent laboratory-passaged assemblage A strain [48*].

The two *Giardia* assemblages are genetically and phenotypically distinguishable [49], sharing less than 70% sequence identity across syntenic regions [50]. Within assemblage B

there is a consistently greater degree of genetic diversity than assemblage A [51, 52]. Additionally, there is ongoing population expansion of *Giardia* haplotype diversity across all continents [53]. Even within a single isolate, *Giardia* displays a high degree of allelic sequence heterozygosity [54]. Proteomics studies reveal that even within the less genetically heterogeneous assemblage A, specific differences in proteins correspond with variable gene families. Notably, differences in variant surface proteins among eight separate isolates aligned with two different profiles that were independent of host origin, sub-assemblage designation, or geographic region [55–57]. With an increasing reference database of different *Giardia* laboratory strains, use of optimized techniques to perform whole genome sequencing from clinical isolates may help identify strain-specific correlates of disease [58*, 59]. Applied to field studies, this additional resolution may help separate out the pathogenic signal from commensal noise.

CONCLUSION

In conclusion, *Giardia* has distinct population-dependent epidemiological patterns. The parasite can cause malabsorptive diarrhea as well as chronic intestinal sequelae when exposures are infrequent and occur later in life, but is at once seemingly inconsequential or even beneficial for diarrhea incidence while contributing to impaired intestinal permeability and growth attainment when infection occurs in early childhood and where exposures are frequent. To consider *Giardia* a commensal would therefore be inappropriate, however, a paucity of clearly defined factors to distinguish virulence strains limits our ability to confidently ascribe pathogenicity. In the meantime, the direct influence of host-immune, nutritional, and microbial factors on experimental giardiasis outcomes may better classify *Giardia* as a eukaryotic pathobiont in high-prevalence settings with varied pathogenic potential across unique isolates and which commonly exists symbiotically but behaves opportunistically when genetic and environmental perturbations allow. As such, defining and addressing the elusive burden of one of the most common intestinal parasitic infections in humans may require broad thinking about complex microbial-host interactions, and careful examination and characterization of long-term sequelae.

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(**highly recommended -- *recommended – references with*s will need annotation)

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KEY POINTS

- New field studies have helped characterize associations between *Giardia* and both diarrhea and growth in children living in high prevalence settings.
- Recent work in *Giardia* pathogenesis is helping reconcile possible mechanisms through which *Giardia* could protect against acute diarrhea, but simultaneously impair growth attainment in these children.
- Advances in comparative genomics may identify novel characteristics that better identify pathogenic *Giardia* strains, which in turn could help refine burden of disease estimates for this complex organism.