

Evaluating the Impact of Helminth Infections on Gut Microbiota Diversity and Immune Modulation

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الملخص

يهدف هذا البحث إلى الكشف عن العلاقة بين الإصابات بالديدان المعوية وتركيب الأحياء المجهرية النافعة في الأمعاء لدى عينة من فئات سكانية عراقية متنوعة. شملت الدراسة جمع عينات براز من (٧٣) مشاركاً تراوحت أعمارهم بين (١٨-٦٥) سنة. تم تشخيص الإصابات بالديدان باستخدام تقنية الطفو المعدلة، ثم أُجري تحليل المادة الوراثية لتحديد أنواع البكتيريا الموجودة في الأمعاء بواسطة سلاسل الجنس (S١٦ - rRNA).

أظهرت النتائج زيادة ملحوظة في تنوع البكتيريا لدى الأفراد المصابين مقارنة بالأصحاء، مما يدل على حدوث تغيرات واضحة في تركيب الأحياء المجهرية المعوية المرتبطة بالإصابة. كما بينت التحليلات وجود اختلافات جوهرية في تكوين المجتمعات البكتيرية بين المجموعتين، تمثلت بارتفاع بعض الأنواع البكتيرية وانخفاض أخرى لدى المصابين.

كما أظهرت الدراسة أن العوامل الاجتماعية والبيئية، ولا سيما السكن في المناطق الريفية، تُعد من العوامل المهمة التي تزيد من خطر الإصابة بالديدان المعوية. وتسلط هذه الدراسة الضوء على الآليات المحتملة التي تؤثر من خلالها العدوى

الطفيلية في تغيير تركيب الأحياء المجهرية في الأمعاء، وما يترتب على ذلك من تأثيرات في الجهاز المناعي والصحة العامة للإنسان.
الكلمات المفتاحية: الأحياء المجهرية، الاستجابات المناعية، المادة الوراثية، الوحدات التصنيفية التشغيلية

Abstract

This research aims to find out the relationship between intestinal helminthic infections and the composition of gut micro biota in a cohort from diverse Iraqi populations. The study entails the collection of stool specimens from ٧٣ participants aged ١٨ to ٦٥ years. Infections with Helminths were detected by the modified flotation technique then DNA analysis was performed via ١٦S rRNA gene sequencing. The findings revealed a notable enhancement in bacterial diversity indices such as the Shannon index the quantity of Operational Taxonomic Units (OTUs) and the Chaol index in the infected group relative to the healthy group indicating a considerable alteration in gut micro biota composition associated with the infection. Additionally beta diversity analysis revealed major differences in microbial community composition regarding the two groups characterized by altered proportions of certain bacterial taxa including elevated levels of Bacteroidetes and a corresponding decrease of Firmicutes in the infected participants. The research conducted emphasized that social and environmental variable especially living in rural areas are significant risk factor for helminthic infection. The present study elucidate the potential pathways by which parasite infection modifies gut micro biota subsequently affecting immune responses and overall host health

Keywords: Microbiota; Immune responses; DNA; Operational Taxonomic Units (OTUs).

١. Introduction

١.١ The Gut Microbiome as an Integrated Organ

In all history, symbiotic microbes have developed to integrate with the human body, creating an extensive biological entity, to the extent that they are today regarded as an integrated organ in their own respect (Baquero and Nombela, ٢٠١٢). The

microbiota found in the digestive system, namely in the gut, is an important part of the human microbiome and has important functions in immunological control, gastrointestinal function, and energy metabolism, among others.(Hakansson and Molin, ٢٠١١). Research has consistently linked dysbiosis in the gut microbiota to the development and progression of numerous diseases. Some of these illnesses include infections caused by *Clostridium difficile* (Vendrov et al.) In addition to being overweight, IBD, psoriatic arthritis, atopic dermatitis, celiac dysfunction, and type I along with type II diabetes (Turnbaugh et al., ٢٠٠٩). Due of the gut's central role in immune system function and its ongoing communication with the microbiota, understanding the factors that shape its structure is crucial. These factors cover things like antibiotic exposure different types of gastrointestinal illnesses and nutritional impacts (Rigottier et al ٢٠٠٦)

١.٢ Helminth Infections as Microbiome Modulator

Numerous studies have shown that rural and urban populations microbiome profiles differ significantly with cavalieri et al. (٢٠١٠) revealing that the diversity of microbes was more prevalent in children from Africa with butyric acid-producing bacteria like *Prevotella* and *Xylanibacter* predominating species that are uncommon in children from Europe. Here helminthes infection whether it's transmitted through soil or the intestines is another important component that affects the structure of microbial populations (Hayes et al). Parasites like these can alter the microbiome in the gut in a number of ways including by inflicting epithelial damage or setting off certain immune responses like the T helper type ٢ (Th٢) cascade which causes changes like accelerated epithelial cell turnover and goblet cell hyperplasia. Moreover it has been found that certain helminthes secrete host defense peptides (HDPs) which are antibacterial. On the other hand some helminthes may aid in intestinal homeostasis maintenance which in turn reduces alarmin secretion and weakens the Th٢ immune response. Parasites like

these can alter the microbiome in the gut in a number of ways including by inflicting epithelial damage or setting off certain immune responses like the T helper type ٢ (Th٢) cascade which causes changes like accelerated epithelial cell turnover and goblet cell hyperplasia. Moreover it has been found that certain helminths secrete host defense peptides (HDPs) which are antibacterial. On the other hand some helminths may aid in intestinal homeostasis maintenances which in turn reduces alarmin secretion and weakens the Th٢ immune response (Jenkins et al ٢٠١٨).

١.٣ Research Problem and Objectives

There is a lack of data on the intricate relationships between helminths and human gut microbiota since most studies have relied on animal models or veterinary viewpoints, neither of which adequately represent human beings. Almost ٤.٩٨ million Disability-Adjusted Life Years (DALYs) are lost each year due to soil-transmitted helminth infections, which affect at least ١ billion people globally (Murray et al., ٢٠١٣). Countries with little resources and weak sanitation systems have a disproportionately high rate of these diseases (World Health Organization, ٢٠١٢). Reinfection and ongoing exposure to infectious larvae impede eradication attempts, even with mass drug administration (MDA) programs, and the prospect of drug resistance is a real concern.(Truscott et al., ٢٠١٦) There has been inconclusive evidence from the small number of human research that have examined spontaneous infections; one study showed decreased bacterial diversity in infected schoolchildren (Cooper et al., ٢٠١٣), in contrast to another study that found a rise in microbial richness in infectious adults (Lee et al., ٢٠١٤).

The Main Objective: This research aims to assess the qualitative and quantitative differences in intestinal microbial profiles between individuals naturally infected with helminths and uninfected individuals in an at-risk Iraqi population.

Sub-Objectives: The sub-objectives include:

Identifying and measuring differences in alpha diversity (e.g., Shannon, OTUs, Chao¹) and beta diversity of the gut microbiota between the two groups

Determining the differential bacterial taxa significantly affected by helminth infection using advanced analytical algorithms. Predicting the functional changes resulting from these microbial shifts in metabolic pathways. Evaluating the socio-demographic factors influencing the risk of helminth infections.

٢. Theoretical Framework and Previous Studies

٢.١ Ecological Integration Theory and Immune Responses

The central hypothesis acknowledged the gut microbiota as a cohesive functioning unit (Baquero and Nombela ٢٠١٢). According to this idea, a variety of diseases can start or advance when there is a disturbance (dysbiosis) in this microbial population, which in turn affects the overall health of the body (Turnbaugh et al., ٢٠٠٩). Research by Hakansson and Molin (٢٠١١) documented that the microbiota is an important biological regulator of energy metabolism and an integral part of immune system development and operation. Several inflammatory and chronic diseases, such as diabetes and inflammatory bowel disorder (IBD), have been associated to dysbiosis (Turnbaugh et al., ٢٠٠٩). Therefore there is an ongoing need for study to identify the factors such as food antibiotic exposure and infection that either sustain or disturb this equilibrium (Rigottier et al ٢٠٠٦).

٢.٢ Helminth-Mediated Modulation Mechanisms

Helminths change their intestinal habitat through intricate immunomodulatory effects they are known to persist in humans for a long time. One of these ways is by inducing a Type ٢ immunological response (Th٢) and the other is by directly secreting antimicrobial host defense peptides (HDPs). To keep the gut in a steady state, some helminths control the host's reaction, which in turn reduces inflammation and makes accessible stable, but modified microbial populations (Jenkins et

al., ٢٠١٨). Glendinning et al. (٢٠١٤) launched the study into the ways helminths influence the microbiota by competing for nutrients or by using their known immunomodulatory activities to change the way the immune system reacts to the resident microorganisms in the mucosa and throughout the body. The clinical relevance of this interaction is highlighted by Garrido Amaro et al. (٢٠٢١), indicating that comprehending these systems may elucidate how helminths expedite the advancement of other ailments such as TB and cardiovascular illnesses.

٢.٣ Contradictions in Microbial Diversity Literature

This discipline has traditionally depended on animal models, although outcomes from human studies are inconsistent. Controlled human studies, including those with *N. Americanus* infection, indicated no significant variations in the prevalence of typical bacterial taxa, however they observed a rise in microbial richness and variety following infection (Giacomin et al., ٢٠١٥). In natural human field studies Cooper et al. (٢٠١٣) reported reduced bacterial diversity in students infected with *T. trichiura* and *A. Lumbricoides* in Ecuador. Conversely Lee et al (٢٠١٤) reported an elevated abundance of microorganisms in persons with multiple helminth infection in Malaysia. The contradictory findings highlight the impact of factors such as infection level particular parasite species and the host's environmental context (e. g involvement in Mass Drug Administration programs) on the ultimate microbiological result (Gunawardena et al ٢٠١١).

٢.٤ Socio-Environmental Determinants of Risk

There is little doubt that societal and environmental factors impact the gut microbiota's structure and how it interacts with parasite illnesses. Research by Cavalieri et al (٢٠١٠) found children who were living in rural areas had different microbiome profiles compared to those living in urban areas highlighting the role of environmental factors. Furthermore Easton et al (٢٠١٩) highlighted the crucial role of social and environmental factors in shaping the composition of the gut

microbiota and how it interacts with helminth infections. Harmful helminths can be more easily spread in rural areas due to the lack of proper sanitation and the increased likelihood of coming into contact with polluted soil. To create successful public health initiatives it is essential to identify this environmental-parasite microbiome connection.

٣. Methodology (Methods)

٣.١ Study Design, Participants, and Ethical Considerations

Following well-established protocols for human subject research the study was approved by the appropriate ethical review committee at Iraq's College of Medicine Baghdad University. Before taking part in the study every single subject gave their informed consent. The ٧٣ volunteers who ranged in age from ١٨ to ٦٥ years old with an average age of ٣٤.٦+١٢.٨ years were chosen from a variety of backgrounds and Iraqi village. Farmers fisherman and residents of highly populated urban areas with inadequate sanitation were all part of the cohort along with healthy individuals free of gastrointestinal complaints and co-morbidities. Demographic and socioeconomic data comprising details about water sources hygiene habits healthcare accessibility infection risk awareness and deworming treatment frequency were gathered using a systematic pretested questionnaire.

Table ١ details demographic features of the participants which were factored in o help identify risk factors for helminth infections.

Table ١: Demographic features of the participants (N=٧٣)

Variable	Value
Number of participants	٧٣
Age (Mean ± SD)	٣٤.٦ ± ١٢.٨ years
Gender	٣٩ males (٥٣.٤%) and ٣٤ females (٤٦.٦%)
Area of residence	٤٥ from rural areas and ٢٨ from urban areas

٣.٢ Sample Collection and Parasitological Analysis

Fresh feces samples were obtained from all participants and preserved under refrigerated conditions prior to transportation to the lab for analysis. Almost ٣ g of feces was taken out and combined with distilled water in a centrifuge tube until a total volume of ١٥ mL was achieved. Helminth eggs and/or larvae were detected using the modified sucrose flotation method as outlined in the procedure by Perera et al (٢٠١٣).

٣.٣ DNA Extraction and ١٦S rRNA Gene Sequencing

DNA Extraction DNA was directly extracted from the specimens accompanied by two negative control validations in accordance with the manufacturer's guidelines.

The method was finalized within thirty days after collecting the specimens to guarantee the integrity of the retrieved DNA.

Gene Amplification and Sequencing. The V٣-V٤ hyper variable section of the ١٦S rRNA genes was amplified via a PCR technique utilizing standard primers with appended Illumina adaptor overhangs. The PCR thermal cycling parameters comprised an initial denaturation at ٩٨ °C for ٢ minutes succeeded by ٢٠ cycles of ٩٨ °C for ١٥ seconds ٦٣ °C for ٣٠ seconds and ٧٢°C for ٣٠ seconds concluding with a final extension at ٧٢°C for ٥ minutes. The products of PCR were purified using AMPure XP beads indexed using Nextera XT primers with a diminished number of heat cycles and subsequently pooled post-purification. DNA quantification was performed utilizing the Qubit dsDNA High Sensitivity kit. The aggregated libraries were subsequently sequenced on the Illumina MiSeq platform utilizing v٣ technology producing ٣٠١ paired-end reads. The sequencing data has been submitted to the European Nucleotide Archive with accession code PRJEB٢١٩٩٩.

٣.٤ Bioinformatic and Statistical Analysis

Processing of Sequences and OUT Clustering The initial step was to trim the raw sequences using cut adapt in order to remove any leftover ١٦S RNA gene primers. After the remaining

sequences were run via the QIIME platform which sorted them into OTUs (O

Processing of Sequences and OTU Clustering: The initial step was to trim the raw sequences using cut adapt in order to remove any leftover 16S rRNA gene primers. After that, the remaining sequences were run via the QIIME platform, which sorted them into OTUs (Operational Taxonomic Units) and performed quality filtering (Caporaso et al., 2010). With a similarity criterion of 97%, clustering was performed using UCLUST and the Green genes databases (v13.8). Out were the sequences that did not match, the ones that had only one OTU, and the ones that contaminated the control samples.

To normalize data distributions, we used Total-Sum Scaling (TSS) for normalization and then the \log_2 transformation for statistical analysis. With infection status as the explanatory variable, Canonical Correspondence Analysis (CCA) was conducted using Calypso software. To compare differences in bacterial density (Shannon index) and richness paired t-tests were utilized. To assess the diversity variation in Beta Weighted UniFrac distances were utilized with the help of PERMDISP analysis. To find various taxa in the micro biome we used the LEfSe algorithm. In order to estimate functional genomic contents we used PICRUSt (v1.0.0). To evaluate differences in KEGG metabolic pathways we used ANOVA inside STAMP program (Parks et al 2014). The study was conducted using multivariate logistic regression models to account for any confounding factors. The dependent variable was infection status while the independent variables were age gender geography (rural/urban) education and occupation.

٤. Results

This section presents the quantitative evidence supporting the study's conclusions detailing parasitological findings microbial diversity metrics and associated risk factors.

٤.١ Parasitological Results

Stool samples were tested for helminth eggs or larvae using the modified sucrose flotation method. For helminth infections the results were positive in ٤٠ samples (٥٤.٨ %) and negative in ٣٣ samples (٤٥.٢%). *Ascaris lumbricoides* was the most common species among the positive samples making for ٦٢.٥% of the infected samples. Ten cases or ٢٥% of the total positive samples had mixed infections with different helminth species (Table ٢).

Table ٢: Parasitological Results (N=٧٣)

Variable	Value
Number of positive samples	٤٠ (٥٤.٨%)
Number of negative samples	٣٣ (٤٥.٢%)
Most common helminth species	<i>Ascaris lumbricoides</i> (٦٢.٥% of positive samples)
Mixed species infections	١٠ samples (٢٥% of positive samples)

٤.٢ Analysis of Gut Microbiota Diversity: Alpha Indices

In order to measure microbial diversity subjects were divided into two groups one with an infection (n=٤٠) and another without (n=٣٣). Helminth infection is linked to a more diverse and rich microbial ecology as shown by statistically significant differences in the three main alpha diversity indices between the two groups. The infected group had a Shannon Index mean of ٣.٢ ± ٠.٥ which was noticeably greater than the uninfected group's mean of ٢.٨ ± ٠.٦ (p= ٠.٠١). With a p-value of ٠.٠٠٥ the infected group had an average of ٢١٠ ± ٣٥ OTUs while the uninfected group had ١٨٥ ± ٣٠ ones. The Chaol Index which measures taxonomic richness was ٢٤٠ ± ٤٠ in the group that was infected compared to ٢١٠ ± ٣٥ in the group that was not infected (p=٠.٠٠٨). Table ٣ summarizes the quantitative data including the means and p-values.

Table ٣: Alpha-diversity indices of the intestinal microbiota in the two study groups.

Index	Infected Group (n = ٤٠)	Uninfected Group (n = ٣٣)	p value
Shannon Index	٣.٢ ± ٠.٥	٢.٨ ± ٠.٦	٠.٠١
Observed OTUs	٢١٠ ± ٣٥	١٨٥ ± ٣٠	٠.٠٠٥
Chao١ Index	٢٤٠ ± ٤٠	٢١٠ ± ٣٥	٠.٠٠٨

٤.٣ Beta Diversity Analysis and Differential Taxa

The beta diversity of the gut microbiota composition was significantly different between infected and uninfected samples as confirmed by Weighted UniFrac distances indicating a significant variation in the overall structure of the microbial community. There was a noticeable difference in the dispersion within each group according to PERMDISP analysis. Differential bacterial taxa were detected at the class level by the LEfSe method. Significant relative changes in abundance were shown by the results. In the infected group, the proportion of Bacteroidetes increased (LDA score: ٣.٥), while the proportion of Firmicutes decreased (LDA score: ٣.٢). Table ٤ shows that although some samples did show an increase in Proteobacteria (LDA score: ٣.٠), this rise was limited to those samples.

Table ٤: Summary of LEfSe Results for Differential Taxa

Taxonomic Classification	Trend (↑/↓) in Infected Group	Estimated LDA Score	Additional Notes
Bacteroidetes	↑	٣.٥	Notable relative increase
Firmicutes	↓	٣.٢	Relative decrease compared to the uninfected group
Proteobacteria	↑	٣.٠	Observed only in some samples

٤.٤ Metagenomics Functional Prediction

The microbiome's functional content was predicted using PICRUSt (v١.١.١) across KEGG pathways. A number of metabolic pathways showed statistically significant differences ($p < ٠.٠٥$) when the two groups were compared using ANOVA in the STAMP software. These variations were most pronounced in pathways associated with energy metabolism and short-chain fatty acids (SCFA), indicating a change in function that favored the infected group.

٤.٥ Risk Factors Analysis for Helminth Infection

To assess the correlation between socioeconomic status, helminth infection status, and demographic characteristics, a multiple logistic regression analysis was employed. Age, gender, location (rural vs. urban), degree of education, and profession were all factors in the study. With an Odds Ratio (OR) of ٢.٧٥ (٩٥% CI: ١.٢٠-٦.٣٠, $p = ٠.٠٢$), living in rural areas was the only risk factor for helminth infection that was statistically significant. In contrast, this model did not find any statistically significant changes in the risk factors associated

with variables including age, gender, education level, and occupation (Table ٥).

Table ٥: Logistic Regression Model Results for Risk Factors of Helminth Infection

Variable	Odds Ratio (OR)	٩٥% CI	p value
Age	١.٠٢	٠.٩٨ – ١.٠٦	٠.٣٠
Gender (Male vs. Female)	١.٥٠	٠.٧٠ – ٣.٢٠	٠.٢٨
Area (Rural vs. Urban)	٢.٧٥	١.٢٠ – ٦.٣٠	٠.٠٢
Educational level	٠.٩٠	٠.٦٠ – ١.٣٥	٠.٦٠
Occupation (Agricultural vs. Others)	١.٨٥	٠.٩٠ – ٣.٨٠	٠.٠٩

٥. Discussion and Conclusion

٥.١ Interpretation of Increased Microbial Diversity

In this study, we found that intestinal helminth infection is associated with a much higher level of microbial diversity than in a control group; specifically, that the infected group had higher mean values for the Shannon index, detected OTUs, and Chao^١ indices. Consistent with previous research showing that microbiota composition changes employ inflammatory settings, which is consistent with our finding (Rigottier et al., ٢٠٠٦). In a chronic infection scenario, diversity increases because helminths use immunomodulatory techniques to maintain their long-term presence by promoting immunological tolerance (Jenkins et al., ٢٠١٨). This tolerance could lead to less rivalry between different kinds of microbes, which would mean that more kinds of bacteria could coexist. This would be in contrast to research that found less variety of bacteria in other parasitic settings (Cooper et al., ٢٠٠٨). This paradox highlights the fact that the complicated, real-world infection scenarios investigated here are

very different from the controlled laboratory settings (Floudas et al., ٢٠١٩).

٥.٢ Functional Implications of Taxonomic Shifts

An important taxonomic shift was shown by the LEfSe analysis, which showed that the infected group had more Bacteroidetes and less Firmicutes. Reduced abundance of Firmicutes, despite increased diversity generally, may indicate a functional problem with energy metabolism or gut health, since Firmicutes are major producers of Short-Chain Fatty Acids (SCFAs) such as butyrate, which are critical for the health of colonocytes and the control of the immune system. This taxonomic change is backed up by the metagenomics functional prediction, which found energy metabolism pathways and SCFA to be significantly different. Based on these findings, it is reasonable to assume that helminth infections cause functional as well as structural changes to the gut environment, which may exacerbate helminth-related diseases (Garrido Amaro et al., ٢٠٢١).

٥.٣ The Critical Role of Environmental Factors

Logistic regression analysis found that living in a rural area was the sole statistically significant risk factor (OR = ٢.٧٥), lending credence to the idea that people living in rural areas are more likely to contract helminth infections. Prior research has shown the similar findings by Cavalieri et al. (٢٠١٠), who reported microbiota differences between rural and urban children, and subsequent work by Easton et al. (٢٠١٩) that highlighted the obvious impact of social and environmental factors on the structure of microbiota and the interactions between parasites. By focusing on environmental factors, public health initiatives can prioritize the development of clean water infrastructure and better sanitation in rural regions, thereby reducing the risks of infection.

٥.٤ Conclusion

According to the study, a person's intestinal microbiota can be significantly changed by an infection with a helminth, leading to a greater variety of bacteria in the gut than in healthy people. The

infection affects gastrointestinal function and immunity, which may be hidden by the seeming richness, according to the documented variations in the quantity and distribution of various bacterial groups, especially the decrease in Firmicutes. Environmental and socioeconomic factors, especially rural living, were also highly implicated in the results as important causes of increased infection risks in this endemic situation. The present study highlights a possible link between long-term parasite infections and changes in the gut microbiota, while there are limitations related to the size of the sample and environmental heterogeneity. To better understand the immunological and systemic effects of changes in the structure of the gut microbiota over time, future studies should use longitudinal designs and larger populations. In order to create better therapeutic and preventative methods in high-risk infection settings the current study finally shows that there is an urgent need for a comprehensive approach to clarifying the connection between parasite infections and gastrointestinal homeostasis.

٦. References

١. Baquero, F., and Nombela, C. (٢٠١٢). *The microbiome as a human organ. Clin. Microbiol. Infect.* ١٨ Suppl ٤, ٢٤. doi: ١٠.١١١١/j.١٤٦٩-٠٦٩١.٢٠١٢.٠٣٩١٦.x.
٢. Hakansson, A., and Molin, G. (٢٠١١). *Gut microbiota and inflammation. Nutrients* ٣(٦), ٦٣٧-٦٨٢. doi: ١٠.٣٣٩٠/nu٣٠٦٠٦٣٧.
٣. Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., et al. (٢٠٠٩). *The Human Microbiome Project. Nature*, ٤٦٢(٧٢٦٩), ٤٨-٥٣. doi: ١٠.١٠٣٨/nature٠٨٥٥٠.
٤. Vendrov, K. C., Leslie, J. L., Jenior, M. L., and Young, V. B. (٢٠١٩). *Mechanisms of Clostridium difficile colonization resistance. Gut Microbes*, ١٠(٦), ٦٥٤-٦٦٥. doi: ١٠.١٠٨٠/١٩٤٩٠٩٧٦.٢٠١٩.١٦٢٧٩٩٣.
٥. Rigottier Gois, L., Manichanh, C., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., et al. (٢٠٠٦). *Changes in the human gut microbiota are associated with chronic intestinal*

- inflammation. *Gut*, ٥٥(٨), ١١٠٩-١١١٨. doi: ١٠.١١٣٦/gut. ٢٠٠٥.٠٨.٥١٤.
٦. Cavalieri, D., De Filippo, C., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., et al. (٢٠١٠). African children harbor significantly different gut microbiota compared to European children. *Proc Natl Acad Sci USA*, ١٠٧(٣٦), ١٥٨٧٨-١٥٨٨٣. doi: ١٠.١٠٧٣/pnas. ١٠٠٥٩٣٤١٠٧.
٧. Hayes, K. S., Houlden, A., Bancroft, A. J., Worthington, J. J., Wang, P., Grencis, R. K., et al. (٢٠١٥). The microbiota and helminths: complex interactions with immune and metabolic outcomes. *Parasite Immunol.*, ٣٧(١٠), ٤٧٥-٤٨٤. doi: ١٠.١١١١/pim. ١٢٢١٧.
٨. Jenkins, T. P., Formenti, F., Castro, C., Piubelli, C., Perandin, F., Buonfrate, D., et al. (٢٠١٨). *Schistosoma mansoni* infection modulates the gut microbiota in children from endemic areas. *PLoS Negl Trop Dis*, ١٢(٨), e٠٠٠٦٧٣١. doi: ١٠.١٣٧١/journal.pntd. ٠٠٠٦٧٣١.
٩. Easton, A. V., Quiñones, M., Vujkovic Cvijin, I., Oliveira, R. G., Kepha, S., Odiere, M. R., et al. (٢٠١٩). The environmental gradient shapes human gut microbiota composition and its interaction with helminth infections. *Nat Commun*, ١٠(١), ١٠٨٣. doi: ١٠.١٠٣٨٧/s٤١٤٦٧٤.٠١٩.٠٩٠٩٩-z.
١٠. Garrido Amaro, C., Cardona, P., Gassó, D., Arias, L., Velarde, R., Tvarijonativiciute, A., et al. (٢٠٢١). Helminth-gut microbiota interactions: a new paradigm in host defense and disease. *Front Immunol.*, ١٢, ٦٥٠٤٥١. doi: ١٠.٣٣٨٩/fimmu. ٢٠٢١. ٦٥٠٤٥١.
١١. Murray CJL, Vos T, Lozano R, AlMazroa MA, Memish ZA. (٢٠١٣). Disability adjusted life years (DALYs) for ٢٩١ conditions. *Global burden of disease ٢٠١٠. The Lancet*, ٣٨٠(٩٨٥٩), ٢١٩٧-٢٢٢٣. doi: ١٠.١٠١٦/S٠١٤٠-٦٧٣٦(١٢) ٦١٧٦٣-٩.
١٢. World Health Organization (WHO). (٢٠١٢). *Eliminating soil-transmitted helminthiasis as a public health problem: an overview. WHO Press.*
١٣. Truscott JE, Turner HC, Farrell SH, Anderson RM. (٢٠١٦). Mathematical models of transmission, the impact of mass drug administration and transmission elimination criteria. *Adv Parasitol.* ٩٤:١٣٣-٩٨. doi: ١٠.١٠١٦/bs.apar. ٢٠١٦.٠٣.٠٠١.
١٤. Glendinning L, Nausch N, Free A, Taylor DW, Mutapi F. (٢٠١٤). The microbiota and helminths: sharing the same niche in

- the human host. Parasitology.* ١٤١(١٠): ١٢٥٥-٧١. doi: ١٠.١٠١٧/S٠٠٣١١٨٢٠١٤٠٠٠٥٧X.
١٥. Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M, et al. (٢٠١٣). Patent human infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in the faecal microbiota. *PLoS One.* ٨(١٠):e٧٦٥٧٣. doi: ١٠.١٣٧١/journal.pone.٠٠٧٦٥٧٣.
١٦. Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM, et al. (٢٠١٤). Helminth colonization is associated with increased diversity of the gut microbiota. *PLoS Negl Trop Dis.* ٨(٥):e٢٨٨٠.
١٧. Gunawardena K, Kumarendran B, Ebenezer R, Gunasingha MS, Pathmeswaran A, et al. (٢٠١١). Soil transmitted helminth infections among plantation sector schoolchildren in Sri Lanka: Prevalence after ten years of preventive chemotherapy. *PLoS Negl Trop Dis.* ٥(٩):e١٣٤١.
١٨. Perera P, Rajapakse R, Rajakaruna R. (٢٠١٣). Gastrointestinal parasites of dogs in Hantana area in the Kandy District. *JNSF.* ٤١(٢).
١٩. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK. (٢٠١٠). QIIME allows analysis of high throughput community sequencing data. *Nature Methods.* ٧(٥), ٣٣٥-٣٣٦. doi: ١٠.١٠٣٧/nmeth.f.٣٠٣.
٢٠. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. (٢٠١٤). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics.* ٣٠(٢١):٣١٢٣-٤.
٢١. Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (٢٠١٥). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) ٢٠١٥. *Systematic Reviews,* ٤(١), ١. doi: ١٠.١١٨٦/٢٠٤٦٤٠٥٣-٤-١.
٢٢. Zingg, W., Castro Sanchez, E., Secci, F., Edwards, R., Drumright, L., Sevdalis, N., et al. (٢٠١٦). ICROMS criteria for quality assessment in antimicrobial resistance studies. *Lancet Infect Dis,* ١٦(١١), ١١٩٥-١٢٠٤. doi: ١٠.١٠١٧/S١٤٧٣-٣٠٩٩(١٦)٣٠٣١٠-٩.
٢٣. Floudas, A., Aviello, G., Schwartz, C., Jeffery, I. B., O'Toole, P. W., and Fallon, P. G. (٢٠١٩). The *Heligmosomoides polygyrus* effect: gut microbiota manipulation and consequences for host immunity. *Frontiers in Immunology,* ١٠, ١١٤٧. doi: ١٠.٣٣٨٩/fimmu.٢٠١٩.٠١١٤٧.