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MULTI-OMICS INVESTIGATION OF THE GUT MICROBIOME–PARASITE–HOST AXIS: LINKING MICROBIAL DYSBIOSIS, IMMUNOMETABOLISM, IMMUNE REGULATION, AND THERAPEUTIC OUTCOMES

Dr Kiran Fatima[1], Zainab Rehman[2], Asma BiBi*[3], Dr. Nazia Akber Mir[4], Hammad Hussain Anjum[5], Dr. Laila Jafri[6], Dr. Ifra Ghori[7], Maria Fareed Siddiqui[8]

[1]Associate Professor, Rawalpindi Medical University, kiran.dr85@hotmail.com
[2]GGDC Khalisa, Higher Education department, zainier.95@gmail.com
[3]Department of Chemical & Life Sciences, Qurtuaba University of Science & Information Technology Peshawar KPK, asmabibi1326@gmail.com
[4]Assistant Professor Microbiology, Rawalpindi Medical University, Rawalpindi, dr.naziamiir@gmail.com
[5]Bahria university Health Sciences Islamabad, hammadhussainanjm7@gmail.com
[6]PhD Biochemistry Program In charge/Associate Professor, Department of Medical Lab Technology, Allied Health Sciences, Health Services Academy, PM Health Complex, Park Road, Chak Shahzad.Islamabad.44000
Laila@hsa.edu.pk
[7]Lecturer, Department of Biotechnology, Fatima Jinnah Women University, ifraghori@fjwu.edu.pk
[8]Faculty of Pharmacy, University of Lahore, ORCID: 0000-0001-8515-5260, maria.siddiqui@pharm.uol.edu.pk
***CORRESPONDING AUTHOR:** Asma BiBi, asmabibi1326@gmail.com

ABSTRACT:
Intestinal parasitic infections disrupt the gut microbiome–host axis, but the underlying immunometabolic mechanisms and post-treatment outcomes remain poorly characterized in endemic South Asian populations. This prospective longitudinal study (N=300) conducted in Islamabad, Pakistan, integrated shotgun metagenomics, LC-MS/MS metabolomics, and host peripheral blood mononuclear cell (PBMC) transcriptomics to comprehensively map the gut microbiome–parasite–host axis. Patients with confirmed *Giardia duodenalis*, *Entamoeba histolytica*, or soil-transmitted helminth infections were evaluated at baseline and 8 weeks post-standard pharmacological treatment, alongside age-matched healthy controls. Infection induced severe microbial dysbiosis, marked by a 26.5% reduction in alpha diversity and the targeted depletion of short-chain fatty acid (SCFA)-producing taxa, notably *Faecalibacterium prausnitzii*. This ecological shift caused a >2-fold collapse in fecal butyrate and secondary bile acids, which strongly correlated with impaired regulatory T cell (Treg) function, reduced IL-10 production, and the hyperactivation of pro-inflammatory *NLRP3* and IFN-γ pathways. Crucially, while standard anthelmintic and antiprotozoal therapy achieved a 95% parasitological cure rate, it failed to restore microbiome resilience (score: 0.68) or resolve systemic immunometabolic inflammation, resulting in persistent post-infectious gastrointestinal symptoms in 23.3% of the cohort. These findings demonstrate that parasite eradication alone is insufficient for holistic mucosal recovery. We conclude that intestinal parasitoses drive profound, multi-layered immunometabolic disruption mediated by microbiome depletion, highlighting the urgent need to integrate adjunctive microbiome-targeted therapies into standard clinical protocols in endemic regions.

KEYWORDS: Gut microbiome, parasitic infections, multi-omics, immunometabolism, microbial dysbiosis, short-chain fatty acids, therapeutic outcomes, inflammatory bowel disease, precision medicine.

INTRODUCTION

The human gastrointestinal tract harbors a complex and dynamic ecosystem of microorganisms, collectively termed the gut microbiome, which comprises bacteria, archaea, fungi, viruses, and protozoa [1]. This microbial consortium, estimated to contain approximately 3.8×10^{13} microbial cells, plays an indispensable role in maintaining host homeostasis through contributions to nutrient metabolism [2], immune system maturation, barrier integrity, and protection against pathogenic colonization [3]. Disruption of the compositional and functional equilibrium of the gut microbiome, known as microbial dysbiosis, has been implicated in a wide spectrum of pathological conditions, including inflammatory bowel disease (IBD), metabolic syndrome, autoimmune disorders, and infectious diseases [4], [5]. Among the diverse array of infectious agents that perturb gut homeostasis, intestinal parasites, including helminths (e.g., *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms) and protozoa (e.g., *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp.), represent a significant yet underappreciated modulator of the gut microbial ecosystem [6]. Parasitic infections affect over one-quarter of the global population, with the highest burden concentrated in low- and middle-income countries (LMICs), where they contribute substantially to morbidity, malnutrition, and impaired cognitive development in children [7]. Despite decades of research on parasite biology and host immune responses, the tripartite interactions among the gut microbiome, parasitic organisms, and the host immune-metabolic system remain incompletely understood [8].

The concept of the gut microbiome – parasite-host axis has emerged as a paradigm that recognizes the bidirectional and multidirectional crosstalk among these three biological entities [9]. Parasites can directly and indirectly alter the gut microbial community structure through physical disruption of the mucosal barrier, secretion of immunomodulatory molecules, and competition for nutrients [10]. Conversely, the resident microbiome can influence parasite establishment, survival, and pathogenicity by modulating the local immune environment and producing antiparasitic metabolites such as short-chain fatty acids (SCFAs) [11], [12]. The host immune system serves as the central mediator of this axis, integrating signals from both the microbiome and the parasite to orchestrate context-dependent immune responses that range from protective type 2 immunity against helminths to inflammatory type 1 and type 17 responses against protozoan pathogens [13].

A critical dimension of the microbiome–parasite–host axis is immunometabolism, which refers to the intersection of metabolic pathways and immune cell function [14]. During parasitic infection, immune cells undergo profound metabolic reprogramming to meet the bioenergetic and biosynthetic demands of mounting an effective response. For instance, the activation of macrophages, dendritic cells, and T lymphocytes during helminth infection is associated with a shift toward oxidative phosphorylation and fatty acid oxidation, which supports the alternatively activated (M2) macrophage phenotype and regulatory T cell (Treg) expansion [15], [16]. The gut microbiome contributes to this immunometabolic landscape by generating metabolites, such as SCFAs, bile acids, tryptophan derivatives, and polyamines, that directly modulate immune cell metabolism and function [17]. Dysbiosis induced by parasitic infection can therefore disrupt these metabolite-mediated immunoregulatory circuits, with consequences for disease severity, chronicity, and therapeutic responsiveness [18].

The advent of high-throughput multi-omics technologies, including metagenomics, metatranscriptomics, metabolomics, proteomics, and single-cell transcriptomics has revolutionized the capacity to interrogate complex biological systems at unprecedented resolution [19]. Integrative multi-omics approaches enable the simultaneous profiling of microbial community composition, functional gene expression, host transcriptomic responses, and the metabolomic milieu, thereby providing a holistic view of the microbiome–parasite–host axis that is unattainable through single-omics investigations [20]. Such approaches have begun to reveal how parasite-driven dysbiosis reshapes the metabolic output of the gut microbiome, which in turn reprograms host immunometabolic pathways and influences immune regulatory networks [21].

Understanding the mechanistic linkages among microbial dysbiosis, immunometabolic reprogramming, immune regulation, and therapeutic outcomes in the context of parasitic infection has profound translational implications. Current antiparasitic therapies, while effective at clearing infections, often fail to restore microbial homeostasis and may inadvertently exacerbate dysbiosis-associated immunopathology [22]. Moreover, the growing recognition that helminth-derived immunomodulatory molecules and microbiome-based interventions (e.g., probiotics, prebiotics, fecal microbiota transplantation) can ameliorate inflammatory and autoimmune conditions underscores the therapeutic potential of harnessing the microbiome–parasite–host axis [23], [24]. A comprehensive multi-omics framework is therefore essential to identify biomarkers of dysbiosis, elucidate immunometabolic checkpoints that govern disease outcomes, and develop precision therapeutic strategies that target the axis at multiple levels.

LITERATURE REVIEW

The Gut Microbiome: Composition, Function, and Ecological Dynamics

The human gut microbiome is a highly diverse and functionally redundant microbial ecosystem that undergoes dynamic changes throughout the host lifespan, influenced by factors such as diet, genetics, age, geography, medication use, and environmental exposures [25]. The dominant bacterial phyla in the healthy adult gut include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* collectively accounting for over 90% of the total bacterial community [26]. The Human Microbiome Project (HMP) and subsequent large-scale metagenomic initiatives have catalogued millions of microbial genes and established reference databases that have been instrumental in characterizing the functional potential of the gut microbiome [27].

Functionally, the gut microbiome contributes to the fermentation of indigestible dietary polysaccharides into SCFAs (principally acetate, propionate, and butyrate), the synthesis of essential vitamins (e.g., vitamin K, B vitamins), the biotransformation of bile acids, the metabolism of xenobiotics and drug [28]., and the maintenance of intestinal epithelial barrier integrity through the stimulation of mucus production and tight junction protein expression [29]. Butyrate, in particular, serves as the primary energy source for colonic epithelial cells and exerts potent anti-inflammatory effects through the inhibition of histone deacetylases (HDACs) and the activation of G-protein-coupled receptors (GPCRs) such as GPR43 and GPR109a [30]. The gut microbiome also plays a critical role in the education and maturation of the host immune system, as evidenced by studies in germ-free animals that exhibit profound defects in lymphoid tissue development, immunoglobulin A (IgA) production, and T cell differentiation [31].

The ecological stability of the gut microbiome, termed colonization resistance, is a key defense mechanism against pathogen invasion and is mediated by competitive exclusion, nutrient competition, production of antimicrobial compounds (e.g., bacteriocins), and immune-mediated clearance [32]. Perturbation of this ecological stability by antibiotics, dietary changes, or infectious agents can lead to dysbiosis, characterized by reduced microbial diversity, loss of keystone taxa, expansion of pathobionts, and altered metabolic output [33].

Parasitic Infections and Gut Microbial Dysbiosis

A growing body of evidence demonstrates that intestinal parasitic infections are associated with significant alterations in gut microbial community composition and function. Early studies using 16S rRNA gene amplicon sequencing revealed that helminth infections, such as those caused by *Trichuris trichiura* and *Ascaris lumbricoides*, are associated with increased microbial diversity and shifts in the relative abundance of specific bacterial taxa [34], [35]. For example, Cooper et al. [36] reported that *T. trichiura* infection in Ecuadorian children was associated with an increased abundance of *Prevotella* and a decreased abundance of *Bacteroides*, consistent with a shift toward a microbiome profile typically observed in non-Westernized populations. Similarly, Lee et al. [37] demonstrated that *Ascaris suum* infection in a porcine model led to significant reductions in *Lactobacillus* and *Clostridium* cluster XIVa, taxa known for their immunoregulatory and barrier-enhancing properties.

Protozoan infections also exert profound effects on the gut microbiome. *Giardia lamblia* infection has been shown to alter the microbial community structure by promoting the expansion of *Proteobacteria* and reducing the abundance of beneficial *Bifidobacterium* species, changes that may contribute to the post-infectious irritable bowel syndrome (IBS) observed in a subset of patients [38]. *Entamoeba histolytica* infection is associated with a marked decrease in microbial diversity and an increase in pro-inflammatory taxa, including members of the *Enterobacteriaceae* family, which may exacerbate tissue invasion and inflammation [39]. *Cryptosporidium* infection in neonatal calves and mice has been linked to delayed microbiome maturation and reduced abundance of SCFA-producing bacteria, potentially contributing to the growth faltering observed in infected children [40].

The mechanisms underlying parasite-induced dysbiosis are multifactorial. Helminths physically traverse and damage the intestinal epithelium, creating microenvironments that favor the colonization of specific bacterial taxa [41]. Parasite-derived excretory-secretory (ES) products contain proteases, glycosidases, and immunomodulatory molecules that can directly alter the mucosal environment and influence bacterial growth [42]. Additionally, the host immune response to parasites, particularly the type 2 immune response characterized by interleukin (IL)-4, IL-5, IL-13, and IgE production, can reshape the microbial community through effects on mucus production, epithelial turnover, and antimicrobial peptide secretion [43]. The increased mucus production driven by IL-13, for instance, creates a favorable niche for mucin-degrading bacteria such as *Akkermansia muciniphila* [44].

Immunometabolic Reprogramming During Parasitic Infection

Immunometabolism has emerged as a fundamental framework for understanding how immune cell function is governed by intracellular metabolic pathways [45]. The concept that distinct immune cell subsets rely on specific metabolic programs for their activation, differentiation, and effector functions has transformed our understanding of immune regulation in health and disease [46]. During parasitic infection, the metabolic demands of the immune system are profoundly altered, and these changes are intimately linked to the nature of the immune response mounted against the parasite.

Helminth infections typically elicit a type 2 immune response, which is characterized by the activation of alternatively activated (M2) macrophages, eosinophils, basophils, mast cells, and T helper 2 (Th2) cells [47]. The metabolic signature of M2 macrophages is distinct from that of classically activated (M1) macrophages; whereas M1 macrophages rely on aerobic glycolysis and a truncated tricarboxylic acid (TCA) cycle for rapid ATP generation and biosynthetic precursor production, M2 macrophages depend on intact oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO), and a complete TCA cycle fueled by glutamine and fatty acids [48]. O'Neill and Pearce [15] demonstrated that the induction of FAO by IL-4 signaling through the STAT6 pathway is essential for M2 macrophage polarization, and pharmacological inhibition of FAO impairs the ability of these cells to mediate tissue repair and parasite expulsion.

Regulatory T cells (Tregs), which are expanded during chronic helminth infections and play a critical role in limiting immunopathology, also exhibit a distinct metabolic profile characterized by high rates of FAO and OXPHOS [49]. The transcription factor Foxp3, which is the master regulator of Treg development and function, promotes mitochondrial fitness and FAO while suppressing glycolysis and mTOR signaling [50]. The gut microbiome contributes to Treg induction and maintenance through the production of SCFAs, particularly butyrate and propionate, which promote Treg differentiation via HDAC inhibition and GPCR signaling [51]. Parasite-induced dysbiosis that reduces SCFA-producing bacteria may therefore compromise Treg-mediated immune regulation, potentially leading to exacerbated inflammation or impaired parasite control.

In contrast, protozoan infections such as those caused by *E. histolytica* and *Toxoplasma gondii* tend to elicit type 1 immune responses dominated by interferon-gamma (IFN-γ)-producing Th1 cells and M1 macrophages, which are metabolically characterized by upregulated glycolysis, glutaminolysis, and a broken TCA cycle with accumulation of metabolites such as succinate and itaconate [52]. Succinate accumulation stabilizes hypoxia-inducible factor 1-alpha (HIF-1α), which drives glycolytic gene expression and IL-1β production, while itaconate, produced by the enzyme IRG1 (ACOD1), exerts anti-inflammatory and antimicrobial effects by inhibiting succinate dehydrogenase and activating the Nrf2 antioxidant pathway [53]. The metabolic rewiring of immune cells during protozoan infections is further influenced by microbiome-derived metabolites; for example, secondary bile acids produced by gut bacteria can modulate the inflammasome activation and cytokine production of macrophages responding to *E. histolytica* [54].

Immune Regulation at the Gut Mucosal Interface

The gut mucosal immune system represents the largest immune organ in the body and must maintain a delicate balance between tolerance to commensal microorganisms and dietary antigens, and the capacity to mount robust

responses against pathogens and parasites [55]. This balance is achieved through a complex network of innate and adaptive immune cells, including intestinal epithelial cells (IECs), dendritic cells (DCs), macrophages, innate lymphoid cells (ILCs), Tregs, Th17 cells, and IgA-producing B cells [56].

Intestinal epithelial cells serve as the first line of defense and play an active role in immune regulation through the production of cytokines (e.g., thymic stromal lymphopoietin [TSLP], IL-25, IL-33), antimicrobial peptides (e.g., defensins, RegIIIγ), and the maintenance of the mucus barrier [57]. Parasite infection can directly alter IEC function; for example, helminth-derived proteases can cleave tight junction proteins and increase epithelial permeability, while protozoan trophozoites can induce epithelial apoptosis and disrupt barrier integrity [58]. The resulting translocation of microbial products across the epithelial barrier can activate mucosal DCs and macrophages, driving inflammatory responses that may contribute to tissue damage [59].

Mucosal DCs play a pivotal role in shaping the adaptive immune response to parasites by sampling luminal antigens and presenting them to T cells in the mesenteric lymph nodes [60]. The functional phenotype of DCs is strongly influenced by microbiome-derived signals; for instance, SCFAs promote the differentiation of tolerogenic DCs that induce Treg responses, while the absence of microbial signals leads to the development of pro-inflammatory DCs that drive Th1 and Th17 responses [61]. During helminth infection, DCs conditioned by both parasite ES products and microbiome-derived metabolites adopt a regulatory phenotype characterized by the production of retinoic acid and TGF-β, which promotes Th2 and Treg differentiation [62].

Group 2 innate lymphoid cells (ILC2s) have emerged as critical early responders to helminth infection, producing large amounts of IL-5 and IL-13 in response to epithelial-derived alarmins (IL-25, IL-33, TSLP) [63]. ILC2s are regulated by the gut microbiome; studies have shown that germ-free mice have reduced ILC2 numbers and impaired type 2 immunity, which can be restored by colonization with specific bacterial species or administration of SCFAs [64]. The interplay between ILC2s, the microbiome, and parasitic infection represents a key node in the microbiome–parasite–host axis that warrants further investigation.

Multi-Omics Approaches to Studying the Microbiome–Parasite–Host Axis

The complexity of the microbiome–parasite–host axis necessitates the application of integrative multi-omics approaches that can capture the dynamic interactions among microbial communities, host cells, and the metabolomic environment [65]. Single-omics studies, while valuable, provide only a partial view of this complex system and often fail to establish causal linkages among microbial changes, metabolic alterations, and immune outcomes [66].

Metagenomics and Metatranscriptomics: Shotgun metagenomic sequencing provides species- and strain-level resolution of the gut microbial community and enables the reconstruction of microbial metabolic pathways, offering insights into the functional potential of the microbiome [67]. Metatranscriptomics, which profiles the actively transcribed microbial genes, adds a layer of functional activity that complements metagenomic data and reveals how parasites alter microbial gene expression in real time [68]. Studies employing these approaches have identified parasite-associated shifts in microbial carbohydrate-active enzymes (CAZymes), bile salt hydrolases, and SCFA biosynthesis pathways, suggesting that parasites can modulate the metabolic output of the microbiome [69].

Metabolomics: Untargeted and targeted metabolomics platforms, including liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy, enable the comprehensive profiling of small-molecule metabolites in fecal, serum, and tissue samples [70]. Metabolomic studies of parasitic infections have revealed alterations in SCFA levels, bile acid profiles, amino acid metabolism, and lipid mediators that correlate with immune cell activation states and disease outcomes [71]. For example, Houlden et al. [72] demonstrated that *Trichuris muris* infection in mice was associated with significant changes in the fecal metabolome, including reductions in butyrate and increases in lysophosphatidylcholines, which modulated mucosal immune responses.

Host Transcriptomics and Proteomics: RNA sequencing (RNA-seq) of host tissues and single-cell RNA sequencing (scRNA-seq) of immune cells provide high-resolution maps of host gene expression changes during parasitic infection [73]. These approaches have revealed the transcriptional programs underlying immune cell metabolic reprogramming, including the upregulation of FAO genes in M2 macrophages and Tregs during helminth infection, and the activation of glycolytic and HIF-1α-dependent pathways during protozoan infection [74]. Proteomic analyses of host tissues and parasite ES products have identified post-translational modifications, protein-protein interactions, and signaling pathway alterations that complement transcriptomic data [75].

Data Integration and Computational Approaches: The integration of multi-omics datasets requires sophisticated computational and statistical frameworks, including multi-block partial least squares (MB-PLS), canonical correlation analysis (CCA), network-based approaches (e.g., weighted gene co-expression network analysis [WGCNA]), and machine learning algorithms [76]. These methods enable the identification of cross-omics correlations, the construction of predictive models of disease outcomes, and the inference of causal relationships among microbial, metabolic, and immune variables [77]. Recent advances in artificial intelligence and deep learning have further enhanced the capacity to integrate heterogeneous omics data and identify novel biomarkers and therapeutic targets [78].

Therapeutic Implications and Outcomes

The recognition that the gut microbiome–parasite–host axis influences disease outcomes has opened new avenues for therapeutic intervention. Current antiparasitic drugs, such as albendazole, mebendazole, and praziquantel, are

effective at reducing parasite burden but do not address the underlying dysbiosis and may even exacerbate microbial imbalances [79]. Studies have shown that anthelmintic treatment can lead to transient increases in pro-inflammatory bacterial taxa and reductions in SCFA-producing bacteria, potentially contributing to the increased risk of allergic and autoimmune diseases observed in populations following deworming campaigns [80].

Microbiome-targeted interventions, including probiotics, prebiotics, synbiotics, and fecal microbiota transplantation (FMT), have shown promise in restoring microbial homeostasis and modulating immune responses in the context of parasitic infection [81]. Probiotic strains such as *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* have been shown to enhance type 2 immune responses and accelerate parasite clearance in animal models of helminth infection [82]. FMT from healthy donors has been explored as a strategy to restore microbial diversity and SCFA production in patients with post-infectious IBS following giardiasis [83].

The therapeutic potential of helminth-derived immunomodulatory molecules has also gained attention, with clinical trials investigating the use of controlled helminth infections (helminth therapy) and purified ES products for the treatment of IBD, multiple sclerosis, and allergic diseases [84]. The efficacy of these approaches is likely influenced by the baseline gut microbiome composition, highlighting the importance of understanding the microbiome–parasite–host axis for patient stratification and treatment optimization [85].

Furthermore, metabolite-based therapies that target immunometabolic checkpoints represent an emerging frontier. Supplementation with butyrate or butyrate-producing bacterial consortia has been shown to enhance Treg function and reduce inflammation in models of colitis and parasitic infection [86]. Pharmacological modulators of metabolic pathways, such as mTOR inhibitors, AMPK activators, and FAO enhancers, are being investigated for their potential to modulate immune responses in infectious and inflammatory diseases [87].

METHODOLOGY:

Study Design & Setting;

Prospective longitudinal cohort study conducted at the Pakistan Institute of Medical Sciences (PIMS) and affiliated diagnostic centers in Islamabad, Pakistan (Jan 2025 – Dec 2025).

Participant Cohorts:

Group A (Infected, n=120): Patients with confirmed intestinal parasitic infection (*Giardia duodenalis*, *Entamoeba histolytica*, or soil-transmitted helminths) via stool microscopy/PCR.

Group B (Post-Treatment, n=120): Same patients sampled 4 and 8 weeks post-standard anthelmintic/antiprotozoal therapy (Albendazole/Metronidazole).

Group C (Healthy Controls, n=60): Age- and sex-matched asymptomatic individuals from the same geographic catchment area in Islamabad.

Sample Collection: Stool:

Fresh fecal samples collected in OMNIgene•GUT kits for immediate stabilization of microbial DNA/RNA and metabolites.

Blood: Peripheral blood mononuclear cells (PBMCs) isolated via Ficoll-Paque density gradient centrifugation for host transcriptomics and immunophenotyping.

Serum: Collected for systemic metabolomic and cytokine profiling.

Metagenomics (Microbiome): Shotgun metagenomic sequencing (Illumina NovaSeq 6000, 2x150 bp). DNA extracted using QIAamp PowerFecal Pro DNA Kit. Reads processed via QIIME 2 and Kraken 2/Bracken for taxonomic profiling; HUMAnN 3 for functional pathway analysis [15].

Metabolomic: Untargeted and targeted Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) on stool and serum samples. Focused on short-chain fatty acids (SCFAs), primary/secondary bile acids, and tryptophan metabolites. Data processed via XCMS and MetaboAnalyst 5.0 [32]

Host Transcriptomics & Immune Profiling:

Bulk RNA-sequencing (RNA-seq) of PBMCs (Illumina). Differential expression analyzed using DESeq2. Complementary high-sensitivity multiplex ELISA (Luminex) for 30-plex cytokine/chemokine panel (e.g., IL-4, IL-10, IL-13, IFN-γ, TGF-β) [43].

Multi-Omics Data Integration: Statistical integration performed using Multi-Block Partial Least Squares (MB-PLS) and Sparse Canonical Correlation Analysis (sCCA) in R (v4.3.1). Network analysis conducted via Cytoscape to map microbe-metabolite-immune interactions [14].

RESULTS

The results of the multi-omics investigation are comprehensively presented in tabular format below, detailing demographic baselines, microbial dysbiosis metrics, immunometabolic shifts, immune regulation markers, and therapeutic outcomes specific to the Islamabad cohort.

TABLE II: DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY COHORT (N = 300)

Variable	Group A: Infected (n=120)	Group C: Healthy Controls (n=60)	p-value
Mean Age (years) ± SD	28.4 ± 12.1	29.1 ± 11.5	0.68
Gender (Male / Female)	68 (56.7%) / 52 (43.3%)	32 (53.3%) / 28 (46.7%)	0.72

Residence (Urban / Peri-urban Islamabad)	75 (62.5%) / 45 (37.5%)	40 (66.7%) / 20 (33.3%)	0.55
Parasite Species Identified	*G. duodenalis* (58%), *E. histolytica* (25%), STH* (17%)	N/A	N/A
Mean BMI (kg/m²) ± SD	21.3 ± 3.4	23.8 ± 2.9	< 0.001
Reported GI Symptoms (Diarrhea, Bloating, Pain)	112 (93.3%)	4 (6.7%)	< 0.001

Table II confirms that the infected and healthy cohorts were well-matched for baseline demographics (age, gender, and residence), ensuring that observed differences are driven by infection status rather than confounding variables. The infected cohort was primarily affected by *Giardia duodenalis* (58%), followed by *Entamoeba histolytica* (25%) and soil-transmitted helminths (17%). Clinically, this parasitic burden translated to significant morbidity, evidenced by a notably lower mean Body Mass Index (BMI) and a 93.3% prevalence of severe gastrointestinal symptoms (such as diarrhea, bloating, and pain) in the infected group compared to the healthy controls ($p < 0.001$).

TABLE III: GUT MICROBIOME DYBIOSIS METRICS (SHOTGUN METAGENOMICS)

Microbial Metric	Group A: Infected	Group C: Healthy Controls	Statistical Significance
Alpha Diversity (Shannon Index)	3.12 ± 0.45	4.25 ± 0.38	$p < 0.001$ (↓ 26.5%)
Alpha Diversity (Chao1 Richness)	145.3 ± 22.1	198.7 ± 18.4	$p < 0.001$
Beta Diversity (Bray-Curtis PERMANOVA)	Distinct clustering observed ($R^2 = 0.18$)	Reference centroid	$p = 0.001$
Depleted Taxa (FDR < 0.05)	*Faecalibacterium prausnitzii*, *Bifidobacterium longum*, *Akkermansia muciniphila*	Baseline abundance	FDR < 0.01
Enriched Taxa (FDR < 0.05)	*Escherichia coli*, *Enterococcus faecalis*, *Proteobacteria* (phylum level)	Baseline abundance	FDR < 0.01
Functional Shift (HUMAnN 3)	↓ Butyrate kinase pathway; ↑ Lipopolysaccharide (LPS) biosynthesis	Baseline metabolic profile	FDR < 0.05

Table III demonstrates that parasitic infection induces severe structural and functional dysbiosis within the gut microbiome. Infected individuals exhibited a significant 26.5% reduction in microbial diversity (Shannon Index) and overall richness compared to healthy controls, resulting in a distinctly altered community structure. Taxonomically, this manifested as the targeted depletion of crucial, barrier-protecting commensals (e.g., *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*) and a concurrent overgrowth of pro-inflammatory pathobionts, such as *Proteobacteria* and *E. coli*. Functionally, this ecological imbalance directly impaired the microbiome's metabolic output, characterized by a downregulation of anti-inflammatory butyrate synthesis pathways and an upregulation of lipopolysaccharide (LPS) biosynthesis, thereby establishing a highly inflammatory gut environment that primes the host for immune dysregulation.

TABLE IV: IMMUNOMETABOLIC PROFILING: KEY METABOLITE ALTERATIONS (LC-MS/MS)

Metabolite Class	Specific Metabolite	Group A (Infected)	Group C (Healthy)	Fold Change (A vs C)	p-value
Short-Chain Fatty Acids (SCFAs)	Butyrate (µmol/g feces)	12.4 ± 3.1	28.6 ± 4.5	-2.3x	< 0.001
	Propionate (µmol/g feces)	18.2 ± 4.0	31.5 ± 5.2	-1.7x	< 0.01
Bile Acids	Secondary Bile Acids (DCA, LCA)	4.1 ± 1.2	15.8 ± 3.4	-3.8x	< 0.001
Tryptophan Metabolites	Indole-3-propionic acid (IPA)	0.8 ± 0.3	2.9 ± 0.6	-3.6x	< 0.001
Systemic Inflammation	Serum Lipopolysaccharide (LPS) (EU/mL)	0.45 ± 0.12	0.18 ± 0.05	+2.5x	< 0.001

Table IV reveals a profound collapse in beneficial, immunoregulatory microbial metabolites in infected individuals, directly linking gut dysbiosis to host immunopathology. The significant depletion of short-chain fatty acids (butyrate and propionate), secondary bile acids, and the tryptophan metabolite IPA strips the gut mucosa of its primary anti-inflammatory and barrier-protecting signals. Concurrently, this metabolic void is accompanied by a 2.5-fold increase in systemic serum lipopolysaccharide (LPS), indicating compromised intestinal barrier integrity ("leaky gut") and the translocation of bacterial endotoxins into the bloodstream, which actively drives systemic inflammation and immune dysregulation.

TABLE V: HOST IMMUNE REGULATION MARKERS (PBMC TRANSCRIPTOMICS & MULTIPLEX ELISA)

Immune Parameter	Group A: Infected	Group C: Healthy Controls	Correlation with Butyrate (r)	p-value
Regulatory T cells (Tregs, % of CD4+)	4.2% ± 1.1%	8.5% ± 1.8%	$r = 0.68$	< 0.001
Serum IL-10 (pg/mL)	15.3 ± 4.2	32.1 ± 6.5	$r = 0.71$	< 0.001
Serum IL-4 / IL-13 (Type 2 response)	Elevated (2.1x baseline)	Baseline	$r = -0.45$	< 0.01
Serum IFN-γ (pg/mL)	45.6 ± 10.2	18.4 ± 5.1	$r = -0.62$	< 0.001
PBMC Gene Expression (RNA-seq)	Upregulation of *HIF1A*, *IL1B*, *NLRP3*	Baseline homeostasis	N/A	FDR < 0.05
PBMC Gene Expression (RNA-seq)	Downregulation of *FOXP3*, *PPARG*	Baseline homeostasis	N/A	FDR < 0.05

TABLE VI: THERAPEUTIC OUTCOMES POST-TREATMENT (LONGITUDINAL TRACKING AT 8 WEEKS)

Outcome Metric	Group A: Baseline (Infected)	Group B: 8 Weeks Post-Treatment	Group C: Healthy Control	Statistical Comparison (Post-Tx vs Baseline)
Parasite Clearance Rate	100% (by design)	114/120 (95.0%)	N/A	N/A
Shannon Diversity Index	3.12 ± 0.45	3.65 ± 0.41	4.25 ± 0.38	$p < 0.01$ (Partial recovery)
Fecal Butyrate (μmol/g)	12.4 ± 3.1	19.8 ± 3.8	28.6 ± 4.5	$p < 0.001$ (Significant improvement, but not full normalization)
Serum IL-10 (pg/mL)	15.3 ± 4.2	24.5 ± 5.1	32.1 ± 6.5	$p < 0.01$
Persistent GI Symptoms (Post-infectious IBS)	112 (93.3%)	28 (23.3%)	4 (6.7%)	$p < 0.001$
Microbiome "Resilience" Score*	N/A	0.68 ± 0.12	1.00	N/A

Resilience Score: Calculated as the Bray-Curtis similarity of the post-treatment microbiome to the individual's theoretical healthy baseline (1.0 = full recovery).

The data presented in Tables V and VI reveal a critical disconnect between parasitological cure and complete immunometabolic recovery. During active infection, the severe depletion of microbiome-derived butyrate strongly correlates with a collapse in regulatory immunity (reduced Tregs and IL-10) and a simultaneous surge in pro-inflammatory pathways (elevated IFN-γ and upregulated *NLRP3* and *IL1B* gene expression). Crucially, while standard pharmacological treatment achieved a 95% parasite clearance rate at 8 weeks, it failed to fully restore the gut ecosystem. The microbiome resilience score remained suboptimal (0.68), and key metabolites like butyrate, alongside immune markers like IL-10, only showed partial improvement. This incomplete ecological and immunological normalization directly explains why nearly a quarter (23.3%) of treated patients continued to experience persistent gastrointestinal symptoms, demonstrating that mere parasite eradication is insufficient for holistic mucosal healing and highlighting the need for adjunctive microbiome-restorative therapies.

CONCLUSION

In conclusion, this prospective multi-omics investigation conducted in Islamabad, Pakistan, provides comprehensive, high-resolution evidence that intestinal parasitic infections induce profound, multi-layered disruptions across the gut microbiome–parasite–host axis. The integration of metagenomic, metabolomic, and host transcriptomic data reveals that infections predominantly driven by *Giardia duodenalis*, *Entamoeba*

histolytica, and soil-transmitted helminths are not merely localized gastrointestinal disturbances, but complex ecological and immunometabolic crises that extend well beyond the immediate presence of the pathogen.

A central finding of this study is the direct mechanistic linkage between parasite-induced microbial dysbiosis and host immune dysregulation. The marked 26.5% reduction in microbial alpha diversity and the specific depletion of keystone short-chain fatty acid (SCFA)-producing taxa, such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, translated into a severe metabolomic collapse. The greater than two-fold reduction in fecal butyrate and secondary bile acids was strongly correlated with a breakdown in mucosal immune tolerance. This metabolite depletion coincided with the depletion of regulatory T cells (Tregs) and IL-10 production, alongside the hyperactivation of pro-inflammatory pathways, including the *NLRP3* inflammasome and IFN-γ signaling. These results confirm that the immunopathology and barrier dysfunction observed in this cohort are heavily mediated by the loss of microbiome-derived immunoregulatory metabolites, rather than being solely a direct result of parasite-host physical interactions.

Crucially, our longitudinal therapeutic tracking exposes a significant limitation in current clinical management paradigms. While standard pharmacological treatment with albendazole and metronidazole achieved a 95% parasitological cure rate, it failed to restore microbial and immunometabolic homeostasis. The post-treatment "Microbiome Resilience Score" of 0.68 indicates persistent ecological instability, which clinically manifested as post-infectious gastrointestinal symptoms in 23.3% of the cohort. This demonstrates that parasite eradication alone is insufficient for holistic patient recovery in the Pakistani clinical setting, leaving a substantial portion of patients vulnerable to chronic post-infectious complications, such as irritable bowel syndrome (IBS).

These findings necessitate a paradigm shift in the therapeutic management of intestinal parasitoses in South Asia. The persistent dysbiosis and unresolved immunometabolic inflammation highlight an urgent need for adjunctive, microbiome-targeted interventions. Future clinical protocols in Islamabad and broader endemic regions should evaluate the incorporation of next-generation probiotics, targeted prebiotics, or postbiotic supplementation (e.g., exogenous butyrate) alongside standard anthelmintic and antiprotozoal therapies. Such combinatorial approaches are essential to accelerate mucosal healing, restore immunometabolic checkpoints, and prevent long-term post-infectious sequelae.

Ultimately, this study establishes a robust, multi-omics baseline for the gut microbiome–parasite–host axis within an endemic South Asian population. By elucidating the specific metabolic and immunological checkpoints disrupted by parasitic infections, this research lays the foundational framework for developing precision medicine strategies that target not only the pathogen but also the compromised host-microbiome ecosystem, thereby improving long-term clinical outcomes in resource-constrained settings.

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