

Review

Biome profiling and clinical parasitology: tools for gut microbiota restoring

Stefania Pane^{1§}, Maria Vittoria Ristori^{1,2,§}, Simone Gardini³, Alessandra Russo¹, Federica Del Chierico², Lorenza Putignani^{1,2,*}

¹ Microbiology and Diagnostic Immunology Unit, Unit of Microbiomic, Bambino Gesù Children's Hospital, IRCCS, 00146 Rome, Italy; stefania.pane@opbg.net (S.P.); mariavittoria.ristori@opbg.net (M.V.R.); alessandra.russo@opbg.net (A.R.); lorenza.putignani@opbg.net (L.P.)

² Multimodal Laboratory Medicine Research Area, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, 00146 Rome, Italy; mariavittoria.ristori@opbg.net (M.V.R.); federica.delchierico@opbg.net (F.D.C.); lorenza.putignani@opbg.net (L.P.)

³ GenomeUp, Via Nimorense 91, 00199 Rome, Italy; simone@genomeup.com (S.G.)

§ Co-first authorship

* Correspondence: lorenza.putignani@opbg.net; Tel.: +39-0668592598 (ext. 4076)

Abstract: A growing body of evidences is showing that dysbiotic gut microbiota may correlate with a wide range of disorders; hence, the clinical use of microbiota maps and fecal microbiota transplantation (FMT) can be exploited in the clinic of some infectious diseases. Through direct or indirect ecological and functional competition, FMT may stimulate decolonization of pathogens or opportunistic pathogens, modulating immune response and colonic inflammation, and restoring intestinal homeostasis, with reduction of host damage. Herein, we discuss how diagnostic parasitology may contribute to design clinical metagenomics pipelines and FMT programs, especially in pediatric subjects.

The consequences of a more specialized diagnostics in the context of gut microbiota communities may improve the clinical parasitology and extend its applications to the prevention and treatment of several communicable and even noncommunicable disorders.

Keywords: parasites; microbiota; faecal microbiota transplantation (FMT)

Introduction

The intestinal environment is an ecosystem where biological and chemical interactions occur at various organizational levels between host, parasites, and microbial communities, greatly affecting human health and physiology.

Considering the gut microbiota at taxonomic level, we can observe a significant variation among individuals, each harboring a unique collection of bacterial species, which may change over time and could be considered such a fingerprint [1–3].

The microbiota at level of the gut microenvironment provides important protective, immune regulatory and metabolic functions. The defensive mechanism against pathogenic bacteria is exerted by the barrier effect of the intestinal epithelium, playing a major role in protecting the host, and really representing an important obstacle to pathogens invasion [4,5].

Moreover, environmental factors, such as age, diet, stress, drugs, strongly influence the composition of the human microbiota [6–9].

The balance of the gut microbial ecosystem, eubiosis, is an important concept. An eubiotic gut microbiota is characterized by a preponderance of potentially beneficial species, belonging mainly to the two bacterial phylum *Firmicutes* and *Bacteroides*, while potentially pathogenic species, such as *Proteobacteria*. In the dysbiosis state, “bad bacteria” predominate “good bacteria” [10–12].

Within this complex scenario, intestinal citizens (e.g., virus, mycetes and parasites) interact with the microbial community modifying the balance between host and gut microbiota [4,13].

The intestinal microenvironment considered as a whole community provides an important protective, mucosal defense mechanism, but there are evidence that change in the composition of the commensal microbiota alters the gut environment making this composition vulnerable to pathogenic organisms [14–16]. Many factors such as antibiotics, psychological stress, physical stress, modern diet and hygiene can affect the microbial stability, and thus, contribute to the intestinal dysbiosis [11]. Indeed, dysbiosis may be harmful for the host, leading to inflammation and mucosal tissue damage that predispose to pathological conditions as in the case of *Clostridium difficile* infections [17].

2. Diseases and gut related microbiota profiling

C. difficile is an infectious Gram-positive spore forming bacillus microorganism of the gastrointestinal tract, and its toxin expression causes gastrointestinal illness with a wide spectrum of severity, ranging from mild diarrhea to pseudomembranous colitis, toxic megacolon, sepsis and death [18,19]. *C. difficile* is considered a member of the normal gut microbiota, however its growth is suppressed by other more dominant anaerobes [17]. The gut colonization of *C. difficile* is reversely related with host age, growing in early infancy and decreasing in adulthood, and it depends on the loss of the commensal microbiota barrier effect following, for example, antimicrobial therapies. In this context, *C. difficile* infection is responsible for initiation of the cascade of inflammatory processes which may play an important and destructive role in initiation and perpetuation of intestinal inflammation [17].

Patients with recurrent *C. difficile* infections are characterized by almost monomicrobial bacterial distributions of the fecal microbiota [20]. A decrease in bacterial diversity and a strong variation in global distribution of operational taxonomic units (OTUs) are, indeed, registered in the fecal microbiota profiling of adult patients presenting with *C. difficile* infections [20]. In particular, a statistically significant increase of Firmicutes and a decrease of Bacteroidetes phyla, compared to healthy subjects or controls (CTRLs) are observed, as well as an increase in Clostridiaceae and Erysipelotrichaceae families (**Figure 1**). Remarkably, in microbiota profiles associated to infectious agents, the dysbiosis index (DI) is usually very high, based on the prevalence of few microbial taxa, as in the case of the maps reported for *C. difficile* infection, for which observed dysbiosis values actually range in very high value intervals (DI>35%) (Lorenza Putignani and Antonio Gasbarrini, oral communication, 24°Congresso Nazionale delle Malattie Digestive, Rome, FISMAD March 23, 2018) (Metagenomic Method for the in vitro diagnosis of intestinal dysbiosis, PTC/IT2017/000119 Patent).

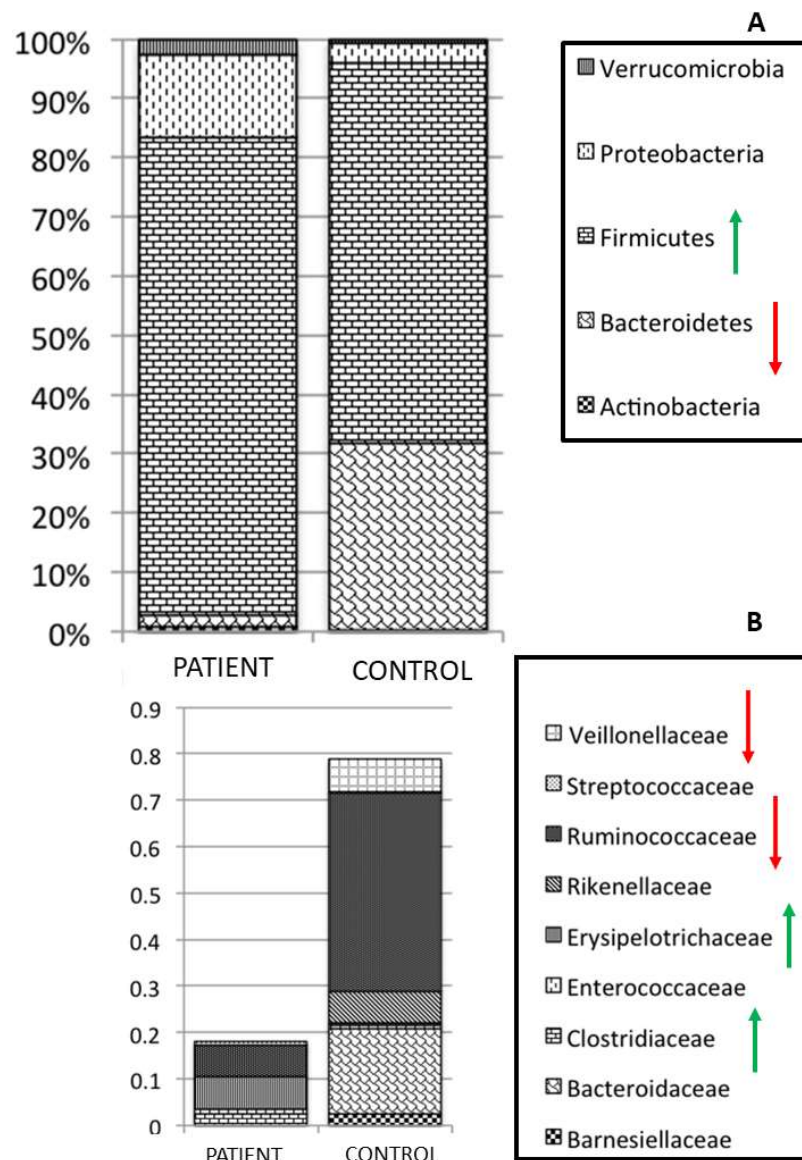


Figure 1. Faecal microbiota map of a patient affected by a *Clostridium difficile* infection. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling.

Another powerful microbial marker associated to different diseases is *Akkermansia muciniphila*. *A. muciniphila* is not a specific disease-related marker, but can be found across disease profiling and depending on host age; it is generally depleted in faecal samples from subjects suffering from a variety of diseases, including obesity, metabolic syndrome, diabetes, cancer, generally characterized by high levels of inflammation [21,22].

The reduction of *A. muciniphila* following antibiotic treatment has been associated to a reduction in the expression of *Muc2* gene encoding the major mucin of the colonic mucus in colonic tissues, as well as reduced mucolysis [23]. Indeed, the analysis of mucosae showed *A. muciniphila* to be abundant in biopsies of healthy subjects but reduced in those of patients with inflammatory bowel disease (IBD), who in contrast, had increased amounts of *Ruminococcus* [24].

The turnover of mucus (synthesis and shedding) [25] plays a crucial role by spatially protecting the intestinal cells of the intestinal barrier by bacteria flow coming from the luminal content, activity reinforced in some cases by the production of secretory immunoglobulin A (sIgA), and secretion of antimicrobial peptides and proteins [26]. The depletion of *A. muciniphila* is observed, as reported above, in several diseases and our group has thoroughly worked on its role in Nonalcoholic Fatty Liver Disease (NAFLD) and liver disease progression, such as non-alcoholic steatohepatitis (NASH) [11,27,28] (Figure 2) and in lung cancer (Figure 3). [29,30]

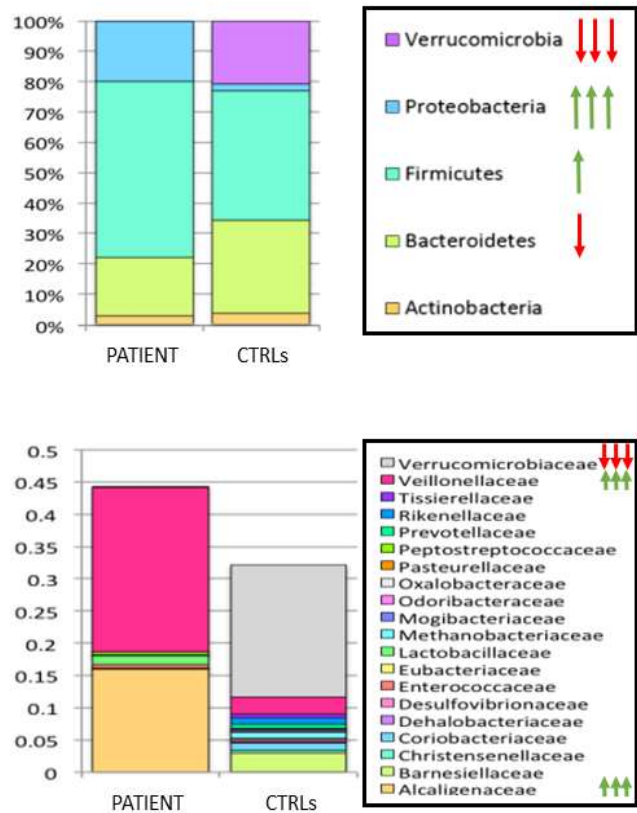


Figure 2. Faecal microbiota map of a patient affected by non-alcoholic steatohepatitis (NASH) and severe insulin resistance. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling. The map is associated to a 27% DI.

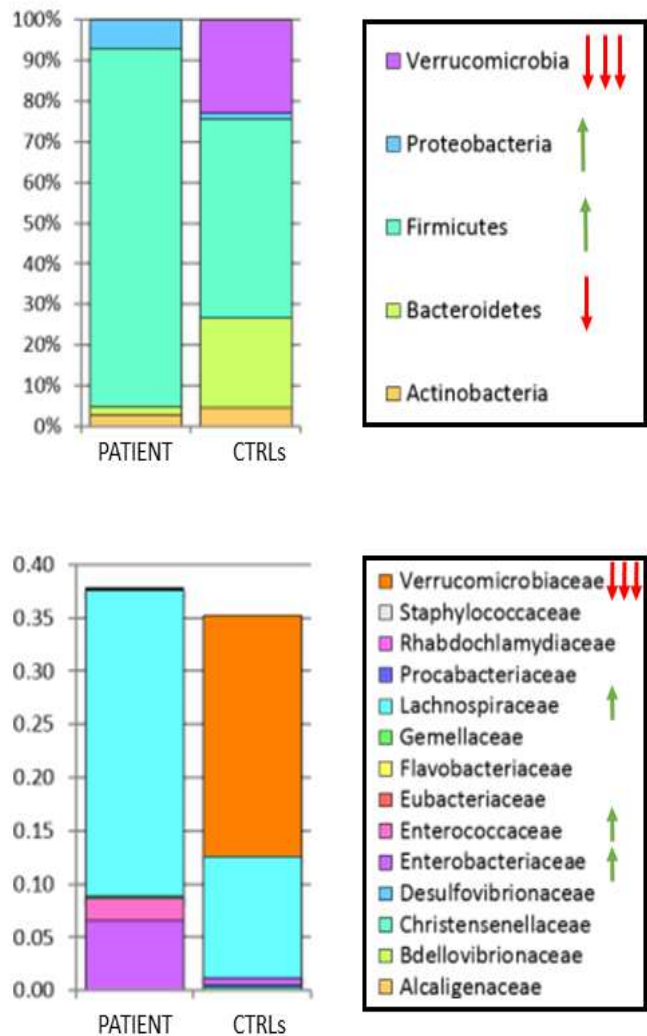


Figure 3. Faecal microbiota map of a patient affected by non-small cell lung cancer undergoing therapy with Nivolumab. Panel A and B describe global distribution of microbiota map at Phylum and Family taxonomic levels. Red and green arrows, refer to decreased and increased, respectively, microbial signatures associated to the disease-driven profiling.

Indeed, the idea to modulate *A. muciniphila* as remodeling intervention for gut dysbiosis or as tool to potentiate chemotherapy or immunotherapy [31] are under the magnifying class of the current advanced research [32]. However, the interplay between bacterial biomarkers, their metabolic and immunological functions and other components of the gut microbiota is the next challenging aim of the translational and clinical studies on microbial communities.

3. Role and Relationship amongst gut microbiota citizens

In addition to bacteria, other key microorganisms, such as yeasts and filamentous fungi, viruses, phages, are present in the gut [33,34]; moreover, for ~25% of the world's population, the gut microbiota also comprises intestinal protozoan and worms, namely meiofauna [35,36].

The diversity of meiofauna living on or in our bodies is associated to all metazoans with dimensions between 30 μm and 1 mm. Many members of the meiofauna significantly affect morbidity and mortality, including fungi (e.g., *Candida*, *Aspergillus*), unicellular protozoa (e.g., *Giardia*, *Entamoeba*), and helminthic worms (e.g., *Ascaris*) (**Figure 4**) [37].

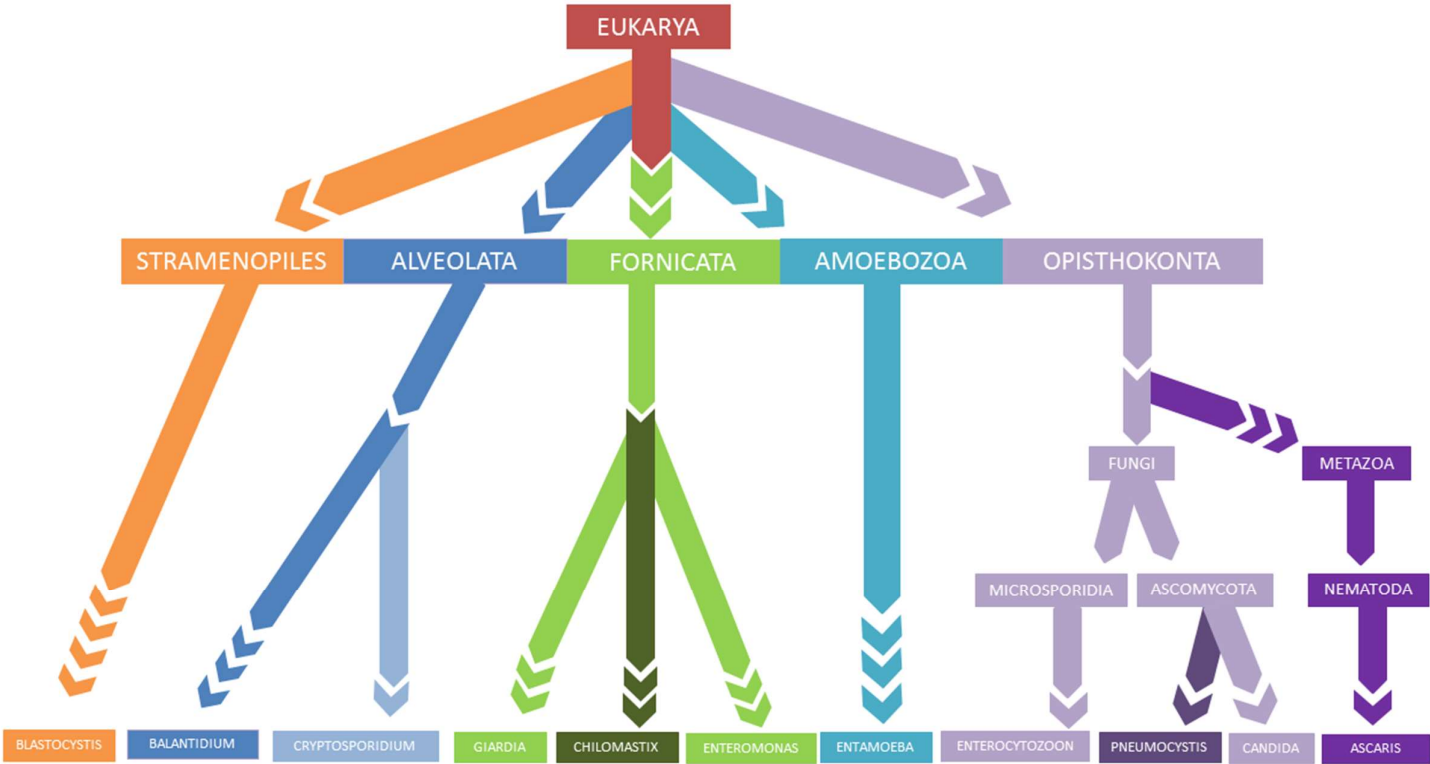


Figure 4. Simplified taxonomic overview of the meiofauna of the human GI tract.



Sequencing technologies are allowing to analyze the global diversity of the meiofauna of the human gastrointestinal tract to intimately demonstrating that commensal gastrointestinal meiofauna may be important in promoting health or disease. Several studies indicate that diet can influence the proportions of the meiofauna and that there is the possibility of trans-kingdom interactions in the gastrointestinal tract [37].

All these microorganisms offer additional dimensions to the investigation of host-microorganisms' and microorganisms-microorganisms' interactions; interplays that can be exploited only by combined models obtained by metagenomics, metabolomics and metaproteomics armonization and integration [38], aiming at producing decision support systems for disease stratification in medicine [39].

All microbial inhabitants may have both beneficial and detrimental roles in the human health, including improvement of microbial resilience, immune evasion, maintenance of physiologic processes, but even alteration of microbial communities [40]. Bacteriophages in the gut are largely unexplored, despite their potential to regulate bacterial communities and thus human health [41,42]; indeed experimental limitations still affect successfully isolation of phages and genome annotation as well as the full characterization of virus human populations [43]. Some studies have highlighted the uniqueness of phage communities in individuals and their capacity to be stable in the healthy gut [44]. Remarkably, the stability of the viral genome is probably responsible for the stableness of bacteria and microbiota metagenomes [45]. However, still there are only few studies carried out on the role of the virome in the host's intestinal microbiota ecosystem [46].

Fungi are normal inhabitants of the mammalian gastrointestinal tract. In fact, the human gut is colonized by more than 50 genera of fungi [47]. In particular, the gut is characterized by the presence of *Candida*, *Saccharomyces* and *Cladosporium* species. Nutritional modification may have an effect on the fungal microbiota; in particular, plant-based diets increase level of *Candida*, whereas animal-based diets improve the presence of *Penicillium* species [48,49]. Indeed, fungi compose a very small portion of gut microbiota, but play determinative roles in homeostasis of the gut bacterial composition and the mucosal immune responses. An interkingdom correlation between bacteria and fungi has been suggested. Alterations in the composition and function of the gut microbiota are a usual event in patients who suffer from IBD. Although the main reason for this alteration is not clear, the interaction between gut bacteria and gut fungi seems to be an important subject in IBD patients [50].

Intestinal parasites, both protozoans and metazoan (helminths and cestoda), interact with the microbial community modifying the balance between host and gut microbiota [51] (Figure 5).

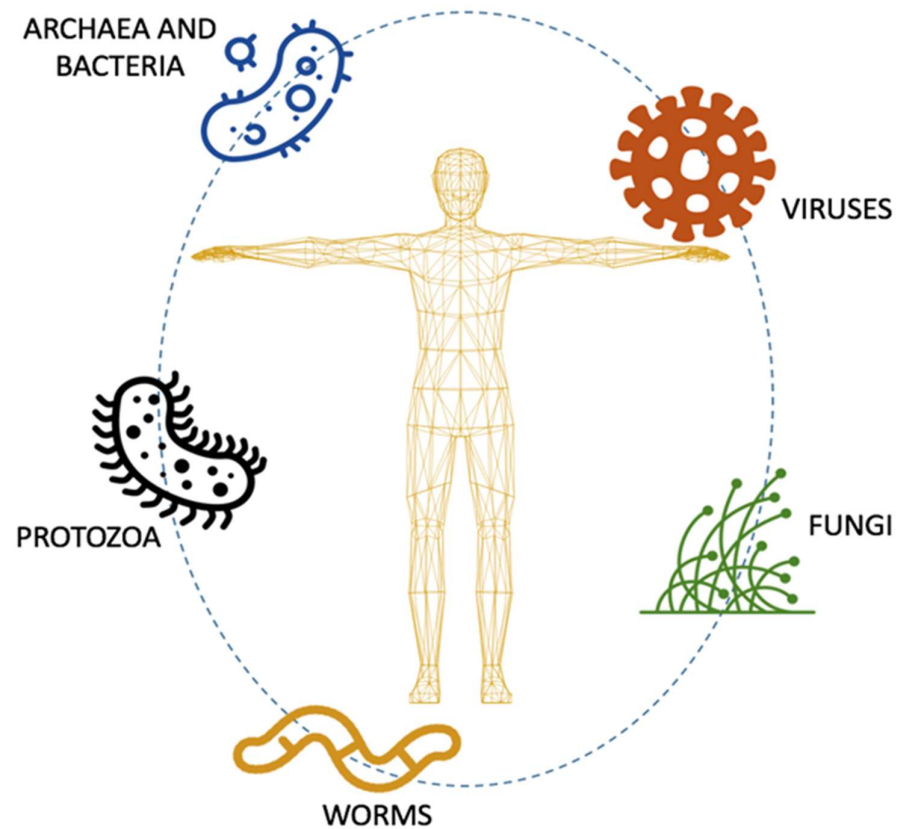


Figure 5. Human gut biome characterized by bacteriome, virome and meiofauna.

The microbiota of host may strongly interfere with the survival and the physiology of many parasites and, consequently, with the outcome of many parasitic infections. A wide range of protozoans are common parasites of the human gastro-intestinal tract (e.g., *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia duodenalis*). The spectrum of clinical manifestations of protozoan infections varies from a chronic diarrhea or weight loss or mild self-limiting illness to acute disease, until malabsorption [36,52,53]. Since the composition of the intestinal bacterial population affects the impact of the infection of protozoans, the modulation of different components of the microbiota could be used to prevent or attenuate intestinal protozoan infection and ultimate outcome of parasitic disease.

4. Parasites and gut microbiota profiling

Parasitic infections represent a significant health problem, particularly in underdeveloped and developing countries. Soil-transmitted helminths (STHs), e.g. *Strongyloides stercoralis* and *Trichuris trichiura*, are usual intestinal parasites, followed by blood flukes (*Schistosoma* spp.) and filarial worms (e.g., *Wuchereria* and *Brugia*). Generally spoken, intestinal parasitic infections have a low-impact in healthy subject; however, they can become a potential major issue in vulnerable groups. In fact, helminths infection produce malnutrition, physical damage and cognitive development in children [54].

In fact, together with trillions of microorganisms, i.e., archaea, viruses, bacteria, and eukaryotes residing in the GI tract, parasitic worms establish the “macrobiota” [55]. During parasites infections, the interaction between the gut microbiota and the helminths, has been associated with the establishment of the infection, the clinical manifestations and even the immune-modulation [56,57].

Experiments led in murine models of intestinal schistosomiasis have suggested the interactions between *Schistosoma* parasites and the host gut microbiota with a direct impact on the intestinal microbial communities [58]. In a study of Alba Cortés *et al.* the gut microbiome composition of the host actually influenced the host susceptibility to *S. mansoni* infection, as well as infection-associated changes in gut microbiota profiles [58]. Some studies have evidenced that *Necator americanus* infection could alleviate chronic inflammation in celiac disease and improve prokaryotic species richness, reestablishing the eubiosis and immune homeostasis [59]. In a case report on *Strongiloides stercoralis* infection, gut microbiota composition triggered an enrichment in *Bifidobacterium*, *Blautia*, *Ruminococcus*, *Bacteroides*, *Corynebacterium*, *Colinsella*, *Streptococcus*, *Coprococcus*, and *Oscillospora* genera, and a decrease in *Staphylococcus*, *Lactobacillus*, and *Pediococcus*. The authors suggested a putative direct or immune-mediated ability of *S. stercoralis* to promote the increase in bacterial diversity [60]. A review of the literature tried to deepen the mutual influences of intestinal nematodes and host-gut microbiota, highlighting the potential benefits effects (*i.e.* promotion of eubiosis) through the production of useful metabolites (*i.e.* short-chain fatty acids, SCFA). In other way, nematodes' infection promote dysbiosis due to promotion of pathogenic bacterial species and decrease of mutualistic commensal. Authors exemplified the nematode-microbiota interactions and their impact on the host immune response [61].

A shotgun metagenomics study on samples of patients with the *Blastocystis* spp. infections, founded a very strong association between the presence of *Blastocystis* spp. and the abundance of archaeal organisms (*Methanobrevibacter smithii*) [62]. Additionally, another study showed a decrease of *Blastocystis* in individuals with the *Bacteroides* enterotype compared to subjects with the *Ruminococcus* or *Prevotella* enterotypes [63].

In a rat model of *Blastocystis* ST3 infection, the colonization altered gut microbiota composition, but not richness, inducing only mild gut inflammation but no clinical symptoms. In addition, the long-term *Blastocystis* exposure appeared to promote a faster recovery from colitis, suggesting that *Blastocystis* may alter the gut ecosystem in a protective way and promote faster recovery [64]. On the opposite, in healthy subjects, the fermentation by anaerobic bacteria and *Blastocystis* induced an increased SCFA production [65].

In the January 2019 started the Parasite Microbiome Project (PMP) to understand the role of parasite-associated gut microbiota on the pathophysiology of helminthiasis [66]. The PMP tried to draw best practices for experimental studies to ensure reliable comparisons between data sets and the introduction of appropriate controls to identify possible environmental microbial contaminants [66]. In a recent review, the authors suggested four elements, which must be considered when the scientist wants to generate reliable and reproducible data [55]. In a first point, they propose to generate an appropriate negative controls ("blanks") in each step of the experiment, followed by the microscopy-based visualization of helminth-associated bacteria, to identify and characterize worm microbiomes across different helminth tissues and developmental stages. Remarkably is the identification of the "core microbiome" in helminth tissues *versus* transiently associated bacteria, in a way to depict the specific function of the only helminth-associated microbiome. These best practices are becoming more and more important, because clinical metagenomic next-generation sequencing (mNGS) is rapidly moving from research to clinical laboratories. Indeed, Chiu *et al.*, focused on the challenges of implementing mNGS in the clinical laboratory and to address potential solutions for maximizing its impact on patient care and public health. Clinical applications of metagenomic sequencing include direct identification of microorganisms from primary clinical samples, antimicrobial resistance prediction by characterization of resistance genes, detection of species-level or strain-level virulence determinants, and antiviral resistance prediction. Furthermore, by means of NGS techniques it is now possible to analyze the onset and progression of infectious diseases in acute and chronic stages [67]. The current challenge will become the agnostic approach through the complete characterization of enteric microbial communities, shallow complete metagenomics and trans-kingdom complete metagenomics (Figure 6).

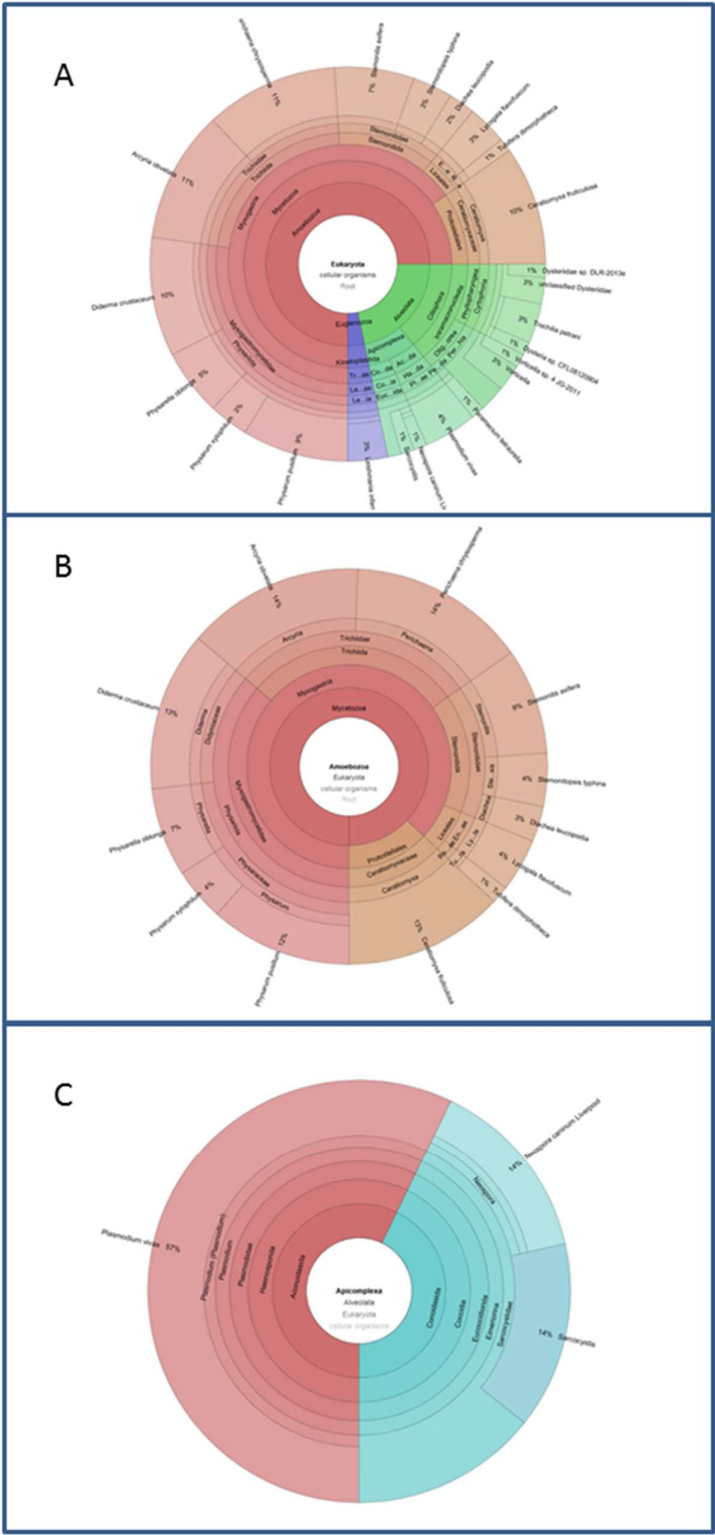


Figure 6. An example of gut parasite kronos graphs obtained by applying agnostic meta-genomics pipelines. Panel A: parasite superkingdom; Panel B: parasite, no rank; Panel C: parasite phylum.

5. Parasites and fecal microbiota transplantation (FMT)

FMT has a major role to manage important diseases such as *C. difficile* infections. In these cases, the transplantation is followed by a re-establishment of diversity; and, in many cases, the percentage of efficacy has been greater than 90% [68]. FMT is considered as the "ultimate probiotics" because it directly changes the intestinal microbial composition of the host, thus restoring eubiosis and intestinal homeostasis. FMT donor screening is a key factor in the safety of the procedure in order to prevent iatrogenic infectious diseases potentially transmittable to the recipient [69]. In fact, the international consensus on stool banking for FMT established that donor stool must be tested for protozoa and helminths, including *Blastocystis hominis*, *Dientamoeba fragilis*, *G. duodenalis*, *Cryptosporidium* spp., *Isospora* and *Microsporidia* [70]. In addition, the donor must be negative for blood nematodes test (i.e. *S. stercoralis*) [70] and a particular attention is necessary for parasitological screening of donors when recipients are children [71]. Remarkably, the fecal material (i.e., emulsion) can be stored frozen in a stool bank for use when needed [71].

Indeed, FMT requires a combination of expertise and appropriate methods, including clinical parasitology, to identify the best donors.

6. Materials and Methods

A literature review was conducted to analyze the role and effects induced by microorganisms (e.g., bacteria, viruses, fungi, worms and protozoa) on the microbial communities of the gastrointestinal tract both in conditions of eubiosis and dysbiosis. The research was conducted on PubMed and using the following terms: "virus" or "fungi" or "worms" or "protozoa" or "diet" or "nutritional status" or "microbiota" or "microbiome" or "dysbiosis" or "eubiosis" and "gastrointestinal symptoms". All articles providing sufficient information about the relationship between the gut microbiota, NGS, clinical parasitology, FMT. The inclusion criteria for study were as follows: (1) observational prospective and retrospective studies, case-control studies, cohort studies or systemic review; (2) study investigating gut microbiota profiles and parasites infection; (3) studies written in English. All the studies that did not fall in the following criteria were excluded from the reviewing process.

7. Conclusions

The consequences of a more specialized diagnostics in the context of the characterization of gut microbiota communities may improve the clinical parasitology and extend its applications to the prevention and treatment of several communicable and even non communicable disorders.

Author Contributions: Conceptualization, S.P., M.V.R., L.P.; resources, L.P.; data curation, A.R., F.D.C., S.G., S.P., M.V.R.; writing—original draft preparation, S.P., M.V.R. and L.P.; writing—review and editing, S.P., M.V.R., F.D.C. and L.P. All authors have read and agreed to the published version of the manuscript.

Funding: Italian Ministry of Health: Ricerca Corrente 2020 and 2021, assigned to LP.

Conflicts of Interest: "The authors declare no conflict of interest."

References

1. Bäckhed, F.; Ley, R.E.; Sonnenburg, J.L.; Peterson, D.A.; Gordon, J.I. Host-Bacterial Mutualism in the Human Intestine. *Science* **2005**, *307*, 1915–1920, doi:10.1126/science.1104816.
2. Eckburg, P.B.; Bik, E.M.; Bernstein, C.N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S.R.; Nelson, K.E.; Relman, D.A. Diversity of the Human Intestinal Microbial Flora. *Science* **2005**, *308*, 1635–1638, doi:10.1126/science.1110591.
3. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; et al. A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing. *Nature* **2010**, *464*, 59–65, doi:10.1038/nature08821.

4. Bancroft, A.J.; Hayes, K.S.; Grencis, R.K. Life on the Edge: The Balance between Macrofauna, Microflora and Host Immunity. *Trends Parasitol* **2012**, *28*, 93–98, doi:10.1016/j.pt.2011.12.001.
5. Mazmanian, S.K.; Liu, C.H.; Tzianabos, A.O.; Kasper, D.L. An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System. *Cell* **2005**, *122*, 107–118, doi:10.1016/j.cell.2005.05.007.
6. Putignani, L.; Del Chierico, F.; Petrucca, A.; Vernocchi, P.; Dallapiccola, B. The Human Gut Microbiota: A Dynamic Interplay with the Host from Birth to Senescence Settled during Childhood. *Pediatr Res* **2014**, *76*, 2–10, doi:10.1038/pr.2014.49.
7. Del Chierico, F.; Vernocchi, P.; Bonizzi, L.; Carsetti, R.; Castellazzi, A.M.; Dallapiccola, B.; de Vos, W.; Guerzoni, M.E.; Manco, M.; Marsegli, G.L.; et al. Early-Life Gut Microbiota under Physiological and Pathological Conditions: The Central Role of Combined Meta-Omics-Based Approaches. *J Proteomics* **2012**, *75*, 4580–4587, doi:10.1016/j.jprot.2012.02.018.
8. Rawls, J.F.; Mahowald, M.A.; Ley, R.E.; Gordon, J.I. Reciprocal Gut Microbiota Transplants from Zebrafish and Mice to Germ-Free Recipients Reveal Host Habitat Selection. *Cell* **2006**, *127*, 423–433, doi:10.1016/j.cell.2006.08.043.
9. Putignani, L.; Dallapiccola, B. Foodomics as Part of the Host-Microbiota-Exposome Interplay. *J Proteomics* **2016**, *147*, 3–20, doi:10.1016/j.jprot.2016.04.033.
10. Zhang, Y.-J.; Li, S.; Gan, R.-Y.; Zhou, T.; Xu, D.-P.; Li, H.-B. Impacts of Gut Bacteria on Human Health and Diseases. *Int J Mol Sci* **2015**, *16*, 7493–7519, doi:10.3390/ijms16047493.
11. Putignani, L.; Del Chierico, F.; Vernocchi, P.; Cicala, M.; Cucchiara, S.; Dallapiccola, B.; Dysbiotrack Study Group. Gut Microbiota Dysbiosis as Risk and Premorbid Factors of IBD and IBS Along the Childhood-Adulthood Transition. *Inflamm Bowel Dis* **2016**, *22*, 487–504, doi:10.1097/MIB.0000000000000602.
12. Mariat, D.; Firmesse, O.; Levenez, F.; Guimarães, V.; Sokol, H.; Doré, J.; Corthier, G.; Furet, J.-P. The Firmicutes/Bacteroidetes Ratio of the Human Microbiota Changes with Age. *BMC Microbiol* **2009**, *9*, 123, doi:10.1186/1471-2180-9-123.
13. Nicholson, J.K.; Holmes, E.; Lindon, J.C.; Wilson, I.D. The Challenges of Modeling Mammalian Biocomplexity. *Nat Biotechnol* **2004**, *22*, 1268–1274, doi:10.1038/nbt1015.
14. Manichanh, C.; Rigottier-Gois, L.; Bonnaud, E.; Gloux, K.; Pelletier, E.; Frangeul, L.; Nalin, R.; Jarrin, C.; Chardon, P.; Marteau, P.; et al. Reduced Diversity of Faecal Microbiota in Crohn's Disease Revealed by a Metagenomic Approach. *Gut* **2006**, *55*, 205–211, doi:10.1136/gut.2005.073817.
15. Darfeuille-Michaud, A.; Boudeau, J.; Bulois, P.; Neut, C.; Glasser, A.-L.; Barnich, N.; Bringer, M.-A.; Swidsinski, A.; Beaugerie, L.; Colombel, J.-F. High Prevalence of Adherent-Invasive Escherichia Coli Associated with Ileal Mucosa in Crohn's Disease. *Gastroenterology* **2004**, *127*, 412–421, doi:10.1053/j.gastro.2004.04.061.
16. Swidsinski, A.; Ladhoff, A.; Pernthaler, A.; Swidsinski, S.; Loening-Baucke, V.; Ortner, M.; Weber, J.; Hoffmann, U.; Schreiber, S.; Dietel, M.; et al. Mucosal Flora in Inflammatory Bowel Disease. *Gastroenterology* **2002**, *122*, 44–54, doi:10.1053/gast.2002.30294.
17. Smits, W.K.; Lyras, D.; Lacy, D.B.; Wilcox, M.H.; Kuijper, E.J. Clostridium Difficile Infection. *Nat Rev Dis Primers* **2016**, *2*, 16020, doi:10.1038/nrdp.2016.20.
18. Dobson, G.; Hickey, C.; Trinder, J. Clostridium Difficile Colitis Causing Toxic Megacolon, Severe Sepsis and Multiple Organ Dysfunction Syndrome. *Intensive Care Med* **2003**, *29*, 1030, doi:10.1007/s00134-003-1754-7.
19. Mylonakis, E.; Ryan, E.T.; Calderwood, S.B. Clostridium Difficile--Associated Diarrhea: A Review. *Arch Intern Med* **2001**, *161*, 525–533, doi:10.1001/archinte.161.4.525.

20. Chang, J.Y.; Antonopoulos, D.A.; Kalra, A.; Tonelli, A.; Khalife, W.T.; Schmidt, T.M.; Young, V.B. Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium Difficile-Associated Diarrhea. *J Infect Dis* **2008**, *197*, 435–438, doi:10.1086/525047.
21. Lopetuso, L.R.; Quagliariello, A.; Schiavoni, M.; Petito, V.; Russo, A.; Reddel, S.; Del Chierico, F.; Ianiro, G.; Scaldaferri, F.; Neri, M.; et al. Towards a Disease-Associated Common Trait of Gut Microbiota Dysbiosis: The Pivotal Role of Akkermansia Muciniphila. *Dig Liver Dis* **2020**, *52*, 1002–1010, doi:10.1016/j.dld.2020.05.020.
22. Lo Presti, A.; Del Chierico, F.; Altomare, A.; Zorzi, F.; Cella, E.; Putignani, L.; Guarino, M.P.L.; Monteleone, G.; Cicala, M.; Angeletti, S.; et al. Exploring the Genetic Diversity of the 16S RRNA Gene of Akkermansia Muciniphila in IBD and IBS. *Future Microbiol* **2019**, *14*, 1497–1509, doi:10.2217/fmb-2019-0175.
23. Ijssennagger, N.; Belzer, C.; Hooiveld, G.J.; Dekker, J.; van Mil, S.W.C.; Müller, M.; Kleerebezem, M.; van der Meer, R. Gut Microbiota Facilitates Dietary Heme-Induced Epithelial Hyperproliferation by Opening the Mucus Barrier in Colon. *Proc Natl Acad Sci U S A* **2015**, *112*, 10038–10043, doi:10.1073/pnas.1507645112.
24. Png, C.W.; Lindén, S.K.; Gilshenan, K.S.; Zoetendal, E.G.; McSweeney, C.S.; Sly, L.I.; McGuckin, M.A.; Florin, T.H.J. Mucolytic Bacteria with Increased Prevalence in IBD Mucosa Augment In Vitro Utilization of Mucin by Other Bacteria. *Am J Gastroenterol* **2010**, *105*, 2420–2428, doi:10.1038/ajg.2010.281.
25. Tailford, L.E.; Crost, E.H.; Kavanaugh, D.; Juge, N. Mucin Glycan Foraging in the Human Gut Microbiome. *Front Genet* **2015**, *6*, 81, doi:10.3389/fgene.2015.00081.
26. Johansson, M.E.V.; Phillipson, M.; Petersson, J.; Velcich, A.; Holm, L.; Hansson, G.C. The Inner of the Two Muc2 Mucin-Dependent Mucus Layers in Colon Is Devoid of Bacteria. *Proc Natl Acad Sci U S A* **2008**, *105*, 15064–15069, doi:10.1073/pnas.0803124105.
27. Nobili, V.; Mosca, A.; Alterio, T.; Cardile, S.; Putignani, L. Fighting Fatty Liver Diseases with Nutritional Interventions, Probiotics, Symbiotics, and Fecal Microbiota Transplantation (FMT). *Adv Exp Med Biol* **2019**, *1125*, 85–100, doi:10.1007/5584_2018_318.
28. Del Chierico, F.; Nobili, V.; Vernocchi, P.; Russo, A.; De Stefanis, C.; Gnani, D.; Furlanello, C.; Zandonà, A.; Paci, P.; Capuani, G.; et al. Gut Microbiota Profiling of Pediatric Nonalcoholic Fatty Liver Disease and Obese Patients Unveiled by an Integrated Meta-Omics-Based Approach. *Hepatology* **2017**, *65*, 451–464, doi:10.1002/hep.28572.
29. Botticelli, A.; Vernocchi, P.; Marini, F.; Quagliariello, A.; Cerbelli, B.; Reddel, S.; Del Chierico, F.; Di Pietro, F.; Giusti, R.; Tomassini, A.; et al. Gut Metabolomics Profiling of Non-Small Cell Lung Cancer (NSCLC) Patients under Immunotherapy Treatment. *J Transl Med* **2020**, *18*, 49, doi:10.1186/s12967-020-02231-0.
30. Vernocchi, P.; Gili, T.; Conte, F.; Del Chierico, F.; Conta, G.; Miccheli, A.; Botticelli, A.; Paci, P.; Caldarelli, G.; Nuti, M.; et al. Network Analysis of Gut Microbiome and Metabolome to Discover Microbiota-Linked Biomarkers in Patients Affected by Non-Small Cell Lung Cancer. *Int J Mol Sci* **2020**, *21*, E8730, doi:10.3390/ijms21228730.
31. Ozaki, Y.; Suzuki, Y.; Nishiyama, K.; Suzutani, T.; Suzuki, H. [Association between Immunotherapy with Immune Checkpoint Inhibitors(Anti-PD-1 Antibodies)and Intestinal Microbiota]. *Gan To Kagaku Ryoho* **2021**, *48*, 1096–1099.
32. Macchione, I.G.; Lopetuso, L.R.; Ianiro, G.; Napoli, M.; Gibiino, G.; Rizzatti, G.; Petito, V.; Gasbarrini, A.; Scaldaferri, F. Akkermansia Muciniphila: Key Player in Metabolic and Gastrointestinal Disorders. *Eur Rev Med Pharmacol Sci* **2019**, *23*, 8075–8083, doi:10.26355/eurev_201909_19024.
33. Parfrey, L.W.; Walters, W.A.; Knight, R. Microbial Eukaryotes in the Human Microbiome: Ecology, Evolution, and Future Directions. *Front Microbiol* **2011**, *2*, 153, doi:10.3389/fmicb.2011.00153.
34. Human Microbiome Project Consortium Structure, Function and Diversity of the Healthy Human Microbiome. *Nature* **2012**, *486*, 207–214, doi:10.1038/nature11234.

35. Hewitson, J.P.; Maizels, R.M. Vaccination against Helminth Parasite Infections. *Expert Rev Vaccines* **2014**, *13*, 473–487, doi:10.1586/14760584.2014.893195.
36. Cacciò, S.M.; Putignani, L. Epidemiology of Human Cryptosporidiosis. *Cryptosporidium: Parasite and Disease* **2014**, 43–79, doi:10.1007/978-3-7091-1562-6_2.
37. Norman, J.M.; Handley, S.A.; Virgin, H.W. Kingdom-Agnostic Metagenomics and the Importance of Complete Characterization of Enteric Microbial Communities. *Gastroenterology* **2014**, *146*, 1459–1469, doi:10.1053/j.gastro.2014.02.001.
38. Nyholm, L.; Koziol, A.; Marcos, S.; Botnen, A.B.; Aizpurua, O.; Gopalakrishnan, S.; Limborg, M.T.; Gilbert, M.T.P.; Alberdi, A. Holo-Omics: Integrated Host-Microbiota Multi-Omics for Basic and Applied Biological Research. *iScience* **2020**, *23*, 101414, doi:10.1016/j.isci.2020.101414.
39. Putignani, L.; Gasbarrini, A.; Dallapiccola, B. Potential of Multiomics Technology in Precision Medicine. *Curr. Opin. Gastroenterol.* **2019**, *35*, 491–498, doi:10.1097/MOG.0000000000000589.
40. Matijašić, M.; Meštrović, T.; Paljetak, H.Č.; Perić, M.; Barešić, A.; Verbanac, D. Gut Microbiota beyond Bacteria-Mycobiome, Virome, Archaeome, and Eukaryotic Parasites in IBD. *Int J Mol Sci* **2020**, *21*, E2668, doi:10.3390/ijms21082668.
41. Zuo, T.; Wong, S.H.; Lam, K.; Lui, R.; Cheung, K.; Tang, W.; Ching, J.Y.L.; Chan, P.K.S.; Chan, M.C.W.; Wu, J.C.Y.; et al. Bacteriophage Transfer during Faecal Microbiota Transplantation in Clostridium Difficile Infection Is Associated with Treatment Outcome. *Gut* **2018**, *67*, 634–643, doi:10.1136/gutjnl-2017-313952.
42. Dalmasso, M.; Hill, C.; Ross, R.P. Exploiting Gut Bacteriophages for Human Health. *Trends Microbiol* **2014**, *22*, 399–405, doi:10.1016/j.tim.2014.02.010.
43. Jonas, O.; Seifman, R. Do We Need a Global Virome Project? *Lancet Glob Health* **2019**, *7*, e1314–e1316, doi:10.1016/S2214-109X(19)30335-3.
44. Minot, S.; Bryson, A.; Chehoud, C.; Wu, G.D.; Lewis, J.D.; Bushman, F.D. Rapid Evolution of the Human Gut Virome. *Proc Natl Acad Sci U S A* **2013**, *110*, 12450–12455, doi:10.1073/pnas.1300833110.
45. Townsend, E.M.; Kelly, L.; Muscatt, G.; Box, J.D.; Hargraves, N.; Lilley, D.; Jameson, E. The Human Gut Phageome: Origins and Roles in the Human Gut Microbiome. *Front Cell Infect Microbiol* **2021**, *11*, 643214, doi:10.3389/fcimb.2021.643214.
46. Mirzaei, M.K.; Maurice, C.F. Ménage à Trois in the Human Gut: Interactions between Host, Bacteria and Phages. *Nat Rev Microbiol* **2017**, *15*, 397–408, doi:10.1038/nrmicro.2017.30.
47. Huffnagle, G.B.; Noverr, M.C. The Emerging World of the Fungal Microbiome. *Trends Microbiol* **2013**, *21*, 334–341, doi:10.1016/j.tim.2013.04.002.
48. David, L.A.; Maurice, C.F.; Carmody, R.N.; Gootenberg, D.B.; Button, J.E.; Wolfe, B.E.; Ling, A.V.; Devlin, A.S.; Varma, Y.; Fischbach, M.A.; et al. Diet Rapidly and Reproducibly Alters the Human Gut Microbiome. *Nature* **2014**, *505*, 559–563, doi:10.1038/nature12820.
49. Hoffmann, C.; Dollive, S.; Grunberg, S.; Chen, J.; Li, H.; Wu, G.D.; Lewis, J.D.; Bushman, F.D. Archaea and Fungi of the Human Gut Microbiome: Correlations with Diet and Bacterial Residents. *PLoS One* **2013**, *8*, e66019, doi:10.1371/journal.pone.0066019.
50. Beheshti-Maal, A.; Shahrokh, S.; Ansari, S.; Mirsamadi, E.S.; Yadegar, A.; Mirjalali, H.; Zali, M.R. Gut Mycobiome: The Probable Determinative Role of Fungi in IBD Patients. *Mycoses* **2021**, *64*, 468–476, doi:10.1111/myc.13238.
51. Ianiri, G.; Iorio, A.; Porcari, S.; Masucci, L.; Perno, C. F.; Gasbarrini, A.; Putignani, L.; Cam-marota, G. How the Gut Parasitome Affects Human Health.
52. Farthing, M.J. Giardiasis. *Gastroenterol Clin North Am* **1996**, *25*, 493–515, doi:10.1016/s0889-8553(05)70260-0.

53. Cryptosporidium - ScienceDirect Available online: <https://www.sciencedirect.com/science/article/pii/B978012818731900080X?via%3Dihub> (accessed on 21 September 2021).
54. Prommi, A.; Prombutara, P.; Watthanakulpanich, D.; Adisakwattana, P.; Kusolsuk, T.; Yoonuan, T.; Poodeepiyasawat, A.; Homsuwan, N.; Prummongkol, S.; Tanita, M.; et al. Intestinal Parasites in Rural Communities in Nan Province, Thailand: Changes in Bacterial Gut Microbiota Associated with Minute Intestinal Fluke Infection. *Parasitology* **2020**, *147*, 972–984, doi:10.1017/S0031182020000736.
55. Formenti, F.; Cortés, A.; Brindley, P.J.; Cantacessi, C.; Rinaldi, G. A Bug's Life: Delving into the Challenges of Helminth Microbiome Studies. *PLoS Negl Trop Dis* **2020**, *14*, e0008446, doi:10.1371/journal.pntd.0008446.
56. White, E.C.; Houlden, A.; Bancroft, A.J.; Hayes, K.S.; Goldrick, M.; Grecis, R.K.; Roberts, I.S. Manipulation of Host and Parasite Microbiotas: Survival Strategies during Chronic Nematode Infection. *Sci Adv* **2018**, *4*, eaap7399, doi:10.1126/sciadv.aap7399.
57. Holzschneider, M.; Layland, L.E.; Loffredo-Verde, E.; Mair, K.; Vogelmann, R.; Langer, R.; Wagner, H.; Prazeres da Costa, C. Lack of Host Gut Microbiota Alters Immune Responses and Intestinal Granuloma Formation during Schistosomiasis. *Clin Exp Immunol* **2014**, *175*, 246–257, doi:10.1111/cei.12230.
58. Cortés, A.; Clare, S.; Costain, A.; Almeida, A.; McCarthy, C.; Harcourt, K.; Brandt, C.; Tolley, C.; Rooney, J.; Berriman, M.; et al. Baseline Gut Microbiota Composition Is Associated With Schistosoma Mansoni Infection Burden in Rodent Models. *Front Immunol* **2020**, *11*, 593838, doi:10.3389/fimmu.2020.593838.
59. Jenkins, T.P.; Pritchard, D.I.; Tanasescu, R.; Telford, G.; Papaiakevou, M.; Scotti, R.; Cortés, A.; Constantinescu, C.S.; Cantacessi, C. Experimental Infection with the Hookworm, Necator Americanus, Is Associated with Stable Gut Microbial Diversity in Human Volunteers with Relapsing Multiple Sclerosis. *BMC Biol* **2021**, *19*, 74, doi:10.1186/s12915-021-01003-6.
60. Pane, S.; Sacco, A.; Iorio, A.; Romani, L.; Putignani, L. Strongyloides Stercoralis Infestation in a Child: How a Nematode Can Affect Gut Microbiota. *Int J Mol Sci* **2021**, *22*, 2131, doi:10.3390/ijms22042131.
61. Midha, A.; Schlosser, J.; Hartmann, S. Reciprocal Interactions between Nematodes and Their Microbial Environments. *Front Cell Infect Microbiol* **2017**, *7*, 144, doi:10.3389/fcimb.2017.00144.
62. Beghini, F.; Pasolli, E.; Truong, T.D.; Putignani, L.; Cacciò, S.M.; Segata, N. Large-Scale Comparative Metagenomics of Blastocystis, a Common Member of the Human Gut Microbiome. *ISME J* **2017**, *11*, 2848–2863, doi:10.1038/ismej.2017.139.
63. Andersen, L.O.; Stensvold, C.R. Blastocystis in Health and Disease: Are We Moving from a Clinical to a Public Health Perspective? *J Clin Microbiol* **2016**, *54*, 524–528, doi:10.1128/JCM.02520-15.
64. Billy, V.; Lhotská, Z.; Jirků, M.; Kadlecová, O.; Frgeleková, L.; Parfrey, L.W.; Pomajbíková, K.J. Blastocystis Colonization Alters the Gut Microbiome and, in Some Cases, Promotes Faster Recovery From Induced Colitis. *Front Microbiol* **2021**, *12*, 641483, doi:10.3389/fmicb.2021.641483.
65. Stensvold, C.R.; van der Giezen, M. Associations between Gut Microbiota and Common Luminal Intestinal Parasites. *Trends Parasitol* **2018**, *34*, 369–377, doi:10.1016/j.pt.2018.02.004.
66. Dheilly, N.M.; Martínez Martínez, J.; Rosario, K.; Brindley, P.J.; Fichorova, R.N.; Kaye, J.Z.; Kohl, K.D.; Knoll, L.J.; Lukeš, J.; Perkins, S.L.; et al. Parasite Microbiome Project: Grand Challenges. *PLoS Pathog* **2019**, *15*, e1008028, doi:10.1371/journal.ppat.1008028.
67. Chiu, C.Y.; Miller, S.A. Clinical Metagenomics. *Nat Rev Genet* **2019**, *20*, 341–355, doi:10.1038/s41576-019-0113-7.

-
68. Guo, B.; Harstall, C.; Louie, T.; Veldhuyzen van Zanten, S.; Dieleman, L.A. Systematic Review: Faecal Transplantation for the Treatment of Clostridium Difficile-Associated Disease. *Aliment Pharmacol Ther* **2012**, *35*, 865–875, doi:10.1111/j.1365-2036.2012.05033.x.
69. Cammarota, G.; Ianiro, G.; Tilg, H.; Rajilić-Stojanović, M.; Kump, P.; Satokari, R.; Sokol, H.; Arkkila, P.; Pintus, C.; Hart, A.; et al. European Consensus Conference on Faecal Microbiota Transplantation in Clinical Practice. *Gut* **2017**, *66*, 569–580, doi:10.1136/gutjnl-2016-313017.
70. Cammarota, G.; Ianiro, G.; Kelly, C.R.; Mullish, B.H.; Allegretti, J.R.; Kassam, Z.; Putignani, L.; Fischer, M.; Keller, J.J.; Costello, S.P.; et al. International Consensus Conference on Stool Banking for Faecal Microbiota Transplantation in Clinical Practice. *Gut* **2019**, *68*, 2111–2121, doi:10.1136/gutjnl-2019-319548.
71. Keller, J.J.; Ooijevaar, R.E.; Hvas, C.L.; Terveer, E.M.; Lieberknecht, S.C.; Högenauer, C.; Arkkila, P.; Sokol, H.; Gridnyev, O.; Mégraud, F.; et al. A Standardised Model for Stool Banking for Faecal Microbiota Transplantation: A Consensus Report from a Multidisciplinary UEG Working Group. *United European Gastroenterol J* **2021**, *9*, 229–247, doi:10.1177/2050640620967898.