



1 **Title: Associations between gut microbiota and common luminal intestinal parasites**

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6

7 **Abstract (100–120 words)**

8 The development and integration of DNA-based methods in research and clinical microbiology
9 laboratories have enabled standardised and comprehensive detection and differentiation of the
10 microbes colonising our guts. For instance, the single-celled parasites *Blastocystis* and
11 *Dientamoeba* appear to be much more common than previously thought, especially so in healthy
12 individuals. While increasing evidence appears to suggest limited pathogenicity of these parasites,
13 next-generation sequencing-based studies have helped us appreciate links between parasite
14 colonisation and certain host phenotypical characteristics and gut microbial profiles. The
15 fundamental question remains as to whether such parasites are merely indicators or active
16 manipulators of gut microbiota structure and function. In this article, we collate existing evidence
17 that these parasites are, as a minimum, indicators of intestinal microbiota structure.

18

19 **Towards mapping of the human gut eukaryome**

20 An increasing body of evidence suggests links between gut microbiota composition and various
21 diseases [1-6]. Application of a holistic view of the structure and function of the gut microbiota

22 requires the inclusion of not only bacteria, but also parasites, fungi, archaea, viruses, and phages [7-
23 12]. To some extent corroborating the “**Old Friends hypothesis**” (see Glossary), Parfrey *et al.*
24 recently produced data exemplifying the “defaunation” of the human gut [13]. Here, diversity
25 patterns of intestinal eukaryotes were compared between individuals with a westernized lifestyle
26 (from the cities of Colorado and Philadelphia in USA) and individuals with an agrarian lifestyle
27 (from rural communities in Malawi). The authors observed that individuals with non-western diets
28 and lifestyles resemble non-human mammals in terms of micro-eukaryotic diversity, while
29 individuals with a western lifestyle have low levels of micro-eukaryotic diversity.

30 The introduction of real-time PCR and other DNA-based technologies including next-generation
31 sequencing in modern clinical microbiology laboratories and research laboratories for detection and
32 differentiation of intestinal parasites has opened up new avenues for exploring how parasites impact
33 our lives [14]. For instance, the high sensitivity of real-time PCR tests has helped us understand that
34 some parasites are—on an overall basis—much more common than previously anticipated, with
35 prevalence rates approaching 100% in some communities, even in developed countries. Examples
36 include *Blastocystis* and *Dientamoeba*, which appear to be more or less obligate eukaryotic
37 members of the gut microbiota in some populations [15-21], while less frequent in others, including
38 individuals with functional and organic bowel diseases and metabolic disorders [13, 22-25]. For
39 instance, we recently showed *Dientamoeba* to be a consistent finding in the stool from children in
40 childcare in Denmark [20], and similarly that *Blastocystis* is a frequent finding in children in
41 Nigeria, with prevalence increasing by age [18]. We have also shown that healthy individuals are
42 more likely to host these parasites than patients with irritable bowel syndrome (IBS) [25] and,
43 especially, inflammatory bowel disease (IBD) [22]. Moreover, it appears that *Blastocystis*,
44 *Dientamoeba* and *Entamoeba* are capable of long-term colonization of the human gut [15, 16, 20,
45 26]. For these parasitic genera, tools to differentiate colonization from infection are not available

46 [27-29]. It is also not unlikely that different genotypes or strains display different levels of virulence
47 [30-32]. More precise molecular diagnostics are therefore required to allow for better mapping of
48 strains/genotypes. However, in order to be able to distinguish clinically relevant strains from mere
49 colonizers, a better understanding of the microbial eukaryotic contribution to the human gut
50 ecosystem is probably required.

51 The above-mentioned types of advances have spurred an interest in mapping and exploring the gut
52 eukaryome [7-10], as well as investigating the interplay between gut parasites and gut bacteria.
53 Interestingly, parasitic genera such as *Blastocystis*, *Dientamoeba* and *Entamoeba*, which are all
54 considered luminal (i.e. non-invasive) intestinal parasites (except for *Entamoeba histolytica*,
55 colonization by which may be invasive), appear more commonly in healthy individuals than in
56 patients with metabolic, organic and functional gastrointestinal disorders [21, 23, 25, 33-36], which
57 has promoted the idea of some parasites being beneficial to the host rather than culprits of disease
58 [37]. Since vast differences in colonization rate are seen across age groups [17], health status [25],
59 and geographical regions [13], which might be the factors driving these differences? While
60 exposure to faecally contaminated matter is most likely one of these factors, another crucial factor
61 might be individual susceptibility to colonization. In this regard, susceptibility to parasite infection
62 may be linked to specific ecological conditions in the gut, including those that have to do with gut
63 microbiota. This line of thinking is supported by several animal experimental studies that have
64 provided evidence of **probiotics** preventing or modulating parasite infection [38]. In fact, the gut
65 microbiota may not only be driving the susceptibility to, but also the outcome of, parasite infection,
66 as suggested by Berrilli *et al.* [39]. Moreover, differences in microbiota signatures, i.e., differences
67 in microbiota taxa, be it on the species, genus, family or even phylum level, may reflect the severity
68 of parasite infections.

69 With the current opportunities for exhaustive gut microbiota profiling using next-generation
70 sequencing, an important step towards fine-tuning our clinical and public health understanding of
71 colonization by intestinal parasites is to study these parasites in relation to their ecological niche,
72 including relationships with gut microbiota. Such steps are already being taken [40]; however, it is
73 also important to develop hypotheses that might explain these relationships.

74

75 **Evidence of links between common intestinal parasites and gut bacterial communities**

76 Over the past few years, specific gut microbiota patterns have been shown to be linked to
77 colonization with common parasitic protists (Table 1). Especially, the relationship between
78 *Blastocystis* and gut bacteria has been a popular research focus [15, 23, 35, 41-47]. In 2011,
79 Arumugam *et al.* launched the concept of **enterotypes** of the human gut microbiome [48].
80 Analysing the gut microbiota of healthy and diseased individuals across nations, they observed a
81 clustering of individuals into one of three microbiota patterns, the so-called “enterotypes”. Each of
82 these three enterotypes were identifiable by the variation in the levels of one of three bacterial
83 genera: *Bacteroides*, *Prevotella*, and *Ruminococcus*. In the study by Arumugam *et al.*, only bacterial
84 data were communicated; no breakdown of the eukaryotic components of the gut microbiota was
85 provided. To mitigate this and taking a retrospective approach to studying the data produced by
86 Arumugam and colleagues, Andersen *et al.* [35] not only identified the prevalence and subtype
87 distribution of *Blastocystis* in various cohorts of healthy and diseased individuals, but also explored
88 links between *Blastocystis* and gut bacteria. They found that *Blastocystis* carriage was significantly
89 less common in individuals with a *Bacteroides*-driven enterotype than in those with a
90 *Ruminococcus*- or *Prevotella*-driven enterotype. They also found that *Blastocystis* colonization was
91 associated with higher bacterial richness (number of individual bacterial taxa) and lower body mass

92 index (BMI) (findings summarized in [28]). A somewhat similar approach was recently taken by
93 Beghini *et al.* [23], who found a strong link between *Blastocystis*, the Archaean
94 *Methanobrevibacter smithii* and several bacterial species across 12 metagenomic datasets.
95 Moreover, similar to observations made by Andersen *et al.* (2015), an inverse relationship was
96 identified between *Blastocystis* carriage and BMI, showing that *Blastocystis* colonization is
97 inversely associated with increasing BMI.

98 To validate the findings by Andersen *et al.* in 2015 [35], members of the same team took to
99 analyzing another set of faecal samples using real-time PCR technology [43]. Again, *Blastocystis*
100 was investigated in relation to major groups of bacteria using a modified **Gut Low-Density Array**
101 (**GULDA**)-approach and *Dientamoeba* was included in the study as well as a “control parasite”.
102 Both *Dientamoeba* and *Blastocystis* were studied in relation to the six bacterial taxa *Bacteroides*,
103 *Prevotella*, the butyrate-producing clostridial clusters IV and XIVa, the mucin-degrading
104 *Akkermansia muciniphila*, and the indigenous group of *Bifidobacterium*, and it was observed that
105 carriers of *Blastocystis* alone or along with *Dientamoeba fragilis* typically had gut microbiota
106 characterized by low relative abundances of *Bacteroides* and clostridial cluster XIVa and high
107 levels of *Prevotella*. Hence, colonization with *Blastocystis* was again linked to a low relative
108 abundance of *Bacteroides*. In fact, when comparing parasite-negative with parasite-positive
109 samples, the relative abundance of *Bacteroides* in the parasite-negative samples was significantly
110 higher compared with parasite-positive samples ($P < 0.001$) [43].

111 Audebert and colleagues performed a cross-sectional study including 48 *Blastocystis*-positive and
112 48 *Blastocystis*-negative patients and performed 16S rDNA sequencing to map *Blastocystis*-
113 associated gut microbiota, identifying higher bacterial diversity in the faecal microbiota of
114 *Blastocystis*-colonized patients, a higher abundance of clostridia, and a lower abundance of
115 Enterobacteriaceae [41]. Earlier on, however, Nourrisson and colleagues had suggested that

116 *Blastocystis* might be linked to microbiota imbalance, observing that, compared with controls,
117 levels of “gut-protective” *Faecalibacterium prausnitzii* were decreased in *Blastocystis*-colonised
118 males and that levels of *Bifidobacterium* sp. were decreased in males with irritable bowel syndrome
119 (IBS) type C [44].

120 In a study comparing the microbiota in individuals with *Giardia*, *Blastocystis*, and *Entamoeba*,
121 **dysbiosis**, as evidenced by a low *F. prausnitzii-Escherichia coli* ratio, was identified in individuals
122 with *Giardia*, while those with *Blastocystis* and *Entamoeba* appeared to be characterized by
123 **eubiosis**, characterized primarily by a high *F. prausnitzii-E. coli* ratio [47].

124 Studying the gut microbiota of African rural populations, Morton and colleagues showed that
125 across populations, intestinal colonisation by the genus *Entamoeba* could be predicted with 79%
126 accuracy based on the composition of an individual's gut microbiota [49]. They moreover observed
127 that several of the taxa critical to distinguishing the absence or presence of *Entamoeba* are in fact
128 signature taxa for autoimmune disorders such as Crohn's Disease. While this appears to suggest that
129 *Entamoeba* is a surrogate marker for gut microbial communities protecting against gut
130 inflammatory diseases, it is not clear whether the parasite *per se* might also play a role in the
131 protection against this and other autoimmune conditions, bringing into memory the idea of the Old
132 Friends hypothesis.

133 Microbial profiling studies have indicated that the gut microbiota of patients with IBD differ from
134 that of healthy individuals [50]. In patients with IBD, a decrease in strict anaerobic bacteria and a
135 shift towards facultative anaerobes such as members of the family Enterobacteriaceae have been
136 suggested to reflect disruption of anaerobiosis, indicating a role for oxygen in intestinal dysbiosis
137 [53, 54]. Although it was known that oxygen concentrations increase in a disturbed gut ecosystem,
138 it was only recently that the molecular mechanism was elucidated [55]. In a healthy gut, bacteria

139 produce butyrate [56], which is the preferred metabolic substrate of colonocytes [57]. Butyrate is
140 used by colonic epithelial cells in the beta-oxidation pathway and yields ATP. This pathway uses
141 molecular oxygen and thereby reduces the oxygen concentration in the intestine. In addition, the
142 produced butyrate is sensed via a human nuclear receptor and subsequently represses the inducible
143 nitric oxide synthase [55], which leads to a reduction of nitrate [58]. This all leads to a reduction in
144 the proliferation of facultative anaerobes [55]. Butyrate is actually required to activate oxidative
145 metabolism [59]. A shift away, caused by antibiotics for example, from obligate anaerobic bacteria
146 from the Firmicutes and Bacteroides phyla towards members of the Enterobacteriaceae disrupts the
147 host's butyrate-linked control mechanism [55] and represses oxidative metabolism [59], increasing
148 luminal oxygen in the human gut. This might partly explain why parasites such as *Blastocystis* are
149 rare in patients with intestinal dysbiosis-linked diseases: *Blastocystis* being a strict anaerobe [60], it
150 is expected that its ability to establish or maintain itself in a micro-aerophilic environment is limited
151 (Figure 1).

152 Taking all these studies into account, it appears that colonization by some intestinal parasites can be
153 predicted with quite a high degree of accuracy merely by studying the composition of gut bacteria
154 [23, 49], and the negative association between *Blastocystis* colonization and levels of *Bacteroides*
155 shown by independent research groups [23, 35, 43] adds further support to this hypothesis. A
156 possible explanation for the negative correlation between *Blastocystis* and *Bacteroides* might be that
157 the latter, unlike the *Firmicutes*, generally are not the main butyrate producers in the gut [56] and
158 therefore contribute to a lesser extent to an environment where *Blastocystis* thrives.

159

160 **Associations between microbial signatures and parasite pathogenicity**

161 A few examples of links between microbes and parasite pathogenicity have been published. One
162 example is the study by Gilchrist and colleagues, who observed that high parasite burden coupled
163 with increased levels of *Prevotella copri* was linked to symptomatic infection with *Entamoeba*
164 *histolytica* in Bangladeshi children [61]. Recently, members of the same group noticed that
165 dysbiosis induced by antibiotic treatment increased the severity of amebic colitis and delayed
166 clearance of *E. histolytica* in an amoebic colitis mouse model [62]. Other examples of links between
167 *E. histolytica* virulence and gut microbiota were recently reviewed by Burgess and Petri [63].
168 Similarly, *Giardia intestinalis*, the most common waterborne cause of diarrhea, was also found to
169 be associated with a perturbed intestinal microbiota in a mouse model system. *Giardia* infection
170 was linked to an increase of facultatively and strictly aerobic bacteria [64]. However, an increase of
171 Enterobacteriaceae normally seen in dysbiosis [54] was not seen in infections with *Giardia*, and
172 strict aerobes belonging to the beta-proteobacteria increased instead suggesting that parasite-linked
173 dysbiosis can lead to different microbiota compositions. Another example demonstrating differences
174 in gut microbial diversity is found in helminth infections [65, 66]. Individuals infected with
175 helminths showed a greater microbial diversity compared to individuals who were not infected. As
176 the populations studied were indigenous Malaysians, their gut flora was not immediately
177 comparable to other intestinal microbial diversity studies that generally focus on individuals
178 consuming Western diets [13, 66]. Interestingly, in an experimental system, introduction of a
179 benign tapeworm into a rat model did not lead to an increase of bacterial alpha diversity [67]. On
180 the other hand, the gut microbiota of mosquitos did contribute to killing of the host when infected
181 with a pathogenic fungus [68], demonstrating that the presence of gut microbiota does not
182 automatically protect its host from a disastrous outcome after an infectious challenge.
183 In many other environments, microbial eukaryotes play an important ecological role as bacterial
184 grazers [69-71]. Nutrient cycling and bacterial protein turnover are important ecological roles

185 played by microbial eukaryotes, observed in e.g. intestinal systems such as the rumen of large
186 herbivores [72]. Intestinal pathogens such as *Entamoeba* are also known to feed on bacteria, and
187 perhaps where evidence of a causative link between disease and the presence of a given parasite is
188 weak, microbial eukaryotes found in the intestine might have roles in the local food web by
189 providing or recycling nutrients. Especially in cases where long-term colonization has been
190 observed [15, 16, 20, 26], it might be prudent to consider alternative roles for microbial eukaryotes
191 in the human gut other than causing disease; eradication of organisms traditionally considered
192 pathogens might not always be advisable, bringing into mind again the “Old Friends” hypothesis
193 [73].

194

195 **How can these links be explored in further detail?**

196 There are several ways in which to explore the above-mentioned links further. Some could involve
197 studies of the parasite itself (including studies of the ecological requirements of the parasites
198 [nutrition, oxygen tension, etc.] through genomic, transcriptomic and metabolomic analyses).
199 Others could involve exhaustive microbiota surveys and experimental studies. Conclusions based
200 on cross-sectional data, which are the data mostly available at present, could be confirmed by using
201 longitudinal data (cohort studies) on individuals losing or gaining parasites to look for simultaneous
202 changes in gut microbiota structure or function, by **16S analysis** and **PICRUSt** analysis,
203 respectively, or by the analysis of metagenomics data. Moreover, *in vitro* and *in vivo* models could
204 be developed for testing the susceptibility to infection in similar hosts with different gut microbiota
205 established by selective use of, for instance, pre- and probiotics, antibiotics, and/or co-infections. *In*
206 *vitro* studies could take advantage of the fact that *Blastocystis* is one of the few parasites that are
207 easily grown in the lab and could therefore make use of cultures where culture-negative stool is

208 grown in culture medium (e.g., Jones' medium [74]) and later spiked with *Blastocystis*, keeping
209 some cultures unspiked as controls. Longitudinal sampling before and after the spiking event could
210 be used for studies of the relative stability of the faecal microbiota during *Blastocystis* colonization,
211 and to explore changes in microbiota due to *Blastocystis* colonisation.

212 One of the major limitations to developing integrative studies of the entire gut microbiota and the
213 effect it exerts on human health and disease is the lack of genomic data on gut parasites. No matter
214 whether metagenomics or amplicon-based methods be used for exhaustive detection and
215 differentiation of gut parasites, problems arise when DNA is annotated to the species—or even
216 genus—level. Some parasites exhibit remarkable genetic diversity, and while genomic data for e.g.
217 *Blastocystis* and *Dientamoeba* is appearing in publicly available databases [75-80], a couple of
218 nuclear ribosomal rDNA sequences is the only available genome data for other intestinal parasites
219 such as *Iodamoeba* and *Endolimax* [81-83] and also for even more common parasites such as
220 *Entamoeba coli* [84]. Therefore, in order to optimize mapping of sequenced parasite DNA, genomic
221 data on such parasites should be made available. Moreover, several common intestinal parasites
222 exhibit remarkably extensive genetic diversity, and the research looking into associations between
223 various genotypes/subtypes/strains and eubiosis/dysbiosis remains limited. Finally, few studies have
224 taken into account colonization by fungi such as yeasts (*Candida*, *Saccharomyces*, etc.), and there is
225 hardly any qualitative or quantitative information on inter-fungal relationships and relationships
226 between fungi and other microbial inhabitants of the gut.

227

228 **Perspectives**

229 Probiotics have been used to prevent but also treat protozoal infections, exemplified in a recent
230 review by Vitetta *et al.* [38], and so future investigations may benefit from looking deeper into how

231 administration of probiotics might interfere with parasite colonization. In this context, it would also
232 appear relevant to mention **faecal microbiota transplantation (FMT)**, which has gained a lot in
233 popularity over the recent years, especially for the treatment of recurrent *Clostridium difficile*
234 infection [85, 86]. Future use of FMT might include alleviation and/or treatment of other diseases,
235 including IBD, type 2 diabetes, metabolic disease, and maybe even neuropsychiatric diseases [87,
236 88]. The presence and effect of e.g. *Blastocystis* and *Dientamoeba* in donor stool is largely
237 overlooked. There seems to be lack of consensus as to which organisms should rule out donors;
238 examples of *Blastocystis* and *Dientamoeba* colonization being obstacles to accepting FMT donors
239 exist [89, 90]; meanwhile, other recommendations do not include these parasites in the panel of
240 pathogens disqualifying donors [91]. Nevertheless, if i) high microbiota diversity is linked to
241 parasite colonization, ii) high microbiota diversity is an attractive asset of FMT donor stool, and iii),
242 parasite colonization is linked to gut health-promoting bacterial communities, future investigations
243 should look into the effect of co-administering common intestinal parasites to patients with
244 conditions for which FMT is used, and also into whether in fact these parasites can be transplanted
245 from a donor to a recipient using FMT.

246

247 **Concluding Remarks**

248 While we still know little about the potential pathogenicity of common luminal intestinal parasites,
249 epidemiological data strongly suggest that at least some of these parasites are linked to
250 gastrointestinal health rather than disease. Based on profiling of gut bacterial communities, we are
251 learning to predict whether or not an individual is colonized with intestinal parasites. This article
252 has provided some hypotheses and ideas for studies that could help us detail and strengthen our
253 knowledge regarding associations between parasites and gut microbiota, with a view to exploring

254 the importance of these parasites in public health and clinical settings and their potential as gut
255 microbiota and overall health indicators and regulators.

256

257

258

259 **Figure legend**

260 **Figure 1. Dynamic interplay between gut flora and human colonocytes.** In a healthy gut,
261 fermentation by obligate anaerobic bacteria and *Blastocystis* results in increased short chain fatty
262 acid production. Butyrate is the preferred metabolic substrate of colonocytes and used in
263 mitochondrial β -oxidation producing ATP, resulting in a decrease of available oxygen. In addition,
264 butyrate is sensed by the nuclear PPAK γ receptor, which suppresses iNOS and thereby nitrate in the
265 intestinal lumen. Butyrate enhances mitochondrial oxidative phosphorylation. Overall, this results
266 in a hypoxic intestinal lumen, which supports a microbiota dominated by health-enhancing
267 microbes and which favours continued colonisation by *Blastocystis*. In dysbiosis, the increase in
268 facultative anaerobes such as Enterobacteriaceae results in a decrease of available butyrate. As a
269 result, colonocyte metabolism shifts away from β -oxidation and oxidative phosphorylation to
270 increased glycolysis and therefore does not reduce the oxygen concentration. The nuclear PPAK γ
271 receptor no longer suppresses iNOS, resulting ultimately in increased nitrate in the gut that can be
272 used by the dysbiotic flora as an electron acceptor to support their growth.

273

274

275

276 **GLOSSARY**

277 **Probiotics:** Live micro-organisms, which, when administered in adequate amounts, confer a health
278 benefit on the host.

279 **Dysbiosis/Eubiosis:** The human gut is colonized primarily by species belonging to the following
280 phyla: Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Actinobacteria and Fusobacteria
281 [92, 93]. To give a distinct definition of dysbiosis and eubiosis is not straightforward, but has
282 nevertheless been attempted: A gut microbiota in a eubiotic state is characterized by a
283 preponderance of potentially beneficial species, belonging mainly to the two bacterial phylum
284 Firmicutes and Bacteroides, while potentially pathogenic species, such as those belonging to the
285 phylum Proteobacteria (Enterobacteriaceae) are present, but in a very low percentage. In the case of
286 dysbiosis, “good bacteria” no longer control the “bad bacteria”, which take over [92]. While the use
287 of this definition might be pragmatic in some situations, it should be noted that a clear dichotomy
288 between beneficial and pathogenic species is unlikely to exist. Secondly, this definition considers
289 beneficial taxa to be mostly Firmicutes and Bacteroides, while pathogenic species would be mostly
290 Proteobacteria; obviously, the situation is more complex. For instance, changes in the
291 Firmicutes/Bacteroides ratio, as for example observed in obese individuals [94], also appears to be
292 associated with dysbiosis. Less concrete and more holistic definitions are given by Miniello *et al.*,
293 “Beyond the microbial richness and diversity, a ‘eubiotic’ gut microbiota is characterized by the
294 presence of the microbes that enhance metabolism, resilience to inflammation, resistance to
295 autoimmunity. Dysbiosis is considered as an alteration in microbiota community structure and/or
296 function, capable of causing/driving a detrimental distortion of microbe-host homeostasis.” [95]

297 **16S analysis:** Also referred to by some as “metagenomic” analysis. In fact, 16S analysis usually
298 refers to the procedure where genomic DNA from a given sample is subject to broad-specificity

299 amplification of bacterial small subunit ribosomal DNA, sequenced by a next-generation
300 sequencing method, and annotated to a taxonomic level by the use of online databases.

301 **Enterotype:** In a study by Arumugam *et al.* [48], application of multidimensional cluster analysis
302 and principal component analysis to fecal metagenomes revealed three distinct clusters, the so-
303 called enterotypes, each of which is characterized by a dominance in abundance of one of three
304 bacterial genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus*
305 (enterotype 3).

306 **Gut Low-Density Array (GULDA):** is a validated, high-throughput real-time quantitative PCR-
307 based analysis platform developed for simultaneous analysis of differences in abundance of 31
308 different microbial 16S gene targets in faecal samples.

309 **‘Old Friends’ hypothesis:** Development of the immune system requires input from at least three
310 sources collectively referred to as the ‘old friends’: (i) the commensal microbiotas transmitted by
311 mothers and other family members; (ii) organisms from the natural environment that modulate and
312 diversify the commensal microbiotas; and (iii) the ‘old’ infections that could persist in small
313 isolated hunter-gatherer groups as relatively harmless subclinical infections or carrier states. These
314 categories of organisms have to be tolerated and hence play a role in the development and
315 regulation of the immune system [96].

316 **PICRUSt analysis:** Phylogenetic Investigation of Communities by Reconstruction of Unobserved
317 States (PICRUSt) is a bioinformatics software package designed to predict metagenome functional
318 content from marker gene (e.g., 16S rRNA) surveys and full genomes
319 (<http://picrust.github.io/picrust/>).

320 **Faecal microbiota transplantation (FMT):** is a procedure in which faecal matter, or stool, is
321 collected from a donor, mixed with a saline or other solution, strained, and infused in a patient

322 (recipient) by, for instance, colonoscopy or an orogastric tube with a view to restoring a healthy
323 intestinal microbiota.

324

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Table 1. Examples of studies on associations between intestinal parasites (protists only) and gut microbial communities.

Method	Parasite	Association(s) observed	Reference
Metagenomics	<i>Blastocystis</i>	Inverse association between body mass index and <i>Blastocystis</i> and strong co-occurrence with archaeal organisms (<i>Methanobrevibacter smithii</i>) and several bacterial species. Negative association between <i>Blastocystis</i> and levels of <i>Bacteroides</i> .	[23]
Real-time PCR	<i>Blastocystis</i>	Linked to eubiosis; significantly higher <i>Faecalibacterium prausnitzii-Escherichia coli</i> ratio in <i>Blastocystis</i> -positive than in <i>Giardia</i> -positive individuals.	[47]
Real-time PCR	<i>Entamoeba</i>	Linked to eubiosis; significantly higher <i>Faecalibacterium prausnitzii-Escherichia coli</i> ratio in <i>Entamoeba</i> -positive than in <i>Giardia</i> -positive individuals.	[47]
Real-time PCR	<i>Giardia</i>	Linked to dysbiosis; significantly lower <i>Faecalibacterium prausnitzii-Escherichia coli</i> ratio in <i>Giardia</i> -positive than in <i>Entamoeba</i> - and <i>Blastocystis</i> -positive individuals.	[47]
Metagenomics	<i>Blastocystis</i>	<i>Blastocystis</i> found in individuals with <i>Prevotella</i> and <i>Ruminococcus</i> enterotypes and not in those with <i>Bacteroides</i> enterotype; <i>Blastocystis</i> colonisation linked to higher bacterial richness.	[35]
Real-time PCR	<i>Blastocystis</i>	Low relative abundances of <i>Bacteroides</i> and clostridial cluster XIVa and high levels of <i>Prevotella</i> in <i>Blastocystis</i> -positive individuals.	[43]
Amplicon-based next-generation sequencing	<i>Entamoeba</i>	Relative abundance of the phyla Cyanobacteria, Elusimicrobia, Euryarchaeota, Firmicutes, Spirochaetes, Tenericutes all higher in <i>Entamoeba</i> -positive individuals. The relative abundance of the phylum Bacteroidetes lower in <i>Entamoeba</i> -positive individuals.	[49]
Amplicon-based next-generation sequencing	<i>Blastocystis</i>	Higher bacterial diversity in the faecal microbiota of <i>Blastocystis</i> -positive patients, a higher abundance of Clostridia, and a lower abundance of Enterobacteriaceae.	[41]
Real-time PCR	<i>Blastocystis</i>	Levels of “gut protective” <i>Faecalibacterium prausnitzii</i> were decreased in <i>Blastocystis</i> -colonised males and levels of <i>Bifidobacterium</i> sp. were decreased in males with IBS type C.	[44]
Amplicon-based next-generation sequencing	<i>Blastocystis</i>	<i>Blastocystis</i> -positive individuals had increased bacterial diversity, but no significant difference in fungal diversity was observed.	[12]

Metagenomics	<i>Blastocystis</i>	The relative abundance of bacteria belonging to <i>Bacteroides</i> was lower in <i>Blastocystis</i> carriers compared with non-carriers, and <i>Blastocystis</i> carriage was also associated with significantly higher bacterial richness.	[97]
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OUTSTANDING QUESTIONS BOX

1. Can current evidence of links between intestinal parasites and microbiota diversity from cross-sectional studies be confirmed and further investigated by data from longitudinal studies?
2. What is the relationship between common parasites and fungi colonizing the intestinal tract in terms of abundance and diversity? Similarly, are intestinal eukaryotic microbial communities found in people consuming non-western diets a potential avenue to exploring how to prevent western diseases?
3. Can hypotheses be generated on links between parasites and certain bacterial taxa, and can *in vivo* and *in vitro* models be developed and included in prospective studies to provide supportive evidence of such links?
4. Are intestinal parasites merely indicators of particular gut microbiota profiles or are they modulators, actively impacting the structure and function of gut microbiota?
5. The answer to 4) will also answer the question as to whether intestinal protists are interesting mainly from a public health view point or also from a clinical/therapeutic view point.
6. Are bacteria driving not only the ability of parasites to establish but also their virulence?

