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# **ABSTRACT BOOK**

# "There will be epidemics..."



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#### IS ENTAMOEBA DISPAR PATHOGENIC?

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*Entamoeba dispar* is generally considered non-pathogenic while *E. histolytica* is known to cause human infections. Because the two species cannot be distinguished based on morphological characteristics, the Wadsworth Center Parasitology Laboratory within the New York State Department of Health, developed a real-time PCR assay to distinguish between the two species. Over 250 specimens were tested using stool samples received in 2017 and 2018. Interestingly, a majority of submitted specimens contained *E. dispar* and not *E. histolytica*. Only 5.3% of the cases submitted were positive for *E. histolytica* whereas a much greater proportion, 83.3%, were positive for *E. dispar*. Samples that were positive for *Entamoeba* based on microscopy but negative for both *E. dispar* and *E. histolytica* by molecular analysis account for 11.4% of specimens tested. Because the specimens were collected from individuals seeking medical attention for intestinal illness, our results suggest that the pathogenicity status of *E. dispar* should be revisited.

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### IMPACT OF *GIARDIA* ON INTESTINAL MICROBIOTA AND VITAMIN B12 BIOSYNTHESIS IN PRESCHOOL CHILDREN

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Depending on the species, parasites can disrupt intestinal bacterial flora affecting nutritional status. Using multi-parallel guantitative real-time PCR (gPCR) and whole genome sequencing analysis for bacterial microbiota and Giardia lamblia (assemblage A or B). Stool samples were collected longitudinally from 100 children sampled at 3 and again at 5 years old from Ecuador. Uninfected versus Giardia only groups were analyzed for microbiome and metagenomic differences and compared to growth delays. For Giardia only infected children, longitudinal sequencing data showed an increase in bacterial biodiversity compared to those uninfected that correlated with increasing Giardia burden (Shannon alpha diversity (Giardia only 2.7; uninfected 2.1, p = 0.0317; Spearman r = -0.5491, p = 0.0244)) within each age group but showed a significant increase in diversity from paired 3 to 5-year-old children (p = 0.01838). In Giardia only infections, microbiome taxonomy shifted to Prevotella copri, with the degree of shift related to the intensity of infection compared to uninfected (43.2 % versus 12%, p = 0.012). Metagenomic analysis of the bacterial microbiota showed the proportion of vitamin B12 producing bacteria (Bifidobacteriaceae) were diminished in the Giardia assemblage A group infected group compared to the non-infected group and also assemblage B group (p = 0.038). Specific genes in the cobalamin synthesis pathway (cobinamide kinase, ATP corrinoid adenosyltransferase) were proportionally decreased with the burden of Giardia infection (p < 0.05). Z-scores for both height and head circumference was decreased in the Giardia infected children at both time points (p < 0.05). The rate of decreased growth was larger in children infected with Giardia at both 3 and 5 years old (p < 0.05). Our data provide evidence for an effect of parasitic infections allowing permissive growth of anaerobic bacteria such as Prevotella and Bifidobacteriaceae, altering capacity of vitamin B12 biosynthesis and impacting growth in children.

#### VALIDATION OF A MULTIPLEX REAL-TIME PCR GASTROINTESTINAL PARASITE PANEL

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Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens in fecal samples, however, it presents numerous challenges including high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We sought to validate a commercial multiplex parasitic real time PCR panel detecting 6 protozoal pathogens: Blastocystis hominis (Bh), Cryptosporidium, Cyclospora, Dientamoeba fragilis (Df), Entamoeba histolytica (Eh) and Giardia lamblia (Gl) in unpreserved fecal specimens submitted for diagnostic parasitology. We analyzed 192 specimens, including 84 banked, frozen known positive specimens containing all of the targeted pathogens (8 Bh, 13 Crytosporidium, 13 Cyclospora, 10 Df, 15 Eh, 13 Gl and 12 mixed protozoal infections) and 108 fresh specimens randomly selected from our prospective parasitology submissions, including 4 Bh, 3 Df, 2 mixed infections, and 99 microscopy negatives. DNA extraction and PCR were setup with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms with stool ELISA as an additional reference assay for Eh. Sensitivity, specificity, positive predictive and negative predictive values of the enteric multiplex were: 96%, 90%, 60%, and 99% for Bh; 100% for Cryptosporidium; 79%, 100%, 100%, and 98% for Cyclospora; 86%, 86%, 86%, and 98% for Df; 81%, 100%, 100%, and 98% for Eh; and, finally, 94%, 85%, 85% and 99% for Gl, respectively. The platform had high sensitivity for Bh, Cryptosporidium and Gl, but suboptimal sensitivity for detection of Cyclospora, Df, and Eh. Low positive predictive value for Bh may reflect challenges to accurate microscopic identification of this organism. Negative predictive value was excellent for all targets, supporting that the platform accurately determines true negatives. This particular enteric multiplex platform provides a useful diagnostic tool for Bh, Cryptosporidium, and Gl. Further optimization of the assay is required for Cyclospora, Df, and Eh prior to clinical use.

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#### GENETIC DIVERSITY OF *TRICHOMONAS VAGINALIS* ISOLATES IN WESTERN AUSTRALIA, THE NORTHERN TERRITORY OF AUSTRALIA AND SOUTHERN GHANA

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Genetic diversity of Trichomonas vaginalis among regional populations has become more evident in studies over the last decade, with increasing cases of treatment failures and variable clinical presentations. We applied next generation-multilocus sequence typing (NG-MLST), comprising seven single-copy housekeeping genes to genetically characterize isolates of T. vaginalis. We examined one hundred and seventy-six archival and recently sampled T. vaginalis isolates from Western Australia, the Northern Territory and female patients visiting selected health care facilities in Southern Ghana, to assess the level of intra- and inter-population genetic diversity of T. vaginalis in these regions. Twenty-two zero-radius operational taxonomic units (ZOTUs) and 106 sequence types (ST) were distinguished among 176 isolates, suggesting diverse T. vaginalis populations within the three geographical regions. Each characterized locus comprised more than one allele and nucleotide diversity for the loci based on pairwise difference averaged 0.0175 differences/site. The number of different alleles for each locus ranged from 2 to 8. Eleven multiple infections with different genotypes were found among 6% of the samples, mostly those from Ghana. ZOTU diversity was greater among isolates from Ghana and one