

EDITORIAL – INTESTINAL AMOEBAE AND BACTERIAL MICROBIOME INTERACTION

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INTERSPECIES MAKE-UP OF THE GUT MICROBIOME

The gut microbiota is a complex ecological system that surrounds the combined genomes and metabolic activities of distinct microbial ecosystems living in the gastrointestinal tract. Beyond the primary bacterial taxa such as *Firmicutes* and *Bacteroides*¹, which are critical in fermenting indigestible dietary fibers into short-chain fatty acids (SCFAs), the microbiome also entails viruses, fungi, archaea, and protozoa².

Bacteriophages, the predominant constituents of the gut virome, control bacterial population dynamics, affect and influence horizontal gene transfer, and contribute to resistance against colonization by pathogens³. The mycobiome, though numerically lesser, wields distinctive immunomodulatory effects, and dysregulation has been woven in conditions such as inflammatory bowel disease and neuropsychiatric disorders⁴. Furthermore, protozoa such as *Blastocystis* and *Entamoeba* species, long remarked as pathogenic, are progressively distinguished as commensal members of the microbiome that may improve bacterial variety and regulate immune responses⁵.

Conserving and maintaining eubiosis, or what is known as microbial balance, is significant for the health of the host, as it sustains nutrient metabolism, the integrity of the epithelial barrier, and immunological homeostasis⁶. Cross-kingdom relations widely serve as protection against opportunistic pathogens by promoting resistance to colonization and nurturing functional strength within the microbial ecosystem⁷.

Therefore, the gut microbiome is not just a bacterial consortium but rather a multidimensional ecosystem, in which bacteria, viruses, fungi, archaea, and protozoa perform mutually dependent and supplementary roles¹. Satisfying this complex equilibrium is vital for maintaining health, while its fear is increasingly acknowledged as a unifying emblem of chronic disease³.

IMPORTANCE OF THE MICROBIOME TO THE HOST

The gut microbiome plays a crucial role in host physiology, especially in nutrient processing and metabolism. It breaks down dietary components that cannot be digested, such as plant polysaccharides, into absorbable metabolites that support nutrition. It also regulates enteroendocrine cell activity and hormone release, which influences metabolism and maintains energy balance.



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In addition to its metabolic functions, the gut microbiome is key to the maturation and regulation of the immune system. It protects against invading pathogens while promoting disease tolerance. This helps lessen the severity of infections even when harmful microorganisms are present^{8,9}.

Furthermore, the gut microbiota helps produce important metabolites like short-chain fatty acids. These metabolites further shape immune responses and overall health. Its effects reach beyond the gut by facilitating communication along the gut, brain, and lung axes, which contributes to host defense, immune regulation, and overall physiological balance¹⁰⁻¹³.

AMOEBAE AS PART OF THE GUT MICROBIOME

Although microbiome research has traditionally focused on prokaryotic bacteria, one of the major groups that comprise the human gut microbiome is diverse, mostly motile, heterotrophic, unicellular eukaryotic microorganisms called the protozoa¹⁴.

Among these, intestinal amoebae portray an especially intriguing subset. Traditionally, *Entamoeba histolytica* (*Eh*) is considered the primary pathogenic amoeba, which is responsible for amoebiasis and manifests in symptoms extending from dysentery to life-threatening abscesses in the liver, mediated by its tissue-lytic virulence factors such as lectins and cysteine proteases¹⁵.

On the contrary, various related species, including *Entamoeba dispar*, *E. moshkovskii*, *E. hartmanni*, *E. coli*, *E. polecki*, *Enolimax nana*, and *Iodamoeba bütschlii*, are known to be non-pathogenic commensals that often appear in stool examinations and serve as indices of environmental hygiene rather than active disease¹⁶. As mentioned by Dubik et al¹⁵, commensal protozoa may contribute significantly to gut homeostasis through interactions with the bacterial microbiota and the host immune system.

In the colon, *Eh* interacts with multiple components that can modulate the course of tissue invasion and destruction. Contact between *Eh* and resident microbiota constitutes the beginning of the first host-parasite interaction that could potentially initiate disease. *Eh*, the outer mucus layer shares a niche rich in a diverse community of microbiota, directly interacting with them and benefiting from their presence.

The colonic microbiota degrades complex carbohydrates into glycans, which can serve as a nutrient source for *Eh*¹⁷. At the same time, as emphasized by Leon-Coria et al¹⁸ in 2020, the pathogenic parasite also feeds on the resident microbiota as its nutritional source. In recent years, researchers have used a metagenomic approach in human stool to identify and analyze bacteria consumed by *Eh*. The study of Iyer et al¹⁹ in 2019 has shown that *Eh* have higher affinity in phagocytosing specific bacterial groups, most notably *Lactobacillus ruminus*, among other members of *Lactobacillales*, *Erysipelotrichales*, *Clostridiales*, and *Bifidobacteriales*. This selective ingestion included *Lactobacillus ruminus*, a commensal and potentially probiotic species previously not known to associate with *E. histolytica*, highlighting the parasite's preference for certain healthy gut bacteria.

Another significant aspect involves interactions with biofilms, particularly extracellular digestion processes. *Entamoeba histolytica* can break down bacterial biofilms using secreted proteases. This process releases nutrients and modifies mucosal communities. Notably, contact with biofilms not only provides nourishment but also improves the resilience of amoebae. It protects the parasites from oxidative stress and influences their harmful potential²⁰. These findings suggest that the dynamics between biofilms and amoebae go beyond nutrition to include stress resistance and regulation of harmful effects.

Lastly, how bacteria influence amoebic virulence and behavior adds further complexity. Certain bacterial partners, which can be gut pathobionts or probiotics, affect amoebic traits, including damage to epithelial cells, movement, and resistance to oxidative stress. Recent studies²¹⁻²³, particularly those on *Eh* and enteropathogenic *Eh* (EPEC) interactions, show that gut bacteria can directly enhance virulence traits, while others might reduce harmful behaviors.

These findings highlight a mutual relationship where amoebae influence and are influenced by the gut microbiota. Through feeding, biofilm changes, and modulation of virulence, intestinal amoebae are not just isolated parasites. They actively participate in the microbial ecosystem, impacting host health and disease.

AMOEBAE AND DYSBIOSIS

Dysbiosis, or microbial imbalance – characterized by reduced microbial diversity, an increased abundance of pathobionts, and disruption of interkingdom homeostasis – has been consistently associated with a wide range of disorders, including metabolic syndrome, inflammatory bowel disease, cardiovascular diseases, and neurological dysfunction^{2,4}.

Recent studies in endemic regions have demonstrated that *Eh* infection significantly alters the composition of the resident gut microbiota^{19,24,25}. In Northern India, *Eh*-positive patients exhibited a dysbiotic profile characterized by reductions in *Bacteroides*, *Clostridium coccooides*, *Clostridium leptum*, *Lactobacillus*, *Campylobacter*, and *Eubacterium*, accompanied by an increase in *Bifidobacterium* species. Similarly, previous scholars²⁴ revealed that *Eh*-infected individuals displayed higher bacterial richness (alpha diversity) but reduced inter-individual variation (beta diversity), with enrichment of *Clostridiales* and *Ruminococcaceae*, alongside a depletion of *Prevotella copri* and *Fusobacteria*.

A longitudinal study in Bangladeshi children associated parasite-induced diarrhea with the expansion of *Prevotella copri*, a bacterium implicated in intestinal inflammation. In contrast, a study of patients with amebic liver abscess (ALA) could not establish direct bacterial associations with disease incidence²⁶; however, most ALA patients were co-infected with multiple bacterial taxa, particularly showing elevated *Klebsiella*. These findings suggest that while *Eh* induces antimicrobial peptide production, its resistance to their cytopathic effects may contribute to microbial shifts during infection. Nonetheless, whether such dysbiosis represents a cause or consequence of infection remains unresolved²⁶.

In parallel, research on other intestinal protozoa reveals additional insights. For instance, *Blastocystis* colonization was associated with increased bacterial richness and selective enrichment of *Prevotella*, particularly in children. By contrast, *Escherichia coli* colonization resulted in microbial profiles similar to those of uninfected controls, but with an enrichment of specific taxa such as *Akkermansia*, which was notably depleted in the *Blastocystis*-positive group²⁷.

ROLE OF THE MICROBIOTA IN IMMUNE RESPONSE AGAINST AMEBIC INFECTION

However, *Eh* interactions with the gut microbiota go well beyond simple predation. Certain gut bacteria modulate the parasite's resistance to oxidative stress and influence its virulence, suggesting a complex interplay that shapes infection outcomes²¹.

Infection with *Eh* induces notable shifts in gut microbial composition, often resulting in dysbiosis characterized by reduction of beneficial bacteria like *Bacteroides*, *Clostridium*, and *Lactobacillus* and enrichment of others like *Bifidobacterium*, highlighting that disease severity may hinge on both microbial and host factors.

Conversely, infection with *Eh* in germ-free animals did not result in disease; however, pathogenicity was restored once bacteria were introduced. Likewise, *in vitro* cultures of *Eh* maintained under axenic conditions showed reduced virulence, which was re-established following inoculation and incubation with live bacteria. More recently, it has been demonstrated that bacteria from the Enterobacteriaceae family stimulate *Eh* gene expression, which is linked to enhanced oxidative stress survival. This effect is not observed when *Eh* is co-cultured with a probiotic strain. While the precise mechanisms through which enteric bacteria enable *Eh* to express virulence-associated genes remain unclear, these findings highlight the intricate interactions between *Eh* and commensal microbes, potentially explaining why only about 10% of individuals infected with *Eh* develop intestinal amebiasis.

The broad spectrum of clinical outcomes following *Eh* exposure, from asymptomatic colonization to severe disease, cannot be fully attributed to host or parasite factors alone, indicating that the intestinal microbiome plays a pivotal role in determining susceptibility and disease severity. The gut microbiota may modulate parasite virulence, provide colonization resistance, drive dysbiosis, or influence host immune responses; however, the exact mechanisms and directionality of these interactions remain poorly defined. A deeper understanding of the tripartite relationship between *E. histolytica*, the microbiome, and host immunity could open new avenues for more effective strategies in the diagnosis, prevention, and management of amebiasis²⁸.

Our recent findings show that a dysbiotic state heightens host sensitivity, leading to exaggerated pro-inflammatory cytokine and chemokine production, as well as hypersecretory responses to *E. histolytica*¹⁸. This is clinically significant, as individuals with dysbiosis—whether due to disease, antibiotic use, or poor diet—are at greater risk of developing severe intestinal amebiasis characterized by acute inflammation, compared to those with a healthy microbiota. This has particular relevance in endemic regions and among vulnerable populations such as children, the elderly, and immunocompromised individuals, where antibiotics are frequently administered, often without prescription. Furthermore, our study demonstrated that in germ-free mice lacking commensal microbiota, normal immune responses to *E. histolytica* infection are profoundly impaired.

CONCLUSIONS

Amoebae, particularly *Entamoeba histolytica*, occupy a unique position within the gut microbiome as both consumers of and contributors to microbial dynamics. Far from being isolated parasites, they rely on resident bacteria for nutrient acquisition, stress resistance, and the expression of virulence traits, while simultaneously reshaping microbial communities through selective predation and biofilm disruption. Commensal amoebae and probiotic interactions highlight the potential for these organisms to support microbial diversity and modulate immune responses, in contrast to the pathogenic outcomes associated with dysbiosis. Collectively, these findings underscore that amoebae are not merely pathogenic invaders but active participants in the gut ecosystem. Recognizing their dual roles, as commensals and potential pathogens, provides deeper insight into the mechanisms of host-microbe interactions and offers opportunities to harness microbiome balance in preventing and managing amebiasis.

Conflict of Interests

All authors declare no conflict of interest.

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Authors' Contributions

All authors contributed to the conceptualization, data curation, and writing of the manuscript. A.P.G. Estocapio was responsible for the first section of the manuscript, and R.K.G. Novero for the second section. M.E. Baladad supervised the overall work, reviewed and edited the final version of the manuscript. All authors have read and approved the final manuscript.

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