Molecular Diagnostics for Gastrointestinal Parasites and Impact on Intestinal Microbiota in Rural Argentinian Children

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Introduction

- >2 billion GI parasite infections worldwide
  - Poorest and resource-deprived communities
- Standard method of diagnosis: Stool microscopy
  - Sensitivity variable depending on prevalence, species, and concentration method
    - 50-90% sensitivity
  - Underestimates polyparasitism
- qPCR is rapid, quantitative, and high-throughput species-specific method
- GI parasites may disrupt normal intestinal microbiota
  - Decreased biodiversity is associated with disease
    - Malabsorption
    - Inflammatory bowel diseases

Materials and methods

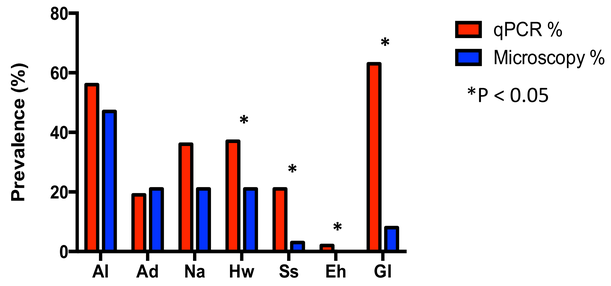
- Field site: Orán, Argentina
  - Peri-urban community
  - Temperate climate
- 99 patient samples
  - Asymptomatic children
  - Ages 2-10 years old
  - No recent antibiotics
- Stool samples evaluated by qPCR and microscopy for presence of :

Ascaris lumbricoides (Al) *Strongyloides stercoralis* (Ss)
Ancylostoma duodenale (Ad) *Giardia lamblia* (Gl)
Necator americanus (Na) *Cryptosporidium* species (C)
Trichuris trichiura (Tt) *Entamoeba histolytica* (Eh)

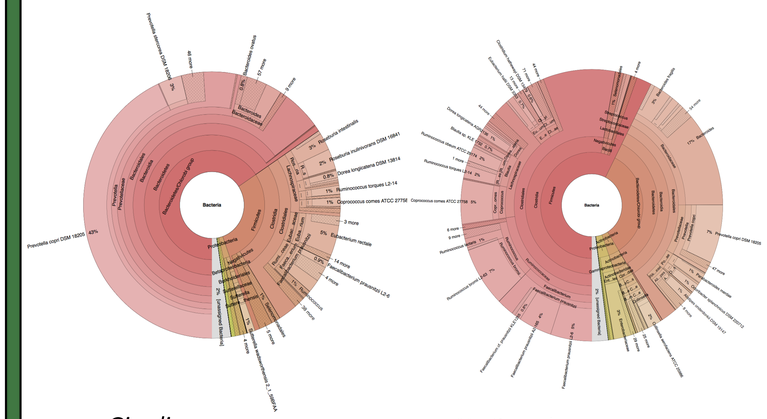
- qPCR required 50 mg samples
- Microscopy required 2 g samples
  - McMaster technique (semi-quantitative)
- NEBNext® Microbiome DNA Enrichment Kit
- NEBNext® Ultra™ DNA Library Prep Kit for Illumina®
- Illumina MiSeq® “shotgun” sequencing

Results

- qPCR (ITS region) (ABI 7500) identified more cases of *Ascaris*, hookworm (Hw), *Strongyloides, Entamoeba histolytica* and *Giardia* infection than microscopy. (Tt, C) no positives

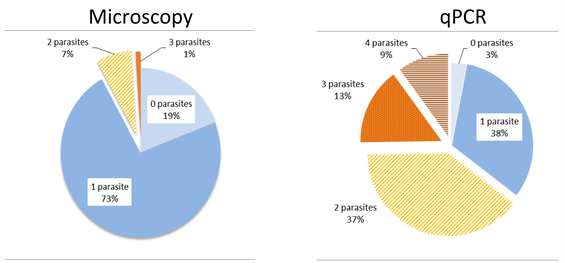


- Decreased intestinal bacterial biodiversity in combined *Giardia* group versus No parasite group

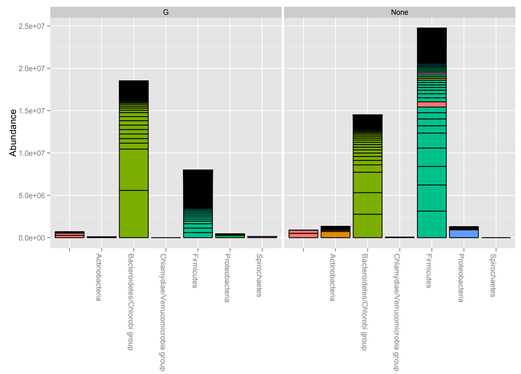


Giardia group No Parasite group

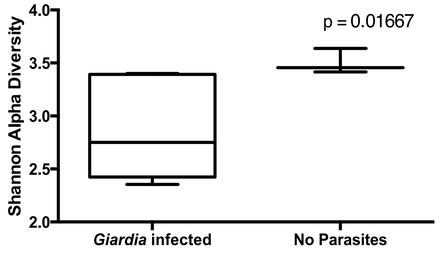
- qPCR identifies more polyparasitism than microscopy



- *Giardia* infected group had higher abundance of *Bacteroidetes* compared to No Parasites group with higher *Firmicutes* ($p < 0.05$)



- *Giardia* infection decreases intestinal bacterial biodiversity



Conclusions

- qPCR can detect more parasites than microscopy
  - *Ascaris* 91.3% Sens, 90.5% NPV
  - Hookworm 95.5% Sens, 98.4% NPV
  - *Strongyloides* 100% Sens, NPV
  - *Giardia* 87.5% Sens, 97.2% NPV
- qPCR can identify polyparasitism better than microscopy
  - Important for treatment selection
- GI parasitic infections at high prevalence
  - qPCR detected *Giardia* 6 x more than microscopy
- *Giardia* infected group had decreased intestinal microbiota biodiversity ($p = 0.01667$)
  - *Giardia* infected group (2.7)
  - No Parasite group (3.45)
- *Giardia* infected group had significant increases in *Bacteroidetes* specifically *Prevotella* species
- Useful for epidemiology and morbidity studies
  - Surveillance after mass drug administration and vaccine programs
  - Expand understanding of morbidity and malnutrition
  - Cost is less than $1.00 US per patient to screen for these parasites
- Future directions
  - Correlate quantity of parasite DNA with clinical outcomes
  - Associate morbidity to changes in microbiome
  - Treat children with anti-parasitics and evaluate changes in microbiome

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