***Review Article***

# Intestinal Microflora Vs Protozoan Parasites: From Interaction to Competition


## ABSTRACT

**Aims:** To reveal the interaction between intestinal microflora and the protozoan parasite organisms and how it affect their host's performance.
**Discussion:** The vertebrate gastro-intestinal system contain wide array of diverse but dynamic bacterial microbiota population that has ubiquitous consequences on its host's well-being including physiology, nutrition, metabolism, and immunity, locally and systematically. Naturally, these bacteria share their milleu with a more or less similar population of parasitic eukaryotes (e.g., protozoan, helminths, and fungi). Both eukaryotes parasites in combination with the prokaryotic microorganisms as inhabitant normal microflora can dynamically shift the bio-physics and immune milleu of the intestine (locally) or even can affect its host as a whole (systematically), creating abundant chances for them to interact to each other; where ideally, both side is in equilibrium state. Beside their function, intestinal normal (commensal) microflora mainly contribute in several activities that control parasite survival and determines the outcome of several, if not many, parasite-base disease. Normal microflora actively limiting the pathogenicity of many parasites. The steadiness among the number and composition of normal microflora and its host seems vital to the host's well-being perpetuation. But unfortunately, this interaction can further shifted into competition that can leads to the dominance of one party in number and probably also strength. Those spectrum of interactions may critically modify infection outcomes (active or dormant/carrier) and in turn affect the overall host condition. Active protozoan invasion may modify interaction between hosts and their normal resident microflora, either supporting or preventing against the condition of dysbiosis and inflammatory disease. Conversely, the microbiota controls parasite's settlement, multiplication, and even virulency; the properties that can modulate the interaction along the parasitism-mutualism sphere.
**Conclusion:** Intestinal microflora composition control the pathogenesis of the protozoan infections.



*Keywords: microflora, symbiont, parasitism, fungi, helminths, protozoans, probiotics, domination*


## 1. INTRODUCTION

Microorganisms interacts with each other and can be physically associated with another organisms in a variety of ways [1]. The microorganism–host or microorganism–microorganism interactions are central for the establishment of non-inhabitant organisms in various condition of environments [1,2]. Synergism between these organisms involve in all important life related ecological aspects, including bio-physico-chemical swapping, metabolite swapping-conversion-signaling, the process of chemotaxis and also genetic

swapping ensuing in genotype selection [3]. furthermore, the accomplishment of certain organism in a specific local milleu based on the diversity of the species, since high functional redundancy in the microbial community elevates the competitive ability of the community, lowering the possibility of an outsider-invader to exhibit themselves in certain milleu [4]. Although there may be internal changes on the host's side that facilitating competition [5].

Competition is a biological interaction between two or more organisms of the same or different species where the species compete with each other for different resources [6,7]. Most of the competitive interaction occurs for nutrition fullfilment [8]. This ultimate need of food sources usually occur in a limited supply when compared to demand, and this is the very basic reason of competition, host-microorganism or microorganism-microorganism and even host-microorganism-parasite [3,5].

The aim of this literature study is to review the spectrum of interaction between intestinal microflora vs parasitic organism and how this relation interfere with their host well being.

## 2. THE HUMAN INTESTINAL MICROBIOTA

Gastro-intestinal system of human constitutes a unique and sophisticated biosphere. It consist of large number of 'good' microorganism community that related with essential functions of the well being of their host's body [9]. The species abundance varies greatly between individuals, with each person containing an eccentric and dynamic cluster of microorganisms, which may fluctuate steadily [7-9].

Genetic factors, to some extent, facilitate the development of intestinal microbiota, although on the host's body [10,11]. Nowadays, the human body along with its 'passenger' normal microflora has been called a "superorganism" due to the reason there is widespead link between nutritional-metabolic-physiological activities that involving the synergism of various organs/systems [12,13].

In the context of intestine, the existence of numerous normal microflora reinforce their host with several crucial roles, namely in (1) First line of defense against microbial pathogens [2], (2) Facilitates digestion process [1,6], (3) Regulating host fat storage [9], (4) Contributes to maturation of the immune system [7], (5) Stimulating intestinal epithelium renewal [1,9], (6) vitamin synthesis-metabolism [15,16], (7) xenobiotics and drug metabolism [17,18].

The equilibrium state among the normal microflora and their human host is important in maintaining health and homeostasis, and the derangement of normal microflora, in number and or configuration, has been purported to be elaborated in a wide array of ailment formation [2,6,7,12,13,19]. Moreover, the commensal microflors devotes to the "barrier properties" of the gut epithelial, which lining the intestinal luminal surface and contributes primarily to protect their host, representing a real hindrance to pathogens assault [4,6,10].

Within this sophisticated framework, due to poor practice of hygiene, intestinal parasites from outer world sometime manage to enter and then reach its predilection site [1]. These parasites interrelate with the existing normal microflora population [3,5,6]. Those meeting can modify the equilibrium condition between normal gut microflora and their host. Each of them have the capability to metabolizes and modifies nutritive compound, interactively.

Resident normal microflora and its by-products may directly affect the survival and the fitness of many parasitic organism and, accordingly, with the clinical progression of many

type of parasitic illness [20]. At the same time, intestinal parasites, e.g., protozoans and helminths, also persistently produce micro-substance that may alter or modify local milleu where they exist; this may shift intestinal microflora, in number and or in compositions. Furthermore, this relation of parasite-normal microflora actually can facilitate the pathogenesis of severe disease in COVID-19; and this phenomenon has been linked to the condition of immune hyperactivation [21].

To some extent, native microflora of the intestine also able to extract and produce energy from nutritive metabolism that can be used for the benefit of the host; even though it is also beneficial to parasitic organisms, whenever they exist [22,23]. Beside vitamin biosynthesis, they involve in their host's macronutrient metabolism by the gut microbiome that affects their host's health by way producing metabolites that interfere with short-chain fatty acids and alcohols (mainly yielded from monosaccharides); ammonia, branched-chain fatty acids, amines, sulfur compounds, phenols, and indoles (derived from amino acids); glycerol and choline derivatives (obtained from the breakdown of lipids); and tertiary cycling of carbon dioxide and hydrogen. [16, 22]. Furthermore, local normal microbiota also involve in trypthopan (Trp) metabolism in the intestine, where the three main Trp metabolism pathways leading to the prodcution of serotonin (5-hydroxytryptamine), kynurenine (Kyn), and indole derivatives are under the control of the inhabitant microbiota, directly or indirectly [23]. It is therefore relevant to consider the intestinal specific milleu as an unique and controlled ecological community where dynamic and persistent bio-chemical interlinkages exist at copious organizational stages affecting the host - normal microbial populations - organisms that behave as parasites [1-3].

## 2.1 protozoans of human gastro-intestinal tract: its general characteristics

A wide array of protozoan organisms are frequently found in the human gastro-intestinal tract [20,21]. Actually, these organisms are not homogenous, or in other words, they cannot simply categorized in one group, because they come from different phylogenetic branches so that their morphologies and characteristics are also different [24]. Basically, their physiology and biochemistry properties are mostly customized to their parasitic properties which has been adapted to the local milleu where these parasites live [25]. They also exhibit different mechanisms of host invasion, some establish an infection through intracellular route (e.g., Cryptosporidium spp.) and some organisms developed a host specialization (e.g., Entamoeba histolytica), and to add complexity to invasion methods, many of them are also developed the ability to infect more than one host (e.g., Giardia lamblia) [26]. Von Huth et al [27] reported that intestinal protozoan infections directly shape fecal bacterial microbiota in children. These findings become an interesting subject for further exploration, for example regarding whether certain bacterial patterns are formed in certain protozoal infections, and whether the duration of parasitic infection then facilitates changes in the character of the microbiota from normal to opportunistic.

Clinical signs and symptoms of intestinal protozoan infections become apparent within one to three weeks post exposure [24,26]. Anatomically, few species of this intestinal protozoan actually caused gradual impairment to their predilection tissue; which if not handled properly can cause continously derangement and ends in a permanent damage. But luckily, what happens more often is a mild infection characterized by a spectrum of symptoms such as: diarrhea, nausea, tomach cramps, gas, greasy stool (because fat absorption is being blocked), and possible dehydration.

Among the lists of protozoans capable conducting intestinal infections, the species G. lamblia could constitute an excellent sample to shed the light on some intermediary related to their initial reciprocity with the intestinal microflora and how they establish themselves and share the same milleu [28]. This flagellate parasite is commonly infect human and also a wide array of animal [29]. The array of clinical signs and symptoms differ from a very minimal lesion, e.g., mild and self-limiting illness, to a more obvious acute or even chronic-persistent diarrhea and also weight depletion, lethargy with malabsorption that can be remain for several months [28,29]. According to kraft et al [30], intestinal protozoan infections are acquired via oral route, largely due to swallowing of cysts in adulterate drinking water. After entering the intestine, stage transformation took place, e.g., into the trophozoite stage, then this protozoans immediately adhere to the gut epithelium surface and start to colonize the duodenum and upper jejunum and in the end followed by the process of immediate replication, vegetatively. The consequences of Giardiasis may vary between individuals, from the condition of simply self-limiting to chronic, and also from the condition of asymptomatic to severe manifestations, with unspecific gastrointestinal complaints.

A condition of asymptomatic infection also widely reported, eventhough Kraft et al [30] proposed the possibility of 'false negative' due to minimum barrier that compromising activities of recent G. Lamblia isolates. The breakdown of intestinal barrier function is one of the proposed mechanism for intestinal protozoan pathogenesis. Analysis via the trans-epithelial electric resistances (TEER) or by indicators of epithelial permeability using labeled sugar compounds in in vitro cell culture systems, mouse models or human biopsies and epidemiological studies are type of studies conducted in order to support the previously mentioned mechanism of pathogenesis. Epithelial cell model infected with protozoan directly actually have the potency to be used as mimicking asymptomatic infection[30]. This perspective leads to the potency of using this model from just simply identification of Giardia virulence factors and shifted to exhibiting disease formation related to non-parasite factors. The underlying origin that determines variability in clinical sign and symptom are still not clear.

Several studies examined the process of invasion at the cellular level and how parasite products contributes to the tissue injury, locally. In the very early phase of invasion, the Giardia enzyme, e.g., cysteine proteinases, break the affected epithelial barrier, and which further arouses the host's inflammatory and immunological responses.31 Host with normal immune armamentarium can easily recognize the protozoans [32]; but when the immune failed, the protozoan inhabitation that parasitizing mucosal facet may aoruse innate immune armamentarium, e.g., toll-like receptors (TLRs) [32,33]. T cells (particularly involving CD8+ cells), macrophages, neutrophils, and antibodies (e.g., IgM, IgG, and IgA) are major components of the acquired immune armamentarium necessary for the battle against giardiasis [34,35].

### 2.2 Interaction between protozoan infection Vs. intestinal microflora

Intestinal microflora portray an additional factor that may strongly prevent the protozoan parasite infections[1,2]. Unfortunately, the actual reciprocity between the normal intestinal microflora and protozoan parasites are still not revealed clearly, yet.

In animal model, e.g., mouse, normal gut microflora was shown to actively reduce the velnerability to Cryptosporidium parvum invasion and changes in the microbiome of cryptosporidium-infected mice correlate to differences in its susceptibility and level of infection (e.g., mild-moderate-severe) [36]. In another animal study using goat, when C. parvum colonization took place, it reduced the affluence of butyrate-producing pathways in bacteria. Low grade of butyrate may stimulate mucosal inflammation and tissue restoration

[37]. This indicates that the intestinal inflammation induced by the protozoan C. parvum is related with the curtailment of butyrate-producing bacteria [38]. These findings strengthen our understanding about the existence and dominance of intestinal microflora that seems to be critical for the pathogenic pronouncement of several enteric protozoans such as Blastocystis hominis, E. histolytica, and different species of other enteric protozoan [39].

Human intestinal normal microflora populations are largely consist of not only bacteria, but also include viruses, fungi, protozoa and archaea, whose play an important role in the intestinal ecosystem. Humans that being colonized by Blastocystis hominid actually contain a more diverse bacterial microflora than individuals not carrying it [40]. The result suggests the beneficial contribution of harboring Blastocystis for the host {41]. There is contrasting microbiota profiles observed in children carrying either Blastocystis spp. or the commensal non-pathogenic amoebas Entamoeba coli or Endolimax nana with an expanded number and diversity-composition shifts in the bacterial microflora in children [42]. Blastocystis hominis that is more commonly isolated in industrialized community, which are otherwise mostly devoid of gut eukaryotes, on contrary among rural “traditional” society, which usually contain a greater diversity of intestinal eukaryotes (whether pathogenic or commensal) [43,44]. This interesting phenomenon must be carefully considered in order to study protozoa interactions in the gut ecosystem, based on their host’s location (rural vs urban).

Even et al [43] profiled the intestinal bacterial microflora of 134 healthy Cameroonian adults utilizing 16S rRNA gene amplicon sequencing data. The pattern of existence and occurence Entamoeba and Blastocystis in a single individuals were decisived using metagenomic shotgun data. When taking into consideration co-occurence of both protozoa simultaneously, Blastocystis was always related with both a higher richness and a higher likeness of the gut bacterial microbiota, while on contrary Entamoeba was associated only with a higher abundance. The existence of these parasitic protozoa affect the bacterial microflora diversity [9,45]. This interaction clearly contribute to the well being of their host [12,19,23]. The abundance of several customary taxa (e.g, Coprococcus, Ruminococcaceae, and Butyrivibrio) diversified due to Blastocystis colonization, but only a single Bacteroides amplicon sequence strain was found profusely between Entamoeba (-)and Entamoeba (+) samples. Based on the result of study conducted by Even et al [43], Blastocystis and Entamoeba have definite interaction with gut bacteria each in its own way.

### 2.3. Hypotheses regarding protozoan pathogenic stimulation by the resident normal microflora

Normal microflora evolves complex mechanisms to restrict pathogen growth, by way of (1) Preventing of attachment [46], (2) Competitive metabolic interactions [47], (3), Niche exclusion [48], (4) Nutrient competition [49] and (5) Induction of host immune response [50], which are collectively termed colonization resistance [51]. On the other hand, pathogens have also developed counterstrategies to expand their population and enhance their virulence to cope with the gut microbiota colonization resistance and cause infection [51,52].

One hypothesis is due to axenization of the parasites. Axenization means the process of isolating a particular organism from all others; in the context of purifying and making pure culture, axenization of certain organism allows concentrated groups to be studied and perhaps countable. In this scenario, the superficial saccharide ligands which located on the protozoan outermost membrane are changed by the attendance of intracellular bacterial symbionts. so the phenomenon seen in axenic protozoa that was being cured of their endosymbionts, resulted in a clear and viable decline in protozoan’s adhesive ability and or

invasive properties. Also in the case of Giardia, a study conducted using murine model revealed the ultrastructural scrutiny of G. muris disclosed endosymbiotic microbes which could be related to disparity in the parasite's stage pathogenicity, rate of infectivity, metabolism, antigenic surface profiles, and even determine host specificity. Based on TEM examination, the occurence of Giardia trophozoites harboring superficial bacterial endosymbionts was also confirmed [53]. only trophozoites which contain endosymbionts were destroyed when in close vicinity of the activated Paneth cells, endorsing the host's preservative role of the bacterial endosymbionts within Giardia trophozoites [54]. Those previously mentioned facts further supporting the idea that intestinal microflora may directly and indirectly affect the pathogenesis of giardiasis.

The second hypothesis come from the result of study conducted by Mirelman et al [55] using non-pathogenic E. histolytica strain. These researchers found out that axenisation of the host that took place at the intestinal level can be involved in the virulence expression of certain intestinal protozoan parasites[56]. Interactions of minor pathogenic amoebae with a variety of Gram-negative bacteria that occupy certain milleu of the intestine, mainly E. coli strains, may be in charge of the increase in amoebic virulence [57]. Galván-Moroyoqui et al. [58] demonstrated that phagocytosis of enteropathogenic bacteria strains (e.g., E. coli and Shigella dysenteriae) in vitro and co-cultured them together with E. histolytica and E. dispar; this mixture turns to multiply the cytopathic effect of E. histolytica and make them more virulent by way of increasing expression of Gal/GalNAc lectin on the amoebic surface and the cysteine proteinase activity, But for E. dispar continued avirulent.

In case of G. lamblia, several previous studies have proved that the normal intestinal microflora may arouse the pathogenic expression of this pathogen, but fortunately not the multiplication effort of parasites [59]. In a gnotobiotic animal model, Torres et al. (2000) apportioned evidence that the microorganism responsible for part of the invigoration of G. lamblia pathogenicity are exist dominantly in the duodenal [60] . Facultative and strictly anaerobic bacterial of the duodenal normal microflora were acquired from biopsy of several individuals with clinical diagnosis of giardiasis. These micro-organisms further challenged for their ability to arouse G. lamblia pathogenicity in animal model (gnotoxenic mice). By quantifying the number of cysts in faecal material and of trophozoites isolation from the small intestine was also accomplished. This approach aim to carefully analyse the protozoan multiplication ability in the different groups of mice. The result revealed that (1) Germ-free mice did not underwent any pathological alterations throughout the course of experimental Giardia infection; (2) Infected gnotoxenic mice exhibited intermediate pathological changes between the group of germ-free and the infected conventional mice group accustomed as controls; (3) No histo-pathological appearance were obtained in the non-infected gnotoxenic or conventional group of animals. As shown also for other intestinal pathogenic protozoans, bacterial sub-population from the intestinal microflora display stimulatory factors for Giardia pathogenicity only but must kept in mind not for protozoan multiplication; because the number of faecal cysts remained similar among the three different groups of mice during the course of experimental infection [60].

The need to reveal the role of parasitome and the metabolome of intestinal normal microflora during chronic-persistent parasitic infection and their relationship with the host's immunoregulatory mechanisms is urgent, because better understanding regarding this topics will help clinicians to improve their clinical management approach while taking care of their patients.

These finding strengthen our understanding about the role of resident normal microflora of the intestine that can stimulate the pathogenicity of some intestinal protozoan. Beside

normal microflora, probiotics have the potency to prevent evolution of some protozoan parasite; which will be discuss in the following section [61].

### 2.4 The potency of Probiotics against protozoan parasites

Probiotics inhibits the advancement of certain intestinal pathogens [61,62]. Probiotics also effective and efficient in the supportive management of gastrointestinal disorders [61,62], infection based respiratory disease [63], and allergic symptoms [64], and also can kill or inhibit or even kills strain-specific pathogens [65] through several mechanisms, namely (1) Competition [66], (2) Molecule secretion [67], and/or (3) Immune induction [62].

Configuration of the intestinal flora was likely involved in the highly variable manifestations in giardiasis in both humans and animals [68]. Pérez et al. [69] analysed the effect of several different probiotic bacteria (six Lactobacillus acidophilus strains, and Lactobacillus johnsonii La1) on G. lamblia strain, in vitro. The result showedus that only L. johnsonii La1 clearly stopped the multiplication of Giardia trophozoites. Data from in vivo experiment support the previously mentioned fact where protection against parasite-induced mucosal damage and a sufficient cellular feedback to Giardia antigens was stimulated in spleen cells from La1-treated animals, bring about a refinement of infection [70].

Furthermore due to an in vivo study using animal model, the addition of L. casei MTCC 1423 strain as well as E. faecium SF68 were both adequate in annihilating Giardia infection in probiotic-fed mice by reducing or avoiding attachment of Giardia trophozoites-mucosal surface and arousing an early humoral response [71].

Previously, the potency of several Lactobacillus species/strains to intercept and even to cure murine Giardia infection has also been reported [72]. In general, all showing the positive effect of the addition of L. casei to Giardia lamblia infected BALB/c mice; most studies corroborate the contribution of adding probiotics to susceptible host in order to minimize the length and severity of infection through the direct action of probiotics organisms on convalescence of the intestine, morphologically and physiologically 73]. Further study need to be conducted regarding the potency and the possibility of probiotics for their therapeutic use for humans.

## 3. CONCLUSION

Human as host, the resident intestinal microflora and the protozoan parasites are connected and interfere each other, and as the result build in a complex ecosystem where alterations in one member of these components may govern a counter response in the remaining ones. Normal microflora have the ability to prevent protozoan infection, and the addition of certain probiotics helps the host recover faster and better.

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