Rural Embodiment and Child Health (REACH) Study:
Macroparasite infection prevalence and associated immune responses among a preliminary sample of children from rural Mississippi

Tara J. Cepon-Robins[1], Elizabeth K. Mallott[2], Isabella C. Recca[1], Theresa E. Gildner[3]

[1]Department of Anthropology, University of Colorado Colorado Springs; [2]Department of Biological Sciences, Vanderbilt University; [3]Department of Anthropology, Washington University in St. Louis

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Introduction

Parasitic infections caused by helminths (i.e., worms) and protozoa (i.e., single-celled eukaryotes) are considered Neglected Diseases of Poverty because they receive little research or medical attention. This categorization leads many to believe they are not common in "wealthy" nations like the United States[1]. Surveys through the 1980s, however, found these infections were endemic in the U.S., with more recent studies suggesting they remain common[2-4].

We present preliminary data on parasitic infection status, white blood cell count, hemoglobin levels, and parent-reported energy/activity levels from 24 children (ages 0.5 to 14 years) from 12 households in a rural Mississippi Delta community experiencing frequent flooding and sewage backups due to infrastructural neglect (Figures 1 and 2)[2]. The goal of this preliminary research is to raise awareness of parasitic infections in the U.S. and understand how they may be affecting child health and development.

Figure 1 (left). A bayou runs through the study community and contributes to flooding. **Figure 2 (right).** The study community has experienced infrastructural neglect, contributing to parasite risk.

Methods

Stool Samples

- **Parasite presence:** 18S rRNA gene amplification and sequencing to assess macroparasite infection status. Infection status is further divided into the following infection types:
  - ❖ **Helminth Infection Status:** Infection with intestinal parasitic worms
  - ❖ **Protozoal Infection Status:** Infection with intestinal protozoa
  - ❖ Multiple types of helminths and protozoa were detected, but due to a small sample size, they are considered collectively.

Finger-prick Blood Samples (for children over 3 years of age; n = 20)

- **White Blood Cell Count (WBCC):** A biomarker of general immune response measured using the Hemocue WBC analyzer. Reported in cells per cubic millimeter (cmm).
- **Hemoglobin (Hb):** A biomarker of iron levels measured using the Hemocue Hb 201+ analyzer. Levels used to determine anemia status.
  - ❖ **Anemia Status:** Anemic vs. not, based on age specific cutoffs: <11 g/dL (ages 3-4 years); <11.5 g/dL (ages 5-11); <12 g/dL (ages 12-14)[5].

Parent Interview Data

- **Age:** in years, confirmed by birth date.
- **Biological Sex:** (0: Female; 1: Male).
- **Child Activity Level:** Rated 0 to 10 (0 – extremely inactive, 10 – extremely active).
- **Child Energy Level:** Rated 0 to 10 (0 – not energetic, 10 – extremely energetic).

Statistical Analyses

- Statistical analyses were conducted using SPSS 28.
- Fisher's Exact Tests compared infection statuses based on sex and anemia status.
- BCa bootstrap ANOVA tests compared age, WBCC, Hb level, Activity Level, and Energy Level based on infection statuses.
- For a conservative interpretation of the results, *p* values (< 0.05), Bca bootstrap 95% confidence intervals, and/or effects sizes are used together when possible to interpret significance. Cutoffs for small, medium, and large effect sizes based on Eta Square (ηp^2) are 0.01, 0.06, 0.14, respectively[6].

Predictions

We predict that:

1. Parasite infections will be detected in stool samples.
2. These infections will be associated with the following markers of immune function, nutritional status, and behavior:
   - Parasitic infections will be positively associated with WBCC.
   - Parasitic infections will be negatively associated with Hb levels.
   - Parasitic infections will negatively impact activity and energy levels.

Results

Table 1 shows descriptive statistics for all participants. Values are presented as mean (SD) or % (n). 38% (n = 9) of children were infected with at least one parasite. More specific types of helminth infections include platyhelminths (flatworms like tapeworms; n = 5) and nematodes (roundworms like hookworm; n = 2). Specific types of protozoal infections included blastocystis (n = 4) and cryptosporidium (n = 1).

Table 1.	
Parent Interview Data/Infection Status	**N = 24**
Female Sex	50% (n = 12)
Age in years	7 (4.09)
Activity Level	8.75 (2.03)
Energy Level	9.08 (1.67)
Infection Status	**N = 24**
Any Parasite	38% (n = 9)
Helminths	25% (n = 6)
Protozoa	21% (n = 5)
Helminth/Protozoa Coinfection	8% (n = 2)
Finger-prick Blood Markers	**N = 20**
White Blood Cell Count (WBCC; cmm)	6.46 (1.49)
Hemoglobin (Hb; g/dl)	11.63 (0.94)
Anemia	50% (n = 10)

No infection variables were associated with age or sex. Infection status also did not cluster based on household. Infections were present in 8 of the 12 households.

Parasite infection in general ($p = 0.05$; $\eta p^2 = 0.30$) and protozoal infection specifically ($p < 0.001$; $\eta p^2 = 0.66$) (**Figure 3**) were associated with higher WBCC. Helminth infection was not associated with WBCC ($p = 0.60$; $\eta p^2 = 0.03$).

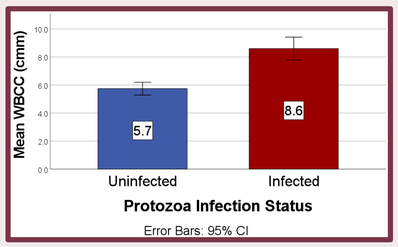

Figure 3. Mean WBCC based on protozoa infection status

Infection variables were not related to Hb level as a continuous variable, but general parasite infection status was significantly associated with anemia status ($p = 0.04$; BCa 95% CI = 0.08, 0.90) (**Table 2**). Helminth infection specifically was also associated with anemia status (**Table 3**) ($p = 0.07$; BCa 95% CI = 0.05, 0.79). Protozoa infection was not associated with anemia status.

Table 2. Anemia status based on general parasite infection status		
	Infected (n = 9)	Uninfected (n = 11)
Anemic	78% (n = 7)	27% (n = 3)
Normal	22% (n = 2)	73% (n = 8)

Table 3. Anemia status based on helminth infection status		
	Infected (n = 6)	Uninfected (n = 14)
Anemic	83% (n = 5)	36% (n = 5)
Normal	17% (n = 1)	64% (n = 9)

There were no significant associations between activity or energy level and any of the infection variables. Protozoa infected children, however, were less active (mean = 7.67) and less energetic (mean = 8.83) than uninfected children (means = 9.11 and 9.17, respectively) ($p = 0.13$; $\eta p^2 = 0.10$, and $p = 0.68$; $\eta p^2 = 0.008$).

Key Findings

- The following parasite infections were detected in stool samples among this small sample (N = 24) of children from rural Mississippi:
  - ❖ Helminths (25%; n = 6): Five platyhelminth infections were detected - likely tapeworm based on parallel microscopic evidence (**Figure 4**). Two nematode infections were also detected, with one categorized more specifically into the category Rhabditida (e.g., hookworm).
  - ❖ Protozoa (21%; n = 5): Blastocystis (n = 4) and Cryptosporidium (n = 1) were detected.
- Protozoal infections were associated with higher WBCC.
- General infection and helminth infection were associated with anemia.
- There were no significant relationships between parasite infection and parent-reported child activity or energy level, although protozoal infections did appear to have a medium ($\eta p^2 = 0.10$) effect on reduced activity level.

Figure 4. Tapeworm egg detected microscopically (400X)

Discussion and Future Directions

This study found preliminary support for our predictions that parasite infection may still be common in regions of the Southern U.S. and may be affecting child health and development related to immune function, nutritional status, and activity level.

Parasites and Immune Function: Protozoal infections appear to increase general immune activity, as evidenced by higher WBCC in infected children. Both types of protozoa detected are associated with diarrheal disease in acute cases but can also be chronic and symptomless[7]. Acute immune responses or chronic immune upregulation could be at play here and more longitudinal data is needed. A larger sample and more immune markers will also increase understanding of how parasitic infections alter immune health and development.

Parasites and Nutrition Status: Parasite infection in general and helminth infection specifically appear to contribute to anemia risk. Probable types of helminths detected here (e.g., tapeworm and hookworm) are known to cause anemia[8]. Importantly, all children in this sample identified as Black and anemia prevalence has been documented at higher levels in Black Americans.[9] **That anemia prevalence appears to be associated with helminth infections in this sample is noteworthy and may speak to the role of environmental inequities in racial health disparities.** A larger comparative sample and more robust measures of nutritional status will allow us to test how parasite infection is affecting child nutritional status with implications for growth and development.

Parasites and Activity Level: Energy demands associated with mounting a costly immune response, combined with reduced nutritional status in association with infection, may result in tradeoffs associated with energy expenditure. **While the results were not significant, the findings that children infected with protozoa were less active might suggest that sickness behaviors and symptoms of infection may be impacting their daily lives.** More measures of activity level in a larger sample could speak to how infection impacts behavior, educational success, and more.

Support

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Citations

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