Molecular Diagnosis of Intestinal Parasites: Impact on Growth among Preschool-Age Children in Rural Ecuador

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MOLECULAR DIAGNOSIS OF INTESTINAL PARASITES: IMPACT ON GROWTH AMONG PRESCHOOL-AGE CHILDREN IN RURAL ECUADOR

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN NURSING

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT HOUSTON

CIZIK SCHOOL OF NURSING

BY

PATRICIA E. BRYAN, PhD(c), MSN, RN

AUGUST, 2020
To the Dean for the School of Nursing:

I am submitting a dissertation written by Patricia Bryan and entitled "Molecular Diagnosis of Intestinal Parasites: Impact on Growth Among Preschool-Age Children in Rural Ecuador." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Nursing.

Cathy L. Rozmus, PhD, RN, FAAN
Committee Chair

We have read this dissertation and recommend its acceptance:

Peter Holzer, MD PhD

[Signatures]

Dean for the School of Nursing

6/11/2020
Date
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Thank you to my loving husband, parents, and brother for your enduring support and sacrifice, and for always believing in me. Thank you for being my biggest fans in all that I do. I love you. Above all, I am thankful to my Lord and Savior, Jesus Christ, for His love and grace and for setting this path before me and giving me the strength and courage to see it through.
Molecular Diagnosis of Intestinal Parasites: Impact on Growth Among Preschool-Age Children in Rural Ecuador

August, 2020

Abstract

Background

Intestinal parasites may have important influences on growth outcomes during early childhood. Previous studies investigating the effects of intestinal parasites on growth have predominantly used microscopy-based diagnostic methods with low sensitivity. Few accurate epidemiologic data are available on intestinal parasitic infections among rural Ecuadorian children. To investigate the impact of single and multiple intestinal parasite infections on growth during early childhood, data from a longitudinal birth cohort in Ecuador were analyzed.

Purpose

The aims of this study were to determine the prevalence of intestinal parasites, including both soil-transmitted helminths and intestinal protozoa, and to describe associations between intestinal parasitic infections and growth among rural Ecuadorian children at 36 and 60 months of age.

Methods

A retrospective longitudinal study was conducted using matched data from 177 children. A rapid, high-throughput, multi-parallel real-time qPCR assay was used to
detect intestinal parasites. Data on parasite infection prevalence, parasite burden, growth measures, and sociodemographic factors were examined. Univariate analyses were carried out to describe the study population. Bivariate analyses including Spearman’s correlation, Chi-square test and Mann-Whitney U test were used to examine differences between categorical and continuous variables. McNemar’s test was used to examine the change in prevalence, while the Wilcoxon signed-rank test was used to examine the change in parasite burden and growth changes. Multivariable regression analyses were carried out to predict growth from intestinal parasite status and sociodemographic factors at each follow-up observation and across age.

**Results**

Over half of the study population was infected with intestinal parasites at 36 and 60 months with 66.7% and 58.2%, respectively. Overall, *Giardia lamblia* was most prevalent parasite followed by *Trichuris trichiura*, and *Ascaris lumbricoides*. Prevalence decrease with age except for *Trichuris trichiura* with a significant change in prevalence across age for *Ascaris lumbricoides* (*p* = .014), *Strongyloides stercoralis* (*p* = .039), and *Giardia lamblia* (*p* = .039). Parasite burden also tended to decrease with age, however, statistically significant increases were found for *Giardia lamblia* (*z* = -2.204, *r* = .20, *p* = .027) and *Strongyloides stercoralis* (*z* = -2.312, *r* = .20, *p* = .021). While single parasite infections were more prevalent, polyparasitism was associated with reduced height (cm) (*U* = 2220.50, *z* = -2.120, *r* = .20, *p* = .034) and HAZ (*U* = 2187.50, *z* = -2.233, *r* = .20, *p* = .026); and co-infection was associated with reduced HAZ (*U* = 1963.50, *z* = -2.094, *p* = .036, *r* = .2) at 36 months. Also, at 36 months, *Trichuris trichiura* was associated with reduced HAZ (*z* = -2.787, *r* = .21, *p* = .005) and WAZ (*z* = -2.324, *r* = .20, *p* = .020). No
associations between intestinal parasites and growth deficits were found at 60 months of age.

**Conclusion**

The true prevalence of intestinal parasites was determined using multi-parallel, real-time qPCR with high sensitivity and specificity contributing accurate epidemiologic data on the distribution of soil-transmitted helminths and protozoa among preschool-age children in rural Ecuador. Intestinal parasitic infections were associated with deficits in growth indicators among children at 36 months of age. Findings indicate that intestinal parasites are highly prevalent and have an important influence on growth during early childhood in rural Ecuador.

Keywords: GI parasites, soil-transmitted helminths, protozoa, early childhood, rural Ecuador
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Summary of Study

Intestinal parasites including, soil-transmitted helminths (STH) and protozoa, are widely distributed throughout tropical and subtropical regions of the world and remain a significant global public health concern. Children are disproportionately affected compared to adults and are most vulnerable to health insults from intestinal parasites including growth delays during early childhood. However, findings on the impact of intestinal parasites on growth in children remain inconsistent and poorly understood. The lack of accurate epidemiologic data due to the wide use of microscopy-based diagnostic methods and the predominant focus on STH in the school-age population have contributed to these research gaps.

In this study, a multi-parallel, real-time qPCR assay was used for detection of eight intestinal parasites, including both STH and protozoa, in a population of preschool-age children in rural Ecuador. Prevalence findings confirm that both STH and protozoa infect children during early childhood. Over half of the study population was infected with at least one parasite species at both follow-up observations and children were infected with up to four parasite species. In this study, *Giardia lamblia* was the most prevalent parasite, followed by *Trichuris trichiura*, and *Ascaris lumbricoides*. Findings revealed that intestinal parasites were associated with linear growth. Multiple parasite infections, primarily co-infection with concurrently occurring STH and protozoa, were found to be associated with reduced HAZ at 36 months of age. Further evaluation indicated that *Ascaris lumbricoides* and *Giardia lamblia* co-infections were associated with reduced HAZ at 36 months. Parasite burden was also associated with growth deficits. Among children with ascariasis who had HAZ < -2 SD (stunted), parasite burden
was higher compared to non-stunted children infected with *Ascaris lumbricoides*. Underweight (WAZ < -2 SD) children with trichuriasis also had increased parasite burden compared to infected children who were not underweight. Findings from this study contribute to the body of research investigating the relationship between intestinal parasitic infections and child growth. This is one of the few studies using a molecular diagnostic approach for identification of both STH and protozoa among a population of preschool-age children in rural Ecuador; thus, contributing to filling the research gap in accurate epidemiologic data for this part of the world. Evidence from this study indicates that both STH and protozoa are present at a high enough prevalence to warrant interventions among preschool age children in rural areas of Ecuador. Furthermore, the association between co-infections with *Ascaris lumbricoides* and *Giardia lamblia* with stunting in the study population suggests treatment needs to target these parasite species.
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Specific Aims

Intestinal parasitic infections remain a significant public health concern of global importance (Mehraj et al., 2008; Saboyá et al., 2013). These parasites, including both soil-transmitted helminths (STH) and intestinal parasitic protozoa, are considered among the most widespread infectious agents worldwide causing significant morbidity and disproportionately affecting children, particularly in early childhood (Hall et al., 2008; Hotez et al., 2009; Hotez et al., 2019; LaBeaud et al., 2015; Saboyá et al., 2013). Compared to adults, children are more often infected and have higher parasite burden; subsequently, experiencing greater morbidity (Bethony et al., 2006; Hotez et al., 2019; Pullan et al., 2014; Saboyá et al., 2013;). Children are at greatest vulnerability for the detrimental health impacts related to intestinal parasitic infections during critical periods of growth and development characteristic of early childhood (Hall et al., 2008; LaBeaud et al., 2015; Pullan et al., 2014). The distribution of intestinal parasites is influenced by critical determinants of transmission including poverty and climate (Al-Delaimy et al., 2014; Jourdan et al., 2018). Endemic to tropical and subtropical regions of the world, intestinal parasites have the highest prevalence among children living in extreme poverty (Al-Mekhlafi et al., 2013; Al-Delaimy et al., 2014; Campbell et al., 2016; Gelaw et al., 2013; Jourdan et al., 2018; Rajoo et al., 2017; Sturrock et al., 2017). Infected children often have chronic morbidity due to frequent reinfections, common in childhood (Weatherhead et al., 2015; Weatherhead et al., 2017b), which can result in impaired growth. However, reported evidence on impaired growth in children infected with intestinal parasites remains inconsistent, with few data reported on the preschool-age population.
The purpose of this study is to generate accurate epidemiologic data on the distribution of intestinal parasites and to provide evidence that will advance current knowledge on morbidity in children infected with intestinal parasites. The specific aims for the proposed study include:

Aim 1: Determine the prevalence and parasite burden for five STH and three protozoan parasite species using a multi-parallel, real-time, quantitative polymerase chain reaction (qPCR) assay among preschool-age children in rural Ecuador.

Aim 2: Describe associations between intestinal parasitic infections and growth among preschool-age children in rural Ecuador.

**Research Strategy**

**Significance**

Intestinal parasites commonly infecting children consist of heterogeneous species of global health importance that can be categorized into two major taxonomic groups, the soil-transmitted helminths (STH) and protozoa (Harhay et al., 2010). The major STH species of medical importance commonly infecting children are a group of parasitic nematodes (worms) including: *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, and Trichuris trichiura* (Schär et al., 2013). The most common protozoa infecting children of medical importance include: *Giardia lamblia, Cryptosporidium* spp. and *Entamoeba histolytica* (Torgerson et al., 2015). Both STH and protozoa are linked to poverty and are endemic to resource-limited countries with tropical and subtropical climate (Al-Delaimy et al., 2014) including regions of Asia, Sub-Saharan Africa, and Latin America (Chammartin et al., 2013). Transmission is
acquired from soil or water contaminated with feces (Jia et al., 2012). Children are at greatest risk for environmental exposure and fecal-oral transmission of intestinal parasites living in areas where sanitation and clean water infrastructure is inadequate or lacking, with the highest prevalence frequently found among children living in extreme poverty.

Intestinal parasites are considered among the most widespread infectious agents disproportionately affecting children (Bethony et al., 2006; Brooker et al., 2004; Hall et al., 2008; LaBeaud et al., 2015). The disease burden imposed by childhood intestinal parasitic infections is attributed to the chronic and debilitating nature of morbidity caused by STH and protozoa, including growth impairment (LaBeaud et al., 2015). Chronic morbidity from intestinal parasites can have long-term implications on childhood growth and development circumventing attainment of full potential and productivity (Hall et al., 2008; Hotez et al., 2019; LaBeaud et al., 2015).

An important research gap contributing to the lack of consistent findings related to the association between intestinal parasites and child growth outcomes is the wide use of microscopy-based diagnostic methods lacking in adequate sensitivity. Subsequently, resulting in underreporting of epidemiologic data on childhood intestinal parasitic infections (Mejia et al., 2013). The accuracy of epidemiologic data is critical for population health investigational efforts in endemic areas that influence the development of treatment and public health intervention approaches. From a clinical and epidemiological standpoint, the accurate and reliable identification of intestinal parasites in children is of paramount importance (Cimino et al., 2015; Llewellyn et al., 2016). However, current standard microscopy-based diagnostic methods have known poor sensitivity, ranging from 50% to 80% (Mejia et al., 2013). Important indications of the
limitations in microscopy are difficulty in detecting parasites when parasite burden is low, as well as, the inability to differentiate between non-pathogenic *Entamoeba dispar* and pathogenic *Entamoeba histolytica* protozoan species and the inability to distinguish between the two hookworm species (*Ancylostoma duodenale* and *Necator americanus*) (Brooker et al., 2004; ten Hove et al., 2009). Furthermore, polyparasitism involving co-infections with concurrently occurring STH and protozoa presents challenges in identification by direct microscopy methods. The public health control of infection transmission and the development of appropriate treatment approaches are dependent on quality epidemiologic data. Without these data, affected children may go undiagnosed and unrecognized for treatment programs. Moreover, investigation of the pathogenic mechanisms related to morbidity associated with intestinal parasites is also dependent on the accurate identification of parasite species and quantification of parasite burden.

Significant improvements to the accuracy of diagnostic and epidemiologic data have come from molecular methods with greater sensitivity and specificity; namely, polymerase chain reaction (PCR) based approaches (Verweij et al., 2004). In addition, newly developed real-time quantitative PCR methodology has important benefits of improved sensitivity and specificity compared to conventional PCR (Mejia et al., 2013; Verweij et al., 2014a; Verweij et al., 2014b). Such methods are also more rapid, resulting in shorter turnaround time (Mejia et al., 2013), have a reduced risk of amplicon contamination from laboratory environment, and have reduced reagent costs (Verweij et al., 2004). However, there remains a pressing need for newer molecular methods utilizing high-throughput technology with the capability for detecting greater numbers of parasite species in a single reaction while preserving high sensitivity and specificity necessary for
detecting parasite species. Evidence from a previous study investigating a peri-urban population of Argentinean children revealed that multi-parallel, real-time qPCR significantly improved detection and quantification accuracy of eight intestinal parasite species compared to microscopy-based methods (Cimino et al., 2015). Furthermore, previously reported findings from rural communities in Honduras, endemic for hookworm and *Trichuris trichiura*, indicated that even lightly infected two to five-year-old children experienced morbidity specific to growth outcomes (Montresor et al., 2015; Sanchez et al., 2013).

Evidence generated from this study will contribute to filling the research gap in accurate epidemiologic data on the distribution of both STH and protozoa in preschool-age children. Furthermore, findings will advance the understanding of morbidity from childhood intestinal parasitic infections and contribute to data on the association between intestinal parasite and growth in preschool-age children. Generating accurate epidemiologic data on the distribution of STH and protozoa in the proposed study population is critical to treatment strategies for mass drug administration and public health intervention approaches for prevention programs, both generating substantial public health gains on a global scale.

**Innovation**

A key innovation of the proposed study is the use of a new multi-parallel, real-time quantitative polymerase chain reaction (qPCR) molecular assay for the diagnosis of intestinal parasites that avoids the constraints of commonly used conventional PCR methods; while overcoming the limitations of microscopy-based diagnostic methods.
This molecular diagnostic approach utilizes high-throughput technology for the detection of an unlimited number of parasite species (Mejia et al., 2013). In this study, five STH and three protozoan parasites commonly infecting children will be analyzed for each child from a single stool sample. Notably, this enables investigation of the corresponding contribution of each parasitic species in addition to quantification of parasite burden. This is the first study to investigate the prevalence and parasite burden of eight intestinal parasites including, both STH and protozoa, using a multi-parallel, real-time qPCR assay and exploring the association between these parasites and growth in this population of rural Ecuadorian children at 36 and 60 months of age.

The innovative approach utilized in the proposed study will provide insights into intestinal parasitic infections during early childhood. This insight can drive future research initiatives advancing knowledge related to morbidity associated with childhood intestinal parasitic infections. The most substantial public health gains that will result from the evidence produced by this study are contributions to improved long-term health outcomes that can circumvent lasting poverty often experienced by children living in endemic areas afflicted with intestinal parasitic infections.

**Preliminary Study**

In 2015, Cimino and colleagues conducted a preliminary study including 116 asymptomatic children living in rural Argentina for the investigation of the detection of intestinal parasites by molecular versus microscopy-based diagnostic methods. This study utilized multi-parallel, real-time qPCR for identification of up to eight intestinal parasites, including both STH and parasitic protozoan species in stool samples (Cimino et al.,
Prevalence findings identified STH infections, including 54.3% for Ascaris lumbricoides, 37.4% for the combined two hookworm species (Necator americanus, Ancylostoma duodenale), 63.6% for Strongyloides stercoralis, 0.8% for Trichuris trichiura; and parasitic protozoa, including 65.5% for Giardia lamblia, 1.7% for Entamoeba histolytica, and 0% for Cryptosporidium parvum (Cimino et al., 2015).

Polyparasitism with two or more parasite species was detected in 70.6% of subjects. This preliminary study demonstrated the feasibility of the proposed study including time and cost to investigate intestinal parasitic infections in children utilizing a multi-parallel, real-time qPCR assay for the detection of eight intestinal parasites.

Methods

Research Design

An observational retrospective longitudinal cohort study design will be used to analyze data collected from children at 36 and 60 months of age within the Ecuador Life (ECUAVIDA) birth cohort study.

Study Site and Sample Size

The study site will be the rural District of Quinindé, Esmeraldas Province, Ecuador. This District is located in the coastal region of northern Ecuador at an elevation of approximately 100 meters above sea level and has an average annual temperature of 30 degrees Celsius and 75% humidity (Cooper et al., 2011). The District of Quinindé has an estimated population of 150,000 people, is largely rural (78%), and is ethnically comprised of Mestizos (90%), Afro-Ecuadorians (7%), and Amerindian (3%) (Cooper et
This is one of the poorest regions in Ecuador, with only 10% of the population having electricity and none with access to basic sanitation services (Cooper et al., 2011).

The sample size was determined using the single population proportion formula, \( N = Z^2 \frac{P(1-P)}{d^2} \) (Arifin, 2013), with an estimated 50% prevalence of intestinal parasitic infections based on previously reported prevalence data (Mehraj et al., 2008; Cooper et al., 2011), 95% confidence interval and 5% bound on the error of estimation for precision. The minimum required sample size was calculated as 385 participants with power \((1-\beta)\) set at 80% and a medium effect size at \(\alpha = .05\). Based on this calculation and available resources for analysis, a total of 400 children who participated in the ECUAVIDA birth cohort study will be randomly selected to be included in the proposed study.

The ECUAVIDA birth cohort study received full ethical approval by the Universidad San Francisco de Quito and the Ecuadorian Health Ministries ethical committees. Eligible newborns in the rural district of Quinindé in Esmeraldas Province, Ecuador, were randomly recruited at birth from November 2005 to December 2009 at the public hospital, Hospital Padre Alberto Buffoni (HPAB).

**Eligibility Criteria**

Inclusion criteria are: (1) being asymptomatic; (2) living in the study geographical district for at least two years prior to each follow-up observation at 36 and 60 months of age; and (3) no antibiotic or anthelmintic treatment prior to each follow-up observation at 36 and 60 months of age.
Data Collection and Instruments

**Anthropometric data.** Anthropometric data will include measures of height and weight obtained at 36 and 60 months of age. At each observation time, height in centimeters (cm) and weight in kilograms (kg) were measured in duplicate and the mean of the two measurements was used as the final measurement (Cooper et al., 2011; Cooper et al., 2015). All research team members involved in measuring growth parameters received standardized training on how to perform anthropometric measurement techniques according to WHO-recommended guidelines (World Health Organization [WHO], 2006). Children were measured without shoes and in light clothing or underwear. Intra-observer discrepancies were reduced by using the same equipment and the same trained personnel to measure growth parameters. All measurements for height and weight were reviewed for possible errors in measurement. Instruments used for growth measurements were selected in accordance with the WHO guidelines for anthropometric measurements and were widely used in local health care settings. The WHO Anthropometric software (version 3.2.2.) (WHO, 2011) for Windows will be used to calculate z-scores for anthropometric indices from height and weight measures including, height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) z-scores. These indices will be used to examine growth differences between infected and non-infected children and to compare overall height and weight with WHO reference population. Children were weighed on a portable electronic scale (Seca, Germany) accurate to within 100 grams. Standing height was measured using an in-house stadiometer accurate to within 0.1 cm (Cooper et al., 2011; Cooper et al., 2015). All instruments used for anthropometric measurements were calibrated daily prior to use. All
growth measurements were performed in Ecuador by trained ECUAVIDA research team members.

**Parasite data.** Stool samples for the detection of intestinal parasites were collected at 36 and 60 month follow-up visits as part of the ECUAVIDA birth cohort study. These stool samples were stored at 4° Celsius to preserve the stability of parasite DNA at the study site laboratory in Quito, Ecuador until being subjected to qPCR assay analysis for the molecular diagnosis of intestinal parasitic infections caused by eight intestinal parasite species. Participant stool samples were analyzed for a total of 8 intestinal parasite species using a modern molecular diagnostic method with a modified version of a validated quantitative real-time polymerase chain reaction assay known as a multi-parallel real-time quantitative PCR (qPCR) (Mejia et al., 2013). This qPCR assay allows for of total reagent volume of 7 µL (Mejia et al., 2013). This accommodated the minimum settings on the Applied Biosystems 7900HT Fast Real-Time PCR System (Mejia et al., 2013). The PCR system calibration and maintenance will be performed according to manufacturer protocol. The qPCR assays will be performed in Ecuador by this investigator who is trained and proficient in performing this assay. Parasite DNA will be extracted from approximately 50 mg of stool via the FastDNA™ SPIN Kit for soil DNA extraction (MP Biomedical, Solon, OH) using a low reagent method developed by Mejia et al. (2013) for use in resource-limited settings. An additional step is required to extract *Trichuris trichiura* DNA, in which the remaining insoluble pellet from the initial DNA extraction will be re-suspended in 200 µL DNA-free water, heated at 90° Celsius for 10 minutes, and centrifuged at 14,000 g for 10 minutes (Mejia et al., 2013). The above described DNA extraction method will then be performed to process the resulting
soluble portion of the sample. Methodology for the qPCR assay will involve testing all control standards in triplicate and all unknown samples in duplicate. A qPCR cycle threshold (Ct) value > 38 will be considered as a negative result. Each primer and probe combination previously demonstrated 100% sensitivity and 100% specificity for the designated parasite species (Mejia et al., 2013). Parasite burden quantifications will be performed by interpolating against parasite species-specific sequence standards and reported as DNA fg/µL. To evaluate parasite burden based on WHO criteria guidelines, estimated egg counts from qPCR DNA fg/µL will be calculated using $Y_{ova/g \text{ feces}} = (0.02748)/(X \text{ fg/µL})$ for hookworm (*Necator americanus* and *Ancylostoma duodenale*); $Y_{ova/g \text{ feces}} = (0.001455)/(X \text{ fg/µL})$ for *Ascaris lumbricoides*; $Y_{ova/g \text{ feces}} = (0.00001095)/(X \text{ fg/µL})$ for *Trichuris trichiura*; and $Y_{\text{larae/gram}} = (0.01471)/(X \text{ fg/µL}) = (Y_{\text{larae/gram}})(0.01505629) = Y_{\text{larae/gram}}$ for *Strongyloides stercoralis*.

**Sociodemographic data.** An interview-led questionnaire was conducted at the 60 month follow-up observation to collect data from the participant’s mother related to demographic and socioeconomic factors. This questionnaire was translated into Spanish and administered by trained research team members. The sociodemographic factors used in this study included: sex, household crowding, birth order, potable water access, maternal education, maternal ethnicity, maternal helminth infection, and socioeconomic status.

**Quality Assurance and Data Management**

The principal investigator (PI) for the present study and all personnel involved in the handling and laboratory analysis of biological samples for this study have received
biosafety and laboratory training. The PI has received training in the form of doctoral level courses on principles and application of biochemistry and molecular biology. The PI also has previous educational background and laboratory training in parasite biology and parasite lifecycles. Training related to the specific intestinal parasite species investigated in this study was received through direct mentorship from senior faculty and Chief of the Laboratory of Clinical Parasitology and Diagnostics with the National School of Tropical Medicine at Texas Children’s Hospital. This mentorship also included laboratory training specifically using the qPCR protocol for identification of the eight parasite species investigated in this study. This mentorship is ongoing and will be in place through the duration of this study.

Anthropometric measurements were obtained by the same trained personnel to minimize between-observer bias. All data collection methodology within the ECUAVIDA birth cohort study was standardized and monitored for adherence by trained research personnel. All data used in the proposed study will be de-identified. Data entry will be carried out using Excel for Office 2013 program software for Windows. All data will be checked by the PI for recording errors. Excel spreadsheet data will be exported to a statistical software program for analysis. A secure computer will be used to save Excel spreadsheets with recorded study data and for the use of statistical analysis software located in the Laboratory for Clinical Parasitology and Diagnostics at Texas Children’s Hospital in Houston, Texas.
Environment

The Ecuador laboratory and the Laboratory of Clinical Parasitology and Diagnostics at Texas Children’s Hospital in Houston, TX, are state of the art laboratories that offer unparalleled opportunities to draw upon the intellectual resources of researchers and experts in the area of laboratory diagnostics for parasitic infections. Additionally, collaborations with faculty mentors at the National School of Tropical Medicine and proximity to Texas Children’s Hospital provides abundant opportunities to obtain rich resources of information related to intestinal parasitic infections and associated morbidity in children.

Data Analysis

All data will be checked for recording errors and verified for accuracy. SPSS version 26 and Prism version 8.1 statistical programs will be used for statistical analysis. Statistical significance will be defined as $p$ value < .05. Only children with complete matched data for both follow-up time points at 36 and 60 months of age will be included in the final analysis. Growth parameter measurements for height (cm) and weight (kg) will be used to calculate $z$-scores for anthropometric indices using WHO Anthro software (Version 3.2.2.) (WHO, 2011). Anthropometric indices to classify growth deficits will include height-for-age $z$-scores (HAZ), weight-for-age $z$-scores (WAZ), and weight-for-height $z$-scores (WHZ), which will then classified as HAZ $\leq -1$ SD $> -2$ SD for impaired linear growth; HAZ $\leq -2$ SD $> -3$ SD for stunting; HAZ $\leq -3$ SD for severe stunting; WAZ $\leq -1$ SD $> -2$ SD for mild underweight; WAZ $\leq -2$ SD $> -3$ SD for underweight; WAZ $\leq -3$ SD for severely underweight; WHZ $\leq -1$ SD $> -2$ SD for mild wasting; WHZ
≤ -2 SD > -3 SD for wasting; WHZ ≤ -3 SD for severe wasting. WHO guidelines were used to classify children as having light, moderate, and heavy infection burden by parasite egg counts per gram of stool (epg) with each parasite as follows: For *Ascaris lumbricoides*, light (1 – 4,999 epg), moderate (5,000 – 49,999 epg), and heavy (≥ 50,000 epg); for *Trichuris trichiura*, light (1 – 999 epg), moderate (1,000 – 9,999 epg), and heavy (≥ 10,000 epg) as heavy; and for hookworm, light (1 – 1,999 epg), moderate (2,000 – 3,999 epg) and heavy (≥ 4,000 epg) (WHO, 2002). Moderate and heavy infection burden were merged into a single category of moderate-to-heavy for estimations of associations with infection burden by epg as categorical variables.

Descriptive statistics will be used to summarize study population characteristics. The mean with standard deviation will be reported for continuous variables. For categorical variables, frequency and proportions will be reported. Normality will be assessed for the distribution of continuous variables by visual inspection of histograms and the Shapiro-Wilk test. Growth indicators and parasite variables will be initially assessed with univariable analyses. Associations between growth indicators and parasite infections, as well as, infection burden will be evaluated using bivariate and multivariable analyses adjusted for potential confounding sociodemographic factors. Correlations between variables will be evaluated using the Spearman rank correlation coefficient when assumptions for the Pearson product-moment correlation coefficient are not met. Categorical variables will be compared using the Chi-square ($X^2$) test of independence or Fisher's Exact test when appropriate. Comparison of variables for associations will be carried out with the Mann-Whitney U test when appropriate for data with a non-normal distribution. Continuous data compared across age will be assessed using the Wilcoxon-
signed rank test. Sociodemographic factors potentially associated with growth deficits and similarly with parasite infection in univariate analysis will be included in multivariable analyses across age. Associations with growth deficits as dichotomous outcomes will be analyzed by logistic regression. Associations with parasite burden by parasite species will be estimated by linear regression with concentrations of parasite DNA in fg/µL as the dependent variable. Due to the overdispersed correlated nature of epg and DNA fg/µL data values, extreme outliers will not be removed from the sample to avoid excluding data points of interest.

Study Limitations

Potential limitations to the proposed study involve the use of retrospectively collected anthropometric data, which may be unreliable. However, reliability in measurement was ensured by using trained personnel, calibrated equipment and standardized techniques. Based on preliminary study success, no difficulties or further limitations are anticipated. A potential limitation is loss to follow-up. Procedures were put into place to ensure high follow-up within the ECUAVIDA birth cohort study. Such procedures included setting up a dedicated team for registering changes in address and locating mothers prior to each scheduled follow-up visit. A significant strength of this study is the utilization of an objective, highly sensitive and specific molecular diagnostic approach for the detection intestinal parasitic infections. A potential limitation is the risk of contamination. Measures will be taken to avoid the contamination of reagents, laboratory equipment and bench space by exercising Good Laboratory Practice at all times.
Future Directions

Evidence from this study will provide true prevalence data for both STH and parasitic protozoa in addition to findings on the impact of intestinal parasitic infection on growth in preschool-age children. These findings will inform future epidemiological studies of co-infections and mixed infections in pre-school and school-age children as well as future studies investigating a link between growth deficits and reduced intestinal microbiota diversity in species-specific intestinal parasitic infections in children.

Protection of Human Subjects

Approval for conducting the proposed study will be sought from the Committee for Protection of Human Subjects (CPHS) at the University of Texas Health Science Center at Houston and the Biomedical Research and Assurance Information Network (BRAIN) at Baylor College of Medicine before initiating any part of the study. The ECUAVIDA birth cohort study protocol (ISRCTN41239086) was approved by the ethics committee of the Hospital Padre Alberto Buffoni (HPAB), Universidad San Francisco de Quito, and Pontifica Universidad Catolica del Ecuador. Informed written consent was obtained from the child’s mother for all children participating in the ECUAVIDA birth cohort study. Children with positive stools for intestinal parasites were treated according to Ecuadorian Ministry of Public Health recommendations.

Potential Risk to Participants and Steps to Minimize Risks

Potential risk to participants is related to loss of confidentiality because this study involves the use of participant data that was previously collected. Every effort will be made to safeguard the security of participant identifiable or protected health information
(PHI). The likelihood of a breach of confidentiality will be minimized by limiting access to participant information; de-identifying and coding participant information; and storing data in a centralized and secure location.

All participant data will be de-identified and coded. Master code keys will be kept by PI. Any written information will be electronically transcribed. All participant information will be electronically stored in a secure file on a computer hard drive with encrypted password access and available only to the PI. The computer used to store participant information will remain in a secure central location that requires restricted ID badge for access.

**Potential Benefits to Participants and to Society**

There are no direct benefits to participants related to the proposed study. However, it is anticipated that the potential benefits in the application of a high-throughput molecular diagnostic method with high sensitivity and specificity for identification of up to eight parasites will contribute to epidemiologic data of public health significance. Furthermore, evidence from this study can inform new treatment and public health intervention approaches with substantial public health gains resulting from the contribution of data in the ultimate elimination of morbidity from childhood intestinal parasitic infections.
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Letter to the Editor

May 27, 2020

Philip J. Rosenthal, MD, FASTMH
Editor-in-Chief, The American Journal of Tropical Medicine and Hygiene
University of California, San Francisco School of Medicine
513 Parnassus Ave.
San Francisco, CA 94143-0410

Dear Dr. Rosenthal,

I am writing to submit the manuscript entitled “Molecular Diagnosis of Intestinal Parasites: Impact on Growth Among Preschool-Age Children in Rural Ecuador” for consideration of publication in The American Journal of Tropical Medicine and Hygiene. In this manuscript, the association between co-infections with soil-transmitted helminth and protozoa is described. The predictors of growth among infected children are also examined. Few studies investigate the association between co-infections and growth during early childhood in rural Ecuador.

This study makes an important contribution to the growing body of literature on growth among children infected with intestinal parasites. This is one of the few studies to examine co-infections with Ascaris lumbricoides and Giardia lamblia and growth in a preschool population of children in rural Ecuador. Findings indicate that among a cohort of 36 and 60 month old children, Ascaris lumbricoides and Giardia lamblia co-infection was associated with reduced height-for-age z-scores compared to children with Ascaris lumbricoides only or Giardia lamblia only infections.

This manuscript has not been published previously and is not under consideration by another journal. We appreciate your consideration of this manuscript for review and look forward to your response. Please feel free to contact me at patricia.bryan@bcm.edu

Sincerely,

Patricia E. Bryan, PhD(c), MSN, RN
Email: patricia.bryan@uth.tmc.edu and patricia.bryan@bcm.edu
MOLECULAR DIAGNOSIS OF INTESTINAL PARASITES: IMPACT ON GROWTH AMONG PRESCHOOL-AGE CHILDREN IN RURAL ECUADOR

DISSERTATION MANUSCRIPT

Patricia Bryan, PhD(c), MSN, RN
Introduction

Infecting nearly one-third of the global population, intestinal parasites remain a significant public health concern (Mehraj et al., 2008; Saboyá et al., 2013; Welch et al., 2017; World Health Organization [WHO], 2014; WHO, 2017). Distinctively, intestinal parasites including soil-transmitted helminths (STH) and intestinal parasitic protozoa are considered among the most widespread infectious agents worldwide causing significant morbidity and disproportionately affecting children, particularly in early childhood (Hall et al., 2008; Hotez, 2009; Hotez & Kamath, 2009; Hotez et al., 2019; LaBeaud et al., 2015; Saboya et al., 2013). Compared to adults, children are more often infected typically, with more than one parasite species and have higher parasite burden; thus, suffering greater morbidity in comparison (Hotez et al., 2019; Pullan et al., 2014; Saboya et al., 2013). Notably, the distribution of intestinal parasites is influenced by critical determinants of transmission including poverty and climate (Al-Delaimy et al., 2014; Jourdan et al., 2018). Endemic to tropical and subtropical regions of the world, intestinal parasites are markedly prevalent where poverty is pervasive, predominantly among children living in extreme poverty (Al-Delaimy et al., 2014; Campbell et al., 2016; Gelaw et al., 2013; Jourdan et al., 2018; Rajoo et al., 2017; Sturrock et al., 2017). Worldwide, an estimated 270 million preschool-age and 600 million school-age children are affected by intestinal parasites (WHO, 2015; WHO, 2017), subsequently suffering significant morbidity.

The overall goals of the present study were to characterize the prevalence of intestinal parasitic infections and their impact on growth among preschool-age children in the rural District of Quinindé, Ecuador. Embedded within these overarching goals are the
following specific aims: (1) to determine the prevalence and parasite burden of five STH and three protozoan parasite species; and (2) to describe associations between intestinal parasitic infections and growth among infected children from 36 and 60 months of age. Additionally, associated sociodemographic factors for intestinal parasitic infections were described.

**Transmission and Epidemiology**

Collectively, intestinal parasites consist of a group of heterogeneous species of global health importance that can be categorized into two major taxonomic groups, the soil-transmitted-helminths (STH) and protozoa (Harhay et al., 2010). The major STH of medical importance commonly infecting children are a group of parasitic nematodes (worms) including, *Ascaris lumbricoides* (roundworm); *Trichuris trichiura* (whipworm); *Ancylostoma duodenale* and *Necator americanus* (hookworm); and *Strongyloides stercoralis* (threadworm) (Jourdan et al., 2018; Schär et al., 2013). The most common intestinal protozoa infecting children of significant importance to child health include *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* (Torgerson et al., 2015). Both STH and protozoa are linked to poverty (Hotez, 2009; Hotez and Kamath, 2009) and are endemic to resource-limited countries with tropical or subtropical climate (WHO, 2020), including regions of Asia, Sub-Sahara Africa, and Latin America. These regions are located within the tropical/subtropical belt spanning a geographical area of 49 degrees latitude north and south of the equator (Chammartin et al., 2013). The intersection of social and environmental factors predisposing children to intestinal parasite exposure, such as poverty and climate, is common where sanitation and clean
water infrastructure are inadequate or lacking. Thus, the prevalence of intestinal parasites is highest among children living in extreme poverty.

Soil-transmitted helminths, alone, are responsible for the most widespread and disabling chronic infections among children worldwide (Hotez, 2009; Hotez & Kamath, 2009; Marocco et al., 2017). STH are macroscopic multi-cellular worms (Bharti et al., 2018) that can typically be seen by the naked eye when in the adult stage. Characteristically, STH require moist warm soil for the successful completion of their lifecycle (Jia et al., 2012); thus, flourishing in tropical and subtropical climate. Furthermore, the survival of STH is dependent on conditions that lead to fecal contamination of the environment. Infection with STH is acquired from infective eggs or larvae in soil contaminated with feces. Transmission of *Ascaris lumbricoides* and *Trichuris trichiura* occurs through the fecal-oral route (Jourdan et al., 2018), not directly from feces, but from contact with soil contaminated with feces (Jourdan et al., 2018). Transmission of both species of hookworm (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis* occurs through transdermal penetration of infective larvae in skin exposed to soil contaminated with feces (Forrer et al., 2017; Jourdan et al., 2018). Notably, *Ancylostoma duodenale* can also be acquired through the fecal-oral route by ingesting infective larvae (Bogitsh et al., 2013). Of the STH species, *Trichuris trichiura* is the only species that does not have a migratory lifecycle phase. The lifecycle of *Trichuris trichiura* is completed entirely in the intestinal tract with the adult worms becoming established in the large intestine (transverse and descending colon) (Despommier et al., 2006). While, *Ascaris lumbricoides*, both hookworm species (*Ancylostoma duodenale* and *Necator americanus*), and *Strongyloides stercoralis* migrate
to the lungs via the circulatory system upon transdermal penetration, then transit through the respiratory tract before becoming established in the small intestinal as adult worms. Depiction of the lifecycle for each STH species can be found in Appendix A. Soil-transmitted helminths affect over 2 billion people worldwide, most of which are children, with over 270 million preschool-age children and over 600 million school-age children are at risk for infection and in need of preventive interventions (Hotez & Kamath, 2009). Among those infected, an estimated 807 million to 1.2 billion are infected with *Ascaris lumbricoides*, 465 million with *Trichuris trichiura*, 439 million infected with hookworm (Centers for Disease Control and Prevention [CDC], 2018; Pullan et al., 2014) and an estimated range of 200 to 370 million are reportedly infected with *Strongyloides stercoralis* (Bisoffi et al., 2013; Forrer et al., 2017). Notably, estimates for *Strongyloides stercoralis* are thought to be largely underestimated because the prevalence of this parasite is often not reported (Bisoffi et al., 2013; Forrer et al., 2017; Krolewiecki, et al., 2013). Among children, STH are responsible for substantial morbidity and disability relative to mortality (Weatherhead et al., 2017a; Weatherhead et al., 2017b). Estimates from the Global Burden of Disease Study indicated that together STH result in more than 6 million disability-adjusted life-years (DALYs) (Global Burden of Disease [GBD], 2016). Although STH are not leading causes of mortality (GBD, 2016, Weatherhead et al., 2017a; Weatherhead et al., 2017b), severe infections result in an estimated 12,000 to 135,000 deaths annually (Bethony et al., 2006; Hotez, 2009; Anuar et al., 2014).

Intestinal protozoa, in contrast to STH, are microscopic single-celled organisms (Yaeger, 1996) with lifecycles that are maintained entirely in the human intestinal tract (Esch & Peterson, 2013). Infection with intestinal protozoa including, *Giardia lamblia,*
Cryptosporidium spp., and Entamoeba histolytica, is acquired through fecal-oral transmission (Muhsen & Levine, 2012; Rogawski et al., 2017a) by ingestion of food or water contaminated with feces (Khalil et al., 2018). Water being the most common reservoir of infection worldwide (Efstratiou et al., 2017; Plutzer and Karanis, 2016; Rosado-García, 2017), particularly in developing countries where poverty is pervasive. Depiction of the lifecycle for Giardia lamblia, Cryptosporidium spp., and Entamoeba histolytica can be found in Appendix B. Of the intestinal protozoa, Giardia lamblia is the most commonly occurring protozoan species infecting children worldwide, with an estimated prevalence of 30% to 45% in developing countries (Fletcher et al., 2012; Kelly, 2014; Minetti et al., 2016; Rogawski & Guerrant, 2017) and as high as 50% among children living in extreme poverty (Al-Mekhlafi et al., 2013). Many of these infections are asymptomatic (Rogawski et al., 2018; Rogawski & Guerrant, 2017b); however, Giardia lamblia is a known cause diarrhea among infants and young children in developing countries (Kotloff et al., 2013; Platts-Mills et al., 2015), with an estimated 280 million symptomatic infections annually (Bartelt and Sartor, 2015; Esch and Petersen, 2013). Cryptosporidium spp., is an important pathogen recognized as a leading cause of diarrhea among children in developing countries, accounting for up to 20% of all cases in developing countries (Khalil et al., 2018; Kotloff et al., 2013; Platts-Mills et al., 2015) and a leading cause of diarrheal mortality in children younger than five years of age (GBD, 2016; Kotloff et al., 2013; Platts-Mills et al., 2015); overwhelmingly, among children living in poverty. Also, predominantly infecting children living in poverty, Entamoeba histolytica causing amoebiasis is responsible for an estimated 480 to 500 million infections (Silva et al., 2014; Spector and Gibson, 2009; Stanley, 2003). Of these,
an estimated 50 million develop invasive disease (Spector & Gibson, 2009), resulting in 40,000 to 110,000 deaths annually (Al-Delaimy et al., 2014; Kelly, 2014).

In Latin America, STH are widely distributed, with approximately 100 million people infected with *Trichuris trichiura*, 84 million with *Ascaris lumbricoides*, and 50 million infected with hookworm (Chammartin et al., 2013). Notably, an estimated 13.9 million preschool-age and 35.4 million school-age children in Latin America are reported to be at risk for infection with STH (Saboyá et al., 2013), with the greatest risk among children living in extreme poverty (Hotez et al., 2008; Saboyá, et al., 2013). Less is known about the distribution of *Strongyloides stercoralis* in Latin American, with few available prevalence data for this parasite (Buonfrate et al., 2015). Data on the distribution of STH in Latin America reported from at-risk regions has predominantly been from Brazil, Honduras, and Venezuela, with few data from other countries including Ecuador (Pullan et al., 2014). Also widely distributed throughout Latin America, are intestinal protozoa; namely, *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* (Rosado-García, 2017). Among available surveillance data, South America was found to be leading in intestinal protozoan infection prevalence compared to Central America (Baldrursson & Karanis, 2011; Efstratíou et al., 2017; Karanis et al., 2007). However, few recent reports of epidemiological data on these waterborne parasites are available from this part of the world (Rosado-García, 2017). Available reported findings on the prevalence of intestinal protozoa in Latin America indicate that *Giardia lamblia* and *Cryptosporidium* spp. occur more often than *Entamoeba histolytica* (Rosado-García, 2017; Speich et al., 2013). The prevalence of *Giardia lamblia* was recently reported as 7.3% in Brazil and 35.2% in Peru (Rogawski et al., 2018). In Latin America, as a whole,
Cryptosporidium spp. was reported to be responsible for 17.5% of infections causing acute diarrhea in children (Damiani et al., 2013). Subsequent to scarcely available surveillance reports, less is known about Entamoeba histolytica in the context of Latin America.

In Ecuador, a recent national survey of STH prevalence among a representative sample of school-age children reported an estimated national prevalence of STH as 28%, with the prevalence of Trichuris trichiura, Ascaris lumbricoides, and hookworm (both species combined) reported as 19.3%, 18.5% and 5.0%, respectively (Moncayo et al., 2018). In this survey, the highest prevalence of STH was found in the rural Amazon region, with 58.9% of children infected, followed by 14.5% and 10.1% in the Coastal and Highlands regions, respectively (Moncayo et al., 2018). A similarly high prevalence pattern was previously reported as 63.4% of STH infections among school-age children in the northcentral Andean region (Cooper et al., 2003). In contrast, only 28.9% of preschool-age children were recently reported to be infected with STH in the northwestern Coastal region (Cooper et al., 2015). Reported prevalence data on STH in Ecuador are primarily focused on the school-age population. Less is known about the distribution of intestinal protozoa in Ecuador, with few available surveillance reports on the prevalence of Giardia lamblia, Cryptosporidium spp., and Entamoeba histolytica (Rosado-García, 2017). A recent study reported the prevalence of intestinal protozoa in the rural Andean region as 3.9% and 12.5% for Giardia lamblia and Entamoeba histolytica/dispar, respectively (Calvopina et al., 2019). In the Highlands region, previous data indicated a prevalence of 21.1% Giardia lamblia and 8.9% Cryptosporidium spp. among a population of preschool-age children (Jacobsen et al., 2007). The prevalence of
intestinal protozoa in preschool-age children from the Coastal region was recently reported as 31.5% *Giardia lamblia*, 5.3% *Cryptosporidium* spp., and 1.0% *Entamoeba histolytica* (Cooper et al., 2015). Since these reports, few recent prevalence data on intestinal protozoa among children are available, suggesting a need for further epidemiological investigation on these parasites.

**Childhood Parasitism**

Because childhood is a critical period for growth and development, vulnerability to intestinal parasites, both STH and protozoa, and associated morbidity are of significant concern. Children are disproportionately affected by intestinal parasites, with a greater prevalence of infection and parasite burden compared to adults (Hotez et al., 2019; Pullan et al., 2014; Saboyá et al., 2013; Sturrlock et al., 2017). Morbidity is reportedly proportional to parasite burden; thus, children also suffer greater morbidity from intestinal parasites (Gabrie et al., 2016; WHO, 2020) compared to adults. This disproportionate vulnerability observed in children is influenced by increased nutritional requirements and immune system immaturity characteristic of childhood (Hall et al., 2008; Hurlimann et al., 2014; Saboyá et al., 2013; Sturrlock et al., 2017). Furthermore, exposure to intestinal parasites and their impact on child health outcomes are thought to be partly rooted in physical and behavioral development characteristic of childhood, particularly early childhood (Belizario et al., 2011; Bryan & Mejia, 2016; Campbell et al., 2016; Harhay et al., 2010; Moya et al., 2004; Weatherhead et al., 2017a). In young children, immune system immaturity and poorly developed hygiene behaviors can increase the risk of intestinal parasitic infection, as well as, contribute to morbidity (Campbell et al., 2016). It is not uncommon for children under five years of age living in
endemic areas to encounter contaminated soil and water as they increasingly interact with their environment, often acquiring primary intestinal parasitic infections during this time (Bryan & Mejia, 2015; Weatherhead, et al., 2017). Frequent oral contact with unclean hands and objects, which is characteristic behavior of early childhood, can further compound environmental influences contributing to a greater risk of exposure to intestinal parasites.

Early childhood is expressly recognized as a critical period of vulnerability to health insults from intestinal parasitic infections and for determining lifelong growth faltering due to high nutritional requirements and immune system immaturity influenced by the health impacts of intestinal parasitic infections (Guyatt, 2000; Adair et al., 2013; Campbell et al., 2016; Devakumar et al., 2018; Gabrie et al., 2016; Victora et al., 2010). Historically, research on child growth has primarily focused on dietary intake and nutrition alone among the school-age population (Vilcins et al., 2018), to the exclusion of environmental influences (Denno et al., 2014; Wondimagegn, 2014) and children younger than five years of age. The interactions between environmental factors and nutritional status play an important role in growth outcomes among younger children. Intestinal parasitic infections are an important example of this interaction (Oliveira et al., 2015; Vilcins et al., 2018), specifically during early childhood when developmental needs and characteristics contribute to the dynamic interplay between nutritional status and intestinal parasites acquired from a contaminated environment. Preschool-age children are an essential population for the investigation of intestinal parasite disease burden due to the potential for long-term health consequences unique to this population. Because interventions in early childhood can circumvent the cumulative exposure to intestinal
parasites and disease burden throughout a critical period of rapid growth and development (Baird et al., 2016; Miguel & Kremer, 2004), measures of growth during this time provide more sensitive detection of health status in response to anti-parasitic treatment and prevention compared to school-age children (Lo et al., 2015; Lo et al., 2018). Subsequently, providing benefits that can have long-term impacts well into adulthood (Baird et al., 2016).

**Diagnostics**

Reporting of accurate epidemiologic data on STH and protozoa is essential to understanding intestinal parasitic infections in children. The prevalence of these parasites has been largely underreported subsequent to the wide use of standard stool microscopy techniques with generally low sensitivity and specificity (Mejia et al., 2013). Historically, the diagnosis of intestinal parasitic infections has relied on microscopy-based methods (O'Connell & Nutman, 2016) due to advantages including, low cost, easy to use in resource-limited settings, and having the capacity to detect several parasite species (Icani et al., 2017). However, microscopy has fundamental disadvantages. Microscopy-based approaches are labor-intensive, time-consuming, and require skilled personnel for accurate interpretation who are often not available in resource-limited settings (Easton et al., 2016; McHardy et al., 2014; O'Connell & Nutman, 2016). Notably, an inherent disadvantage of microscopy-based methods is low sensitivity (Easton et al., 2016; Incani et al., 2017; O'Connell & Nutman, 2016), particularly in low prevalence areas (Easton et al., 2016), ranging from 53 – 80% (Easton et al., 2016) and as low as ≤ 30% (Gerace et al., 2019). Specificity is also a limiting factor in microscopy, in that, species-specific distinctions cannot be made between parasite eggs that are morphologically
indistinguishable (O'Connell & Nutman, 2016; ten Hoven, 2009). Given these
disadvantages, microscopy continues to be the cornerstone of diagnostic testing for both
STH and intestinal protozoa (McHardy et al., 2014). Subsequently, infected children can
go undiagnosed and untreated, and infection prevalence is underreported for
epidemiologic surveys. This has significant impacts for treatment decision making and
the implementation of mass drug administration programs.

In light of the shortfalls with microscopy, efforts in the use of molecular
diagnostic methods as an alternative have increased (O'Connell & Nutman, 2016). In
particular, real-time quantitative polymerase chain reaction (qPCR) with high-throughput
technology has shown to have significant advantages (Incani et al., 2017) for the accurate
detection of both STH and protozoa. Compared to microscopy, growing evidence has
indicated that qPCR platforms have high specificity and markedly improved sensitivity
(Mejia et al., 2013; O'Connell & Nutman, 2016), with up to a 10-fold increase in
detection of infection with two or more distinct parasite infections in a single stool
sample (polyparasitism) (Cimino et al., 2015; Mejia et al., 2013) or co-infection with
concurrently occurring STH and protozoa. This is a significant factor in treatment
decision making for selection of appropriate anti-parasitic drugs (Mejia et al., 2013).
Moreover, qPCR can detect parasites that are difficult or impossible to detect by
microscopy with 100% specificity. For example, the detection of *Strongyloides stercoralis*
(Basuni et al., 2011; Shär et al., 2013); parasites with day-to-day variation in
fecal shedding like *Giardia lamblia* (Flecha et al., 2015; Liu et al., 2013; Phuphisut et al.,
2014; Taniuchi et al., 2011; Van Lint et al., 2013); and parasites that are morphologically
indistinguishable by microscopy, such as the pathogenic *Entamoeba histolytica*, and the
nonpathogenic *Entamoeba dispar* (Incani et al., 2017); as well as, the distinction between the two hookworm species *Ancylostoma duodenale* and *Necator americanus.* As a relatively new qPCR platform, multi-parallel real-time qPCR (singleplex) is a high throughput assay that has greater sensitivity and specificity and less costs (Cimino et al., 2015; Easton et al., 2016; Liu et al., 2013; Mejia et al., 2013; Pilotte et al., 2016) compared to other platforms such as multiplexed qPCR (Basuni et al., 2012; Gordon et al., 2015; Hu et al., 2015; Liu et al., 2013; Phuphisut et al., 2014; Taniuchi et al., 2011; Verweij et al., 2007), in which reagents can be a limiting factor (O'Connell & Nutman, 2016). In using multi-parallel real-time qPCR, consumable costs have been estimated to be almost half of the cost of microscopy (Mejia et al., 2013) and requires less time and labor compared to microscopy (Basuni et al., 2011). Thus, providing an optimal alternative to microscopy providing a more accurate and cost-effective approach for the diagnosis of an unlimited number of intestinal parasites that has been implemented for capacity building in resource-limited settings.

**Impact of parasitism**

Childhood intestinal parasitic infections often result in significant morbidity given that children have a propensity for high parasite burden. Children living in highly endemic areas typically have chronic morbidity due to frequent reinfection, which is exceedingly common in children. Childhood intestinal parasitic infections are characteristically chronic and insidious, often with subclinical symptoms, resulting in detriments to health with long-term sequela, often not evident until later in life (Rogawski & Guerrant, 2017b; Weatherhead et al., 2017a; Weatherhead et al., 2017b; ), such as impaired linear growth (Campbell et al., 2016; Gabrie et al., 2016; LaBeaud et al., 2015).
Thus, circumventing achievement of full potential and productivity that can result in lifelong implications influencing adult workforce capacity and wage potential, perpetuating a cycle of poverty (Pullan et al., 2014; Weatherhead et al., 2017a).

Because STH reside in the intestinal tract, these parasitic worms are associated with abdominal manifestations and can interfere with nutrient utilization (Campbell et al., 2016). Infections with moderate-to-heavy parasite burden often cause acute symptoms. Children infected with *Ascaris lumbricoides* (roundworm) can experience abdominal pain, abdominal distention, reduced appetite, vitamin A deficiency, wheezing or asthma (Weatherhead et al., 2017a) and intestinal obstruction with severe infections (Weatherhead et al., 2015). While, *Trichuris trichiura* (whipworm) infection can result in colitis, *Trichuris* dysentery syndrome, and rectal prolapse (Weatherhead et al., 2015). Children infected with hookworm (*Ancylostoma duodenale* and *Necator americanus*) experience chronic iron-deficiency anemia due to blood loss resulting from worm blood-feeding (Hotez et al., 2004). STH infections with light parasite burden, and in some cases with moderate parasite burden, are often asymptomatic without overt disease (Al-Delaimy et al., 2014; O’Connell & Nutman, 2016). Although STH infections can be asymptomatic in children, they have been associated with indirect chronic morbidity, such as impaired growth (de Gier et al., 2014). The association between STH and growth in children has been widely investigated; however, study findings remain inconsistent (Campbell et al., 2016; Gyorkos et al., 2011; LeBeaud et al., 2015).

A series of Cochrane systematic reviews and meta-analyses (Taylor-Robinson et al., 2012; Taylor-Robinson et al., 2015; Taylor-Robinson et al., 2019) examined the impact of mass drug administration for STH infection (deworming) on growth,
hemoglobin levels, and school performance. In these reviews, the authors concluded that
deworming did not significantly improve growth (Taylor-Robinson et al., 2012; Taylor-
Robinson et al., 2015; Taylor-Robinson et al., 2019). This evidence contributed to the
ongoing controversy in the sharply debated question on whether the evidence
demonstrates reduced STH morbidity from deworming (Campbell et al., 2016; de Silva et
al., 2015; Lo et al., 2018; Majid et al., 2019). In contrast to the Cochrane reviews
(Taylor-Robinson et al., 2012; Taylor Robinson et al., 2015), a recent systematic review
and meta-analysis expanded the sample used in the Cochrane reviews and found
improvements in weight following deworming (Croke et al., 2016). Additionally, Lo and
colleagues (2018) recently examined the association between deworming and growth
among preschool-age children using data from 45 STH endemic countries. In this study,
findings indicated that children treated for STH were less likely to have impaired growth
but showed no consistent effect on improved weight (Lo et al., 2018). In contrast,
previous randomized controlled trials reported significant improvements in weight but
not in height following treatment for STH (Awasthi et al., 2008; Stoltzfus et al., 2004).
Furthermore, a recent randomized controlled trial with a one year of follow-up reported
no effect of deworming on growth in a population of Peruvian preschool-age children
(Joseph et al., 2015). In contrast, deficits in growth measures for both height and weight
were reported by studies among school-age children living in rural communities in
Honduras (Sanchez et al., 2013) and southwest China (Liu et al., 2015). These
inconsistencies in reported findings on the relationship between STH infection and
growth in children highlight the need for continued research. Furthermore, shortfalls of
the previous Cochrane reviews, including deficits in reliably differentiated parasite
species (Majid et al., 2019) or species-specific effects (Campbell et al., 2016; de Silva et al., 2015) and a lack of evaluation of morbidity with consideration of parasite burden (de Silva et al., 2015) identify significant research gaps. These deficits in research are particularly important to child health given that parasite burden is proportional to morbidity (Anderson & May, 1991; Bundy & Medley, 1992) and considering that morbidity varies depending on STH species (Majid et al., 2019). Thus, lending support to the importance of utilizing highly sensitive and specific diagnostic methods in STH research studies. Another critical gap in research on the association between STH infection and growth in children is the predominant focus on the school-age population compared to children younger than five years of age (Thayer et al., 2017). The preschool age group is an important population due to increased vulnerability to health insults given critical periods of growth and development that are characteristic to early childhood.

Intestinal protozoa, including *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*, like STH, also contribute significantly to childhood morbidity. These parasites are recognized as pathogens causing diarrhea among infants and young children, particularly those living in poverty throughout developing regions of the world (Mmbaga et al. 2017; Mondal et al., 2006). Because infections with these intestinal protozoa are often asymptomatic or occurring in the absence of diarrhea (Bernin et al., 2014; Kantor et al., 2018; Kattula et al., 2017; Korpe et al., 2016; Kotloff et al., 2013; Platts-Mills et al., 2015), their impact is unclear in endemic regions where frequent reinfection is common among children, particularly among those living in extreme poverty (Rogawski et al., 2018). Findings from recent birth cohort studies in low-to-middle income countries indicated that *Giardia lamblia* (Platts-Mills et al., 2015;
Rogawski et al., 2017) and Cryptosporidium (Korpe et al., 2016; Kotloff et al., 2013; Platts-Mills et al., 2015) infections during the first two years of life were associated with reduced growth measures, whether children experienced diarrheal illness or not (Rogawski et al., 2017). A previous longitudinal study conducted in Bangladesh, found that *Entamoeba histolytica* was associated with reduced height and weight among children from two to five years of age, but *Giardia lamblia* and *Cryptosporidium* were not associated with reduced growth measures (Mondal et al., 2006). Similarly, recent birth cohort studies in Kenya (LeBeaud et al., 2015) and Bangladesh (Korpe et al., 2016) found that *Entamoeba histolytica* was associated with reduced mean height-for-age *z*-scores (Rogawski et al., 2017). In contrast, a recent study in Mexico found no association between *Entamoeba histolytica* and height or weight measures among school-age children (Rojas et al., 2016). Findings from studies on the association between intestinal protozoa (*Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*) and growth among children remain mixed. These studies raise the question of the role of intestinal protozoa in causing covert damage contributing to growth deficits (McCormick et al., 2015; Rogawski et al., 2017). The lack of consistent findings suggests the need for further research on this relationship.

Polyparasitism is exceedingly common among children (Mutombo et al., 2019). Children with polyparasitism are infected with more than one parasite species, either STH or protozoa; or infection with both STH and protozoa occurring concurrently as a co-infection. The high prevalence of STH and protozoa in resource-limited regions of the world and their overlap in geographic distribution indicates that the potential for polyparasitism or co-infection is high (Gordon et al., 2015; Howard et al., 2001; Viney et
al., 2013). These infections are of significant concern among children, especially during early childhood, given that multiple species infections can exacerbate and compound morbidity (Sayasone et al., 2015; Steinmann et al., 2010) at a time of particular vulnerability to health insults. Although it has been increasingly recognized that both STH and intestinal protozoa commonly infect preschool-age children causing significant morbidity in this population (LeBeaud et al., 2015), by and large, research studies on the relationship between polyparasitism and growth among preschool-age children have been limited compared to studies on the school-age population (Aiemjoy et al., 2017; Brooker et al., 1999; Campbell et al., 2016; Garzón et al., 2018; Gyorkos et al., 2011; Harhay, et al., 2010; LeBeaud et al., 2015; Pullan et al., 2009). Importantly, there is also a paucity of high-quality epidemiologic data to elucidate the extent of polyparasitism and associated morbidity, such as impaired growth among children (Steinmann et al., 2010). A study conducted in Southeast Asia among children between six months and 12 years of age found no evidence of an association between STH polyparasitism and impaired growth or being underweight (Sayasone et al., 2015). Although polyparasitism and co-infections are highly prevalent in endemic areas, limited large-scale studies have been conducted evaluating true infection status and parasite burden in association with child health (Howard et al., 2001; Keiser et al., 2002), including growth outcomes. Furthermore, studies have predominantly focused on the identification of a single parasite group, either STH or protozoa, with identification of polyparasitism and co-infections likely limited by the wide use of microscopy-based diagnostic methods with generally poor sensitivity and specificity. Because clinical manifestations may be similar between intestinal parasite species, the lack of accurate identification of polyparasitism or co-infection is further
complicated (Weerakoon et al., 2018). Thus, molecular diagnostic methods, such as real-time qPCR are paramount for more accurate identification of intestinal parasites with high sensitivity and specificity for the detection of multiple parasite species and concurrently occurring STH and protozoa (Al-Shehri et al., 2016; Gordon et al., 2015). This is critical for treatment decision making and implementation of mass drug administration, which can have a global impact.

Early childhood is a vulnerable time for exposure to intestinal parasites and disease burden from STH and protozoa due to the potential for long-term consequences and the contribution to ongoing transmission (Lo et al., 2015). Because growth during early childhood is an important determinant of future health (Matos et al., 2017), the preschool age group is an important population for investigation of morbidity from intestinal parasites in endemic regions. Differences in study design, type of diagnostic method used, and study populations between studies have led to a wide variation of prevalence and parasite burden estimates for STH and protozoa infections, including polyparasitism. The lack of consensus in findings among studies investigating the relationship between intestinal parasites and growth in young children raises the question of the role of STH and protozoa in causing chronic and covert physiological damage contributing to long-term health detriments including growth deficits (McCormick et al., 2015; Rogawski et al., 2017). Furthermore, inconsistent diagnostic methods across studies present challenges for surveillance to reliably measure the impact of mass drug administration programs (Easton et al., 2016). Thus, suggesting that further research is needed to better understand the relationship between intestinal parasites and child growth outcomes. In light of the challenges presented in the evaluation of childhood growth
given the dynamic characteristics of growth changes over time, the study design is an important consideration for further research.

**Methods**

**Study Design**

An observational retrospective longitudinal cohort study design was used to analyze data collected from children at 36 and 60 months of age within the Ecuador Life (ECUAVIDA) birth cohort study, which followed children through eight years of age.

**Study Population**

The study site was the rural District of Quinindé, Esmeraldas Province in Ecuador. This District is located in the coastal region of northern Ecuador at an elevation of approximately 100 meters above sea level and has an average annual temperature of 30 degrees Celsius and 75% humidity (Cooper et al., 2011). The District of Quinindé has a population that is largely rural (78%) and is ethnically comprised of Mestizos (90%), Afro-Ecuadorians (7%), and Amerindians (3%) (Cooper et al., 2011). Esmeraldas Province is one of the poorest regions in Ecuador. In the rural areas of the District, only 10% have access to electricity, and none have access to basic services such as sanitation and clean water (Cooper et al., 2011).

**Sample Size**

The sample size was determined by using the single population proportion formula, \( N = \frac{Z^2 P (1-P)}{d^2} \) (Arifin, 2013), with an estimated 50% prevalence of intestinal parasitic infections based on reported prevalence data (Cooper et al., 2011; Mehraj et al., 2008), 95% confidence interval and 5% bound on the error of estimation for precision. The minimum required sample size was calculated as 385 participants with power (1-\( \beta \))
set at 80% and a medium effect size at $\alpha = .05$. Based on this calculation and available
resources for analysis, a total of 400 children were randomly selected. Of these, only
those participating in both follow-up observations with complete data were used in the
present study for a final total population of $n = 177$. This sample size allowed for
estimation of the prevalence of intestinal parasites and for evaluation of associations
between parasite infections and anthropometric measures in rural Ecuadorian children at
36 and 60 months of age.

**Data Collection**

*Anthropometric data.* Anthropometric data included measures of height and
weight obtained at 36 and 60 months of age. At each observation, height in centimeters
(cm) and weight in kilograms (kg) were measured in duplicate and the mean of the two
measurements was used as the final measurement (Matos et al., 2017). All research team
members involved in measuring growth parameters received standardized training on
how to perform anthropometric measurement techniques according to WHO
recommended guidelines (WHO, 2006). Children were measured without shoes and in
light clothing or underwear. Intra-observer discrepancies were reduced by using the same
equipment and the same trained personnel to measure height and weight. All
measurements for height and weight were reviewed for possible errors in measurement.
Instruments used for growth measurements were selected in accordance with the WHO
guidelines for anthropometric measurements and were widely used in local health care
settings. The WHO Anthropometric software (WHO Anthro version 3.2.2.) for Windows
was used to calculate z-scores for anthropometric indices for height and weight, including
height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) z-scores
These anthropometric indices were used to examine growth differences between infected and non-infected children and to compare overall height and weight with WHO reference population. Children were weighed on a portable electronic scale (Seca, Germany) accurate to within 100 grams. Standing height was measured using an in-house wooden stadiometer accurate to within 0.1 cm (Matos et al., 2017). All instruments used for anthropometric measurements were calibrated daily prior to use.

**Parasite data.** Stool samples for the detection of intestinal parasites were collected at 36 and 60 month follow-up visits as part of the ECUAVIDA birth cohort study. These stool samples were stored at 4° Celsius to preserve the stability of parasite DNA at the study site laboratory in Quito, Ecuador until being subjected to qPCR assay analysis for the molecular diagnosis of intestinal parasitic infections. Participant stool samples were analyzed for a total of eight intestinal parasite species using a modern molecular diagnostic method with a modified version of a validated quantitative real-time polymerase chain reaction assay known as a multi-parallel real-time quantitative PCR (qPCR) (Mejia et al., 2013). This qPCR assay allowed for a total reagent volume of 7 µL (Mejia et al., 2013). This accommodated the minimum settings on the Applied Biosystems 7900HT Fast Real-Time PCR System (Mejia et al., 2013). Parasite DNA was extracted from approximately 50 mg of stool via the FastDNA™ SPIN Kit for soil DNA extraction (MP Biomedical, Solon, OH) using a low reagent method developed by Mejia and colleagues (2013) for use in resource-limited settings. An additional step was required to extract *Trichuris trichiura* DNA, in which the remaining insoluble pellet from the initial DNA extraction was re-suspended in 200 µL DNA-free water, heated at 90° Celsius for 10 minutes, and centrifuged at 14,000g for 10 minutes (Mejia et al., 2013).
The above described DNA extraction method was then be performed to process the resulting soluble portion of the sample. Methodology for the qPCR assay involved testing all control standards in triplicate and all unknown samples in duplicate. A qPCR cycle threshold (Ct) value > 38 was considered as a negative result. Each primer and probe combination previously demonstrated 100% sensitivity and 100% specificity for the designated parasite species (Mejia et al., 2013). Parasite burden quantifications were performed by interpolating against parasite species-specific sequence standards and reported as DNA fg/µL. To evaluate parasite burden based on WHO criteria guidelines, estimated egg counts from qPCR DNA fg/µL were calculated using $Y_{\text{ova/g feces}} = (0.02748)/(X \text{ fg/µL})$ for hookworm (Necator americanus and Ancylostoma duodenale); $Y_{\text{ova/g feces}} = (0.001455)/(X \text{ fg/µL})$ for Ascaris lumbricoides; $Y_{\text{ova/g feces}} = (0.00001095)/(X \text{ fg/µL})$ for Trichuris trichiura; and $Y_{\text{larvae/gram}} = (0.01471)/(X \text{ fg/µL}) = (Y_{\text{larvae/gram}})(0.01505629) = Y_{\text{larvae/gram}}$ for Strongyloides stercoralis.

**Sociodemographic data.** An interview-led questionnaire was conducted at the 60 month follow-up to collect data from the participant’s mother related to demographic and socioeconomic factors. This questionnaire was translated into Spanish and administered by trained research team members. The sociodemographic and maternal factors used in this study included: sex, household crowding, birth order, potable water, maternal education, maternal ethnicity, maternal helminth infection, and socioeconomic status.

**Data Analysis**

All data were checked for recording errors and verified for accuracy. Both SPSS (version 26) and GraphPad Prism (version 8.1) were used for statistical analysis and graphs. Statistical significance was defined as $p$ value < .05. Only children with complete
matched data for both follow-up observation time points at 36 and 60 months of age were included in the final analysis (n = 177). Growth parameter measurements for height (cm) and weight (kg) were used to calculate z-scores for anthropometric indices using WHO Anthro software (Version 3.2.2.) (WHO, 2011). Anthropometric indices used to classify growth deficits included height-for-age z-scores (HAZ), weight-for-age z-scores (WAZ), and weight-for-height z-scores (WHZ), which were then classified as HAZ ≤ -1 SD > - 2 SD for linear growth faltering; HAZ ≤ -2 SD > -3 SD for stunting; HAZ ≤ -3 SD for severe stunting; WAZ ≤ - 1 SD > -2 SD for mild underweight; WAZ ≤ -2 SD > -3 SD for underweight; WAZ ≤ -3 SD for severely underweight; WHZ ≤ -1 SD > -2 SD for mild wasting; WHZ ≤ -2 SD > -3 SD for wasting; WHZ ≤ -3 SD for severe wasting. WHO guidelines were used to classify children as having light, moderate, and heavy parasite burden by parasite egg counts per gram of stool (epg) with each parasite as follows: For *Ascaris lumbricoides*, light (1 – 4,999 epg), moderate (5,000 – 49,999 epg), and heavy (≥ 50,000 epg); for *Trichuris trichiura*, light (1 – 999 epg), moderate (1,000 – 9,999 epg), and heavy (≥ 10,000 epg) as heavy; and for hookworm, light (1 – 1,999 epg), moderate (2000 – 3,999 epg) and heavy (≥ 4,000 epg) (WHO, 2002). Moderate and heavy parasite burden were merged into a single category of moderate-to-heavy for estimations of associations with infection burden by epg as categorical variables.

Descriptive statistics were used to summarize study population characteristics. The mean with standard deviation was reported for continuous variables. For categorical variables, frequency and proportions were reported. Normality was assessed for the distribution of continuous variables by visual inspection of histograms and the Shapiro-Wilk test. Growth indicators and parasite variables were initially assessed with
univariable analyses. Associations between growth indicators and parasite infections, as well as, parasite burden were evaluated using bivariate and multivariable analyses adjusted for potential confounding sociodemographic factors. Correlations between variables were evaluated using the Spearman rank correlation coefficient when assumptions for the Pearson product-moment correlation coefficient were not met. Categorical variables were compared using the Chi-square ($X^2$) test of independence or Fisher's Exact test when appropriate. Comparison of variables was carried out with the Mann-Whitney U test when appropriate for data with a non-normal distribution. Continuous data compared across age was assessed using the Wilcoxon-signed rank test. Sociodemographic factors associated with growth deficits and similarly with parasite infection in univariate analysis were included in multivariable analyses across age. Associations with infection burden by parasite species were estimated by linear regression with concentrations of parasite DNA in fg/μL as the dependent variable. Due to the overdispersed correlated nature of epg and DNA fg/μL values, extreme outliers were not removed from the sample to avoid excluding data points of interest. Multivariable regression analyses were used to identify potential predictors of growth. Beta ($\beta$) coefficient and its standard error and 95% confidence interval were reported for significant variables. All statistically significant variables in bivariate analysis were used in multivariable regression for the most parsimonious model.

**Ethical Considerations**

The study protocol was reviewed and approved by the Committee for Protection of Human Subjects (CPHS) at the University of Texas Health Science Center at Houston and the Biomedical Research and Assurance Information Network (BRAIN) at Baylor.
College of Medicine. The study protocol for the ECUAVIDA birth cohort study was approved by the ethics committee for the Hospital Padre Alberto Buffoni (HPAB), the Universidad San Francisco de Quito, and Pontifica Universidad Catolica del Ecuador.

Results

Study Population Characteristics

During the two-year study period, 177 children participated in both follow-up observations at 36 and 60 months of age. Of these, 48.6% (86) were male and 51.4% (91) were female. The study population was ethnically mixed, comprised of Mestizos (73.4%), Afro-Ecuadorian (26.0%), and Amerindian (1%) children. Socioeconomic status (SES) relative to poverty level was reported as 40% low SES (high poverty), 32.8% medium SES (moderate poverty), and 27.1% high SES (low poverty). Sociodemographic and maternal characteristics are summarized in Table 1. In this study population, the mean for height (cm) fell below the 50th percentile on WHO growth charts at both 36 and 60 months of age. Among these children, 44.6% and 32.8% at 36 and 60 months of age, respectively, were found to have linear growth faltering (HAZ ≤ -1 SD > -2 SD). While, 11.9% at 36 months and 5.1% at 60 months were stunted (HAZ < -2 SD). Overall, growth measures indicated that boys were on average taller than girls and girls were heavier than boys; however, growth differences by sex were not statistically significant. Anthropometric characteristics and frequency of growth delays are summarized in Table 2. At 36 months of age, comparison of anthropometric indices by ethnicity indicated statistically significant differences in WAZ but not for HAZ between Afro-Ecuadorian and Mestizo children (p = .026 and p = .060, respectively). This same analysis for comparison of growth indices at 60 months of age indicated statistically significant
differences in both HAZ and WAZ between Afro-Ecuadorian and Mestizo children ($p = .047$ and $p = .014$, respectively). Anthropometric characteristics by ethnicity are summarized in Table 3.

**Parasite Prevalence and Parasite Burden**

Eight intestinal parasites, five STH and three protozoa, were detected by multi-parallel, real-time qPCR from a single stool sample collected at each follow-up observation. Over half of the study population was infected with at least one parasite species at both 36 and 60 months, with a prevalence of 118 (66.7%) and 103 (58.2%), respectively. The frequency of detection for all parasite species in this study decreased with age, except for *Trichuris trichiura* and *Ancylostoma duodenale*, which increased with age. However, the difference in proportion for parasite species across age was only statistically significant for *Ascaris lumbricoides* ($p = .014$), *Strongyloides stercoralis* ($p = .039$) and *Giardia lamblia* ($p = .001$). Among infected children the most prevalent parasite species at both follow-up observations were *Giardia lamblia*, followed by *Trichuris trichiura* and *Ascaris lumbricoides*. The most frequently detected single parasite was *Giardia lamblia*. Polyparasitism, infection with more than one parasite species, was predominantly comprised of concurrently occurring STH and protozoa or co-infections. The most common co-infections were *Ascaris lumbricoides* and *Giardia lamblia*, as well as, *Trichuris trichiura* and *Giardia lamblia*. In this study population single parasite infections were significantly more prevalent than polyparasitic infections at both 36 months ($p = .001$) and 60 months ($p = .001$). Of those with polyparasitism, up to four different parasites were detected. Parasite infection prevalence stratified by infection type and parasite species across age, including change in parasite prevalence, is
summarized in Table 4. Parasite burden by each parasite species, measured as the concentration of parasite DNA in femtograms per microliter (fg/µL), were found to have highly skewed non-normal distributions. Thus, non-parametric tests were used for the evaluation of these variables. Analysis for change in parasite burden across age from 36 to 60 months of age indicated a statistically significant difference in parasite burden for *Ascaris lumbricoides*, which decreased with age ($p = .045$). Because parasite burden measured in DNA fg/µL is correlated with parasite burden measured in eggs per gram of stool (epg), *Ascaris lumbricoides* burden was predominantly moderate-to-heavy at 36 months and predominantly light-to-moderate at 60 months of age based on WHO criteria (WHO, 2002). Parasite burden for *Ascaris lumbricoides* at 36 and 60 months of age measured as DNA fg/µL and epg is illustrated in Figure 1.

**Sociodemographic Factors and Intestinal Parasites**

Correlations between parasite burden and sociodemographic factors were analyzed using the Spearman’s correlation coefficient for the most frequently detected parasites, *Giardia lamblia, Ascaris lumbricoides*, and *Trichuris trichiura*. This analysis indicated that *Giardia lamblia* parasite burden (DNA fg/µL) and household crowding were positively correlated at 36 months, $r = 0.226, p = .030, 95\% \text{ CI} [0.016 \text{ to } 0.417]$, and at 60 months, $r = 0.257, p = .023, 95\% \text{ CI} [0.301 \text{ to } 0.459]$. This correlation is illustrated in Figure 2. Further correlation analyses indicated that *Ascaris lumbricoides* was also positively correlated with crowding at 36 months, $r = .506, p = .005, 95\% \text{ CI} [.206 \text{ to } .7510]$, but not at 60 months. In addition, parasite burden for *Giardia lamblia* was negatively correlated with SES, $r = -.229, p = .028, 95\% \text{ CI} [-.386 \text{ to } -.081]$, and maternal education, $r = -.249, p = .017, 95\% \text{ CI} [-.463 \text{ to } -.040]$ at 36 months, but not at
60 months. No other significant correlations were found between parasite burden and sociodemographic factors. Child sex was not statistically significantly associated with parasite burden or parasite infection status and was not a significant predictor of any infection type; therefore, male and female variables were analyzed together.

The relationship between sociodemographic factors and parasite status was explored using a Chi-Square test with Yates’ Correction for Continuity for two by two cross-tabulation. At 36 months, the most common sociodemographic factor found to be associated with parasite status was maternal helminth infection, which was associated with the following: infection with any parasite, $X^2 (1, 177) = 6.080, p = .021, \phi = .185$, with OR = 2.278 and 95% CI [1.176, 4.413]; polyparasitism, $X^2 (1, 177) = 8.600, p = .003, \phi = .234$, with OR = 3.060 and 95% CI [1.487, 6.295]; and co-infection, $X^2 (1, 177) = 4.837, p = .028, \phi = .179$, with OR = 2.457 and 95% CI [1.160, 5.205]. At 60 months, maternal helminth infection was also significantly associated with polyparasitism, $X^2 (1, 177) = 12.163, p = .0005, \phi = .276$, with OR = 3.960 and 95% CI [1.846, 8.497]; and co-infection, $X^2 (1, 177) = 11.235, p = .001; \phi = .266$, with OR = 4.075 and 95% CI [1.809, 9.180].

Differences in Growth Measures by Parasite Status and Parasite Burden

The Mann-Whitney U test was used to determine whether parasite burden differed between children with and without growth deficits based on anthropometric indices.

Parasite burden for the most prevalent parasites, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Giardia lamblia* were evaluated. Findings indicated that parasite burden (DNA fg/μL) for *Ascaris lumbricoides* was significantly different between infected children with and without stunting or HAZ < -2 SD ($U = 40.00, z = -2.147, p = .032$) at
36 months (Figure 3), where stunted children had higher median parasite burden (Md = 81.05 DNA fg/µL, n = 8) compared to non-stunted children (Md = 4.11, n = 21). A large effect size for this difference was determined as $r = .4$ based on Cohen’s criteria (Cohen, 1988). Parasite burden for *Trichuris trichiura* also differed between infected children at 36 months who were underweight (WAZ < -2 SD) (Md = .202, n = 3) and those who were not underweight (Md = .013, n = 30), $U = 9.00$, $z = -2.254$, $p = .024$, with a large effect size as $r = .4$. At 60 months, no significant associations were found between parasite burden and growth deficits. The Mann-Whitney U test results for differences in parasite burden between children with and without growth deficits based on anthropometric indices at 36 months are summarized in Table 5. Further evaluation using the Mann-Whitney U test was used to determine associations between parasite status and growth for differences in growth measures. Findings indicated that both measures of height (cm), $U = 2220.50$, $z = -2.120$, $p = .034$; and HAZ, $U = 2187.50$, $z = -2.233$, $p = .026$, differed between children with and without polyparasitism, where children with polyparasitism had lower HAZ, (Md = -1.39, n = 42) compared to children without polyparasitism (Md = -1.0, n = 59). Children with co-infection status were also found to have statistically significant differences in HAZ compared to children with co-infection, $U = 1963.500$, $z = -2.094$, $p = .036$, where children infected with both STH and protozoa (co-infection) had lower HAZ (Md = -1.45, n = 36) compared to children without co-infection (Md = -1.06, n = 141). Evaluation of associations between species-specific parasite status and growth were carried out. A Mann-Whitney U test indicated a statistically significant differences in HAZ between children with *Ascaris lumbricoides* single parasite infection (Md = -0.40, n = 7) and non-infected children (Md = -1.18, n =
Children infected with *Ascaris lumbricoides* and *Giardia lamblia* co-infected had lower HAZ compared to children without this co-infection, with this difference only approaching significance, $U = 1032$, $z = -1.934$, $p = .053$, $r = .14$. No other growth differences by parasite status were found at 36 months. No associations between parasite status and growth were found at 60 months of age. The Mann-Whitney U test results are summarized for differences in growth measures between infected and non-infected children based on parasite status Table 6 and species-specific parasite status Table 7. Further evaluating growth differences by co-infection, the most common co-infections including *Ascaris lumbricoides/Giardia lamblia* and *Trichuris trichiura/Giardia lamblia* co-infections were evaluated. The Kruskal-Wallis test was used to answer the question of whether there were differences in HAZ among children with *Ascaris lumbricoides* single parasite infection, *Giardia lamblia* single parasite infection, and *Ascaris lumbricoides/Giardia lamblia* co-infection. Results indicated a statistically significant difference in HAZ between children across these parasite infections, $X^2 (2, n = 77) = 7.373$, $p = .025$. Children infected with both *Ascaris lumbricoides* and *Giardia lamblia* (co-infection) had statistically significantly lower HAZ ($M = -2.03$) compared to *Ascaris lumbricoides* only infection ($M = -.76$) and *Giardia lamblia* only infection ($M = -1.62$). These difference in HAZ are illustrated in Figure 4. The association between these parasite infections and risk of stunting was explored. A Fisher’s Exact test indicated that children infected with both *Ascaris lumbricoides* and *Giardia lamblia* had 3.44 greater odds of being stunted compared to children without this co-infection, OR = 3.44, 95% CI [1.08, 10.89], $p = .044$. Similar significant findings were not found for *Trichuris trichiura* and *Giardia lamblia* co-
infection or *Ascaris lumbricoides*, *Trichuris trichiura* and *Giardia lamblia* single parasite infections. Cross-tabulation results for risk of stunting are summarized in Table 8.

**Multivariable Regression for Predicting Growth**

Multivariable regression was used to determine significant predictors of HAZ at both 36 and 60 months, while adjusting for potential confounding variables. Potential predictors included parasite status and sociodemographic factors. For the most parsimonious multivariable regression model, parasite status and sociodemographic factors found to be significant predictors of HAZ in bivariate analyses were included in the multivariable model at 36 and 60 months. Maternal helminth infection, maternal ethnicity, and *Ascaris lumbricoides/Giardia lamblia* co-infection at 36 months were found to be significant predictors in bivariate analysis. At 60 months, only maternal helminth infection and maternal ethnicity were found to be significant predictors. Given the results of bivariate analyses, maternal helminth infection, maternal ethnicity, and *Ascaris lumbricoides/Giardia lamblia* co-infection were included as the most parsimonious multivariable regression model at 36 months. When considered together, each of these variables were found to be statistically significant predictors of HAZ at 36 months, $F(3, 175) = 5.664, p = .001$, with maternal ethnicity making the greatest contribution to this model. At 60 months, maternal helminth and maternal ethnicity were included in the multivariable model. Both remained statistically significant predictors of HAZ at 60 months, $F(3, 175) = 3.588, p = .010$, with maternal ethnicity making the greatest contribution to this model. Multivariable regression models for 36 and 60 months are summarized in Table 9. Furthermore, multivariable regression was used to determine significant predictors of HAZ at 60 months from parasite status at 36 months, while
controlling for potential confounding variables. Multivariable regression models included all sociodemographic factors as covariates. None of the multivariable regression models were statistically significant, indicating that parasite infection status (single, polyparasite, helminth, and protozoa) at 36 months was not a significant predictor of HAZ at 60 months.

**Discussion**

The present study investigated retrospective data for prevalence and parasite burden of intestinal parasites and their associations with growth in a population of asymptomatic preschool-age children living in the rural District of Quinindé in tropical Ecuador. Findings from this study contribute to filling the research gap in epidemiologic data on the distribution of intestinal parasites and furthers the understanding of the impacts of intestinal parasites on growth during early childhood. The ethnic distribution of the study population was representative of the population in the study District, with Mestizos comprising the ethnic majority (73.4%) followed by Afro-Ecuadorian (26.0%), and fewer Amerindian children (0.6%). Children in this study were nearly equally distributed between males (48.6%) and females (51.4%). Overall, the study population reflected a growth pattern falling below the median for the WHO reference population relative to HAZ and WAZ (Matos et al., 2017). Below-average growth among children in this study is consistent with a recent national growth survey in Ecuador, reporting that on average, 15% of preschool and school-age children experience growth restrictions (Freire et al., 2013). Although there were no appreciable growth differences between boys and girls, boys tended to be taller than girls; while, girls tended to be heavier than boys. In contrast, significant differences were found relative to ethnicity, with Afro-Ecuadorian
children being taller and heavier than Mestizo children. Overall, this rural study population was poor, lacking basic services for sanitation and clean water, with the majority experiencing a high level of poverty (40.1%). Given the tropical climate and poverty in this rural setting, environmental conditions were favorable for the survival and transmission of both STH and protozoa.

**Intestinal Parasite Prevalence**

A multi-parallel, real-time qPCR assay was used to detect eight intestinal parasite species including STH and protozoa commonly infecting children. Over half of the study population was infected with at least one parasite species, with 66.7% infected at 36 months and 58.2% infected at 60 months. The most prevalent parasite species were *Giardia lamblia*, followed by *Trichuris trichiura*, and *Ascaris lumbricoides*. In contrast, a previous study from rural Ecuador reported a predominance of STH species versus *Giardia lamblia* (protozoa) among preschool and school-age children (Sackey et al., 2003). Because this previous study was conducted in the northern Sierra region of rural Ecuador (Sackey et al., 2003); while, the present study was conducted in the northern Coastal region of Ecuador may be a possible explanation for this discrepancy. At both 36 and 60 months of age, *Trichuris trichiura* was more prevalent than *Ascaris lumbricoides*. This predominance is consistent with reported findings from a recent national survey in Ecuador on STH infections in school-age children (Moncayo, 2018). In this survey, the prevalence of STH (27.9%) reported as an aggregate group (Moncayo et al., 2018) was higher than what was found in the present study at 36 and 60 months of age (19.5% and 21.4%, respectively). Because this survey used a microscopy-based diagnostic approach with generally poor sensitivity and specificity, STH prevalence may have been
overestimated. In the present study, an overall decreasing trend in prevalence across age was observed, with a significant prevalence pattern for *Ascaris lumbricoides* (*p* = .014), *Strongyloides stercoralis* (*p* = .039), and *Giardia lamblia* (*p* = .001). A similar decreasing trend was also found for parasite burden, but was only significant for *Ascaris lumbricoides* burden (*p* = .045). In contrast, previous studies reported increasing prevalence trends for children up to two years of age in Sub-Saharan Africa (Garzón et al., 2018) and up to three years of age in Ecuador (Menzies et al., 2014). This contrast may be evidence indicating that the prevalence of intestinal parasites may peak at three years of age. A peak prevalence at this young age indicates a high transmission rate among children in this study (Woolhouse, 1998) and supports the vulnerability to infection with STH and protozoa in this young age group. The prevalence of *Trichuris trichiura* combined with a very low prevalence of both hookworm species (< 3%) is consistent with previously reported epidemiologic data from South America among populations of children with a greater than 20% overall prevalence of STH (Chammartin et al., 2013). Furthermore, previously reported findings from a study in the same rural Ecuadorian setting indicated a lower prevalence for STH among preschool-age children (Menzies et al., 2014). This previous study used microscopy-based diagnostic methods with low sensitivity, supporting a potential underestimation of prevalence compared to that determined using the highly sensitive molecular, real-time qPCR approach used in the present study. Although the prevalence of *Strongyloides stercoralis* was very low at 36 months (8.5%), this is an important finding because it confirms that this parasite infects children in early childhood. Furthermore, this contributes accurate epidemiologic data on the distribution of this parasite among preschool-age children, which is largely poorly
understood due to the scarcity of available accurate epidemiologic data in this young population of children.

In this study, single parasite infections were significantly predominant compared to polyparasitic infections. Overwhelmingly, *Giardia lamblia* was the most prevalent single parasite infections. A similar predominance of *Giardia lamblia* was reported from a previous study in Argentina among a population with a mean age of 7.2 years (Cimino et al., 2015). In the present study, approximately half as many children with single parasite infections were infected with multiple parasite species (polyparasitism).

Although polyparasitism was less prevalent, infections with more than one parasite species are an important concern for this young age group due to the physiological vulnerability to health insults inherent of early childhood. Because parasite burden is proportional to morbidity, multiple parasite species subsequently increase parasite burden compounding and exacerbating morbidity (Steinmann et al., 2010; Sayasone et al., 2015). Thus, representing a substantial contribution to chronic morbidity in young children. In this study, children with polyparasitism were infected with up to four parasite species. The most frequent STH polyparasitic infection was *Ascaris lumbricoides* and *Trichuris trichiura*. This was not unexpected because these two STH species commonly occur together in children living in endemic regions due to similar transmission patterns (Weatherhead, 2017b). However, the most common dual infections in this study population were co-infections with concurrently occurring STH and protozoa. In contrast, *Ascaris lumbricoides* and *Trichuris trichiura* dual infection was previously reported as the most common polyparasitic infection among a school-age population in rural Malaysia (Al-Delaimy et al., 2014). Notably, polyparasitic infections were largely
comprised of concurrently occurring STH and protozoa (co-infections) with 85.7% and 87.1% of the total polyparasitic infections found at 36 and 60 months, respectively. Thus, providing evidence of overlapping geographic prevalence and similar transmission dynamics (Cimino et al., 2015); thus, highlighting the vulnerability of this young age group to infection with both STH and protozoa. Subsequently, suggesting children in this study were at greater risk of exposure to both contaminated soil and water. This dual exposure is not uncommon in rural resource-limited settings with a high level of poverty consistent with the study population. The identification of co-infections in this study further highlights the important need for utilizing highly sensitive and specific diagnostic methods for reporting accurate epidemiologic data. Another study utilizing a molecular qPCR approach previously reported co-infections to be common in a population of children in a rural region of the Philippines (Weerakoon et al., 2018).

In the present study, co-infections were mostly comprised of *Ascaris lumbricoides* and *Giardia lamblia* or *Trichuris trichiura* and *Giardia lamblia*. Similar findings for *Ascaris lumbricoides* and *Giardia lamblia* co-infection were previously reported among a population ranging in age from seven months to 57 years of age in Argentina (Cimino et al., 2015). Also, similar finding for *Trichuris trichiura* and *Giardia lamblia* co-infection were previously reported among school-age children in rural Malaysia (Al-Delaimy et al., 2014). Co-infections during early childhood are an important concern, not only because multiple parasite species can compound morbidity, but also because STH and protozoa occurring together reportedly have complex interactions with the host immune system allowing for persistent infection and subsequent greater chronic morbidity (Weatherhead et al., 2017b).
be higher (94.4%) than single parasite infections (84.2%) across follow-up observations over a span of two years supporting evidence for persistent exposure to an environment favoring transmission of both STH and protozoa over time.

**Intestinal Parasites and Sociodemographic Factors**

Although, over half of the study population was infected with intestinal parasites, a significant association between the overall prevalence of intestinal parasitic infections and sociodemographic factors was not found. However, parasite burden and parasite status were found to be associated with sociodemographic factors related to poverty in this population. Parasite burden for *Ascaris lumbricoides* and *Giardia lamblia* were found to be positively correlated with overcrowding (p = .005 and p = .030, respectively), indicating that as crowding conditions increase, parasite burden also increases. Thus, suggesting that children infected with *Ascaris lumbricoides* or *Giardia lamblia* living in crowded household conditions tended to have greater parasite burden compared to children living in less crowded conditions. This is not surprising because household overcrowding is common in poor rural populations and children often share beds with siblings and other household members. Previously reported findings indicated that with a high level of poverty, intestinal parasites are closely associated with overcrowding (Karan et al., 2012). Parasite burden for *Giardia lamblia* was also found to be negatively correlated with socioeconomic status (SES) (p = .028) and maternal education (p = .017). Thus, parasite burden was higher with lower levels of socioeconomic status (SES) and maternal education. Because this study population was overall very poor, SES was stratified by poverty level; such that, as SES decreased, poverty level increased. Suggesting, that children infected with *Giardia lamblia* who were living in conditions of
high poverty (low SES), tended to have greater parasite burden compared to less poor children. Recent studies reported a similar association between *Giardia lamblia* and socioeconomic status among preschool- and school-age children in the Philippines (Heimer et al., 2015). In contrast, a previous study conducted in Peru among preschool- and school-age children reported *Giardia lamblia* infection to be independent of socioeconomic status (Nundy et al., 2011). A more recent study found the same lack of association in a population of school-age children in Lebanon (Osman et al., 2016). Overall, child sex was not associated with either parasite status or parasite burden. However, significant associations were found between ethnicity and parasite status. In this study population, children of Mestizo ethnicity were more likely to be infected with any parasite species and had greater odds of having polyparasitism including co-infection with both STH and protozoa compared to Afro-Ecuadorean or Amerindian children. Maternal STH infection had similar associations with greater odds of children being infected with intestinal parasites. Maternal STH infection was also found to be significantly associated with polyparasitism and co-infection in children. Although the associations between sociodemographic factors found in this study provide evidence to support that intestinal parasitic infections are poverty-related diseases, further analyses not included in this study will further elucidate the relationship between sociodemographic factors and parasite infections.

**Intestinal Parasites and Growth**

Evidence from this study indicated that intestinal parasitic infections were associated with significant differences in growth between infected and non-infected children. Among infected children, linear growth faltering (HAZ ≤ -1 SD > -2 SD) was
more prevalent than stunting (HAZ < -2 SD); however, intestinal parasitic infections were only associated with stunting. The prevalence of stunting among infected children was significantly higher at 36 months (13.6%) compared to 60 months of age (5.1%). A prevalence of stunting among children in a population that is greater than 2.5% has been reported to be an indicator of a deficient growth environment (Leroy & Frongillo, 2019). Overall, growth deficits among infected children were largely relative to measures of linear growth versus weight. Study data indicated that growth effects were more often significant at 36 months compared to 60 months of age. Similar findings were reported from a previous birth cohort study among children up to 36 months in rural Coastal Kenya (LaBeaud et al., 2015). Given that the deleterious effects of intestinal parasites on growth are chronic and occur over time, these findings support the increased vulnerability of younger children to health insults from intestinal parasites and provides evidence that these children were likely infected prior to 36 months of age (LaBeaud et al., 2015).

Because linear growth captures long-term cumulative health effects and is known to be correlated with later life outcomes (WHO, 2006; Zhang et al., 2017), deficits in linear growth were of important interest.

In this study, children infected with more than one parasite species (polyparasitism) and co-infection with both STH and protozoa at 36 months were found to have growth deficits compared to any other parasite status. Children with polyparasitism and co-infections had significantly reduced HAZ compared to children who did not have these infections. Similar findings were previously reported among infants and preschool-age children in Kenya (LeBeaud et al., 2015) and among a population of polyparasitized school-age children in the Colombian Amazonian region.
(Ordonónez & Angulo, 2002). Because polyparasitic infections were predominantly comprised of infections with concurrently occurring STH and protozoa, co-infections likely had the greatest impact.

Previous studies have largely reported associations with growth and STH as an aggregate group and predominantly in the school-age population (Prado et al., 2005; Rajoo et al., 2017). In this study, species-specific associations with growth were evaluated. Evidence indicated that *Ascaris lumbricoides* infection was significantly associated with HAZ in children at 36 months but not at 60 months of age. Among stunted children with Ascariasis, parasite burden (DNA fg/µL) was significantly greater compared to those who were not stunted at 36 months ($p = .032$). Similar increases in parasite burden were found among underweight children with trichuriasis. In contrast, no associations were previously found between parasite burden and anthropometric indices among preschool and school age children in a study conducted in Southeast Asia (Sayasone et al., 2015). In children concurrently infected with *Ascaris lumbricoides* and *Giardia lamblia* co-infection at 36 months, reduced measures for both HAZ and WAZ were found. However, differences in HAZ between infected and non-infected children only approached significance ($p = .053$). Similar growth deficits were not found in children with *Trichuris trichiura* and *Giardia lamblia* co-infection, which was also common in this study population. Furthermore, children with *Ascaris lumbricoides* and *Giardia lamblia* co-infection had significantly greater odds of being stunted compared to children with *Ascaris lumbricoides* and *Giardia lamblia* single parasite infections (Table 7). Further exploring growth measures in children with *Ascaris lumbricoides* and *Giardia lamblia* co-infection, comparisons of growth in children infected with *Ascaris*
Ascaris lumbricoides and Giardia lamblia as single parasite infections indicated that children had significantly lower HAZ (Md = -2.02, \( p = .025 \)) (Figure 5). Notably, these findings on Ascaris lumbricoides and Giardia lamblia co-infection may provide evidence of potential interactions between parasite species or potential synergistic effects that require further investigation when considering health impacts during early childhood in this study population. Despite their potential for interaction, very few studies have examined the relationship between Giardia lamblia and STH (Blackwell et al., 2013). In evaluating sociodemographic factors and parasite status as predictors of growth, Ascaris lumbricoides and Giardia lamblia co-infection, maternal helminth infection, and maternal ethnicity were included as the most parsimonious multivariable regression model given that these variables were significant in bivariate analysis. When considered together, each of these variables were found to be significant predictors of HAZ at 36 months. However, only maternal helminth infection and maternal ethnicity were significant predictors of growth at 60 months. Further multivariable regression analysis indicated that parasite status at 36 months was not a significant predictor of growth at 60 months.

Findings from this study confirmed that intestinal parasitic infections were common in a young age group from 36 to 60 months of age in a resource-limited rural setting in tropical Ecuador. This is one of the few studies to determine the prevalence for both STH and protozoa among preschool-age children in this study setting. The high prevalence of intestinal parasites, which included co-infection with both STH and protozoa is noteworthy in an asymptomatic population of preschool-age children. In this study, growth deficits were found to be associated with intestinal parasitic infections,
predominantly at 36 months of age. Preschool-age children are an important population for intestinal parasitic infection due to their physiological vulnerability and subsequent potential for long-term health consequences, their contribution to ongoing transmission, and their need for treatment outside of school-based mass drug administration programs (Lo et al., 2015; Lo et al., 2018). Reducing morbidity associated with STH and protozoan infections requires accurate and reliable epidemiologic data on prevalence and parasite burden. However, this is difficult to accomplish with widely used microscopy-based methods known to have low sensitivity and specificity (Cimino et al., 2015). The highly sensitive and specific multi-parallel, real-time qPCR assay used in this study for the detection of eight intestinal parasite species including both STH and protozoa was developed to overcome the limitations and challenges of microscopy and other previously developed qPCR approaches in order to provide accurate epidemiologic data on childhood intestinal parasitic infections. Notably, infection with *Strongyloides stercoralis* was found in this young population. Infections with *Strongyloides stercoralis* among children are often not reported due to difficulty in the detection of this parasite with standard microscopy methods. Another advantage of using multi-parallel qPCR in this study was the ability to accurately detect multiple parasite species, including concurrently occurring STH and protozoa from a single stool sample more accurately than microscopy methods. Data from a previous study showed that multi-parallel qPCR was able to detect multiple parasite species at a higher rate compared to microscopy (Cimino et al., 2015). This has important population health implications for pharmacological treatment decisions in the implementation of mass drug administration (MDA) based on the most accurate epidemiologic data (Hotez et al., 2011; Mejia et al., 2013, Cimino et al., 2015).
Findings from this study indicated that *Giardia lamblia* had an overwhelmingly high prevalence in this population of children and confirmed that co-infections with both STH and protozoa where prevalent in this young rural population. However, protozoan infections may not be addressed with traditional community-based MDA programs that primarily target STH (Ferreira et al., 2015; Cimino et al., 2015). In this study, giardiasis commonly occurred with ascariasis or trichuriasis as co-infections. Previously reported evidence on co-infections with *Giardia lamblia* and *Ascaris lumbricoides* or *Trichuris trichiura* is conflicting (Blackwell et al., 2013). The interactions between parasite species in co-infection and the resulting health implications involving immune modulation through antagonistic or synergistic effects have been previously described, further supporting the need for the accurate diagnosis of intestinal parasitic infections among children (Hagel et al., 2011; Blackwell et al., 2013).

The present study had limitations that should be considered when interpreting the findings. First, this exploratory study has a retrospective longitudinal observational design. Thus, limited control existed over (1) participant assessment and follow-up between and within observations, (2) construction of the cohort, and (3) data on nutritional factors that could have had a confounding effect on growth parameters such as chronic anemia, micronutrient deficiencies, and nutritional intake (LeBeaud et al., 2015), which were not available. However, the anthropometric indices used in this study are considered to be reliable tools for growth assessment when growth parameters were measured by trained individuals (de Onis & Blossner, 1997; de Onis et al., 2006; LeBeaud et al., 2015). Second, measurement tools for growth parameters and human error could have introduced both random and systematic error (LeBeaud et al., 2015). To
overcome this limitation, inter-rater variability was reduced in the parent study because the same trained research team members were used to measure growth parameters, means for duplicate measures were used for all final recorded growth parameter measures, and measurement tools were calibrated daily. Third, extreme outliers were not excluded from statistical analyses, and the species-specific sample size was small, which decreased the precision of the significant findings generating wide confidence intervals and required non-parametric statistical tests with reduced power for data analyses. However, this is comparable to other studies investigating parasite data (LeBeaud et al., 2015) because parasite data is highly aggregated with most infected individuals having very low parasite burden and few individuals having very high parasite burden and often few species-specific infections for some parasites by population (Shaw and Dobson, 1995; Shaw et al., 1998).

A significant strength of the present study was the use of a highly sensitive and specific multi-parallel, real-time qPCR assay for the detection of intestinal parasites. This approach overcomes the limitations of widely used microscopy-based diagnostic methods with poor sensitivity and specificity; as well as, other molecular diagnostic methods, which are more expensive and complex presenting greater challenges for their use in resource-limited settings. Another important strength of this study was the detection of a total of eight intestinal parasites commonly infecting children from two distinct taxonomic groups, the STH and protozoa. Subsequently, narrowing the research gap on the distribution of STH and protozoa, including co-infections in a resource-limited region of rural Ecuador. Overall, the accurate epidemiologic data from this study is critical for informing treatment decisions for the implementation of mass drug administration for the
preschool-age population in rural Ecuador. The multi-parallel, real-time qPCR assay used in this study for the accurate diagnosis of intestinal parasitic infections during early childhood provides insight to drive future research initiatives for advancing knowledge related to morbidity associated with childhood intestinal parasitic infections. This can lead to substantial public health gains to improved long-term child health outcomes that can circumvent lasting poverty.

Future directions for this research involve investigating the impact of intestinal parasites on growth beyond the two follow-up observations in this present study. Furthermore, including application of advanced analytical approaches in order to capture the cumulative effects of intestinal parasites and to capture growth changes across the childhood age continuum accounting for the wide variability of growth during this time. An additional future research direction includes pursuing an investigation of the interactions between *Giardia lamblia* and STH, namely *Ascaris lumbricoides*, and the influences of potential interactions between these parasite species on the gut microbiome for implications on growth in preschool-age children and possible insight to the mechanisms involved in reduced growth among children infected with intestinal parasites.
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Table 1

Sociodemographic Characteristics \((n = 177)\)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(n)</th>
<th>%</th>
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</thead>
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<tr>
<td><strong>Sex</strong></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>89</td>
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<tr>
<td>Female</td>
<td>91</td>
<td>51.4</td>
</tr>
<tr>
<td><strong>Birth order</strong></td>
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<td></td>
</tr>
<tr>
<td>First</td>
<td>51</td>
<td>28.8</td>
</tr>
<tr>
<td>Second</td>
<td>39</td>
<td>22.0</td>
</tr>
<tr>
<td>Third</td>
<td>41</td>
<td>23.2</td>
</tr>
<tr>
<td>Fourth</td>
<td>18</td>
<td>10.2</td>
</tr>
<tr>
<td>Fifth</td>
<td>12</td>
<td>6.8</td>
</tr>
<tr>
<td>Sixth</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td>Seventh</td>
<td>4</td>
<td>2.3</td>
</tr>
<tr>
<td>Eighth</td>
<td>2</td>
<td>1.1</td>
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<tr>
<td><strong>Crowding</strong></td>
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<td></td>
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<tr>
<td>Yes</td>
<td>64</td>
<td>36.2</td>
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<tr>
<td>No</td>
<td>113</td>
<td>63.8</td>
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<td><strong>Potable water access</strong></td>
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<tr>
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<td>58</td>
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<tr>
<td>No</td>
<td>119</td>
<td>63.8</td>
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<tr>
<td><strong>Socioeconomic status</strong></td>
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<tr>
<td>Medium</td>
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<tr>
<td>high</td>
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</table>

Maternal Characteristics \((n = 177)\)

<table>
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<tr>
<th>Characteristics</th>
<th>(n)</th>
<th>%</th>
</tr>
</thead>
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<tr>
<td>Maternal helminth infection</td>
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</tr>
<tr>
<td>yes</td>
<td>77</td>
<td>43.5</td>
</tr>
<tr>
<td>No</td>
<td>100</td>
<td>56.5</td>
</tr>
<tr>
<td><strong>Maternal ethnicity</strong></td>
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<tr>
<td>Afro-Ecuadorian</td>
<td>46</td>
<td>26.0</td>
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<tr>
<td>Mestizo</td>
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<tr>
<td>Amerindian</td>
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<td>0.6</td>
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<td><strong>Maternal education</strong></td>
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<tr>
<td>Illiterate</td>
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<td>Primary education</td>
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<td>59.3</td>
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<tr>
<td>Secondary education</td>
<td>48</td>
<td>29.9</td>
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</table>
Table 2

*Anthropometric Characteristics by Age (n = 177)*

<table>
<thead>
<tr>
<th>Growth Measures</th>
<th>36 months of age</th>
<th>60 months of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( M ) (SD)</td>
<td>( M ) (SD)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>92.42 (3.89)</td>
<td>106.96 (4.61)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>13.49 (1.72)</td>
<td>17.77 (2.74)</td>
</tr>
<tr>
<td>HAZ</td>
<td>-0.98 (1.04)</td>
<td>-0.58 (0.98)</td>
</tr>
<tr>
<td>WAZ</td>
<td>-0.50 (1.00)</td>
<td>-0.29 (1.02)</td>
</tr>
<tr>
<td>WHZ</td>
<td>0.05 (0.95)</td>
<td>0.04 (1.03)</td>
</tr>
</tbody>
</table>

*Note:* cm = centimeters; kg = kilograms; HAZ = height-for-age z-scores; WAZ = weight-for-age z-scores; WHZ = weight-for-height z-scores; \( M \) = mean; SD = standard deviation.

*Frequency of Linear Growth Deficits (n = 177)*

<table>
<thead>
<tr>
<th>Growth Index</th>
<th>36 months n (%)</th>
<th>60 months n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear growth faltering</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ ( \leq ) -1 SD &gt; -2 SD</td>
<td>79 (44.6%)</td>
<td>58 (32.8%)</td>
</tr>
<tr>
<td>Stunting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ &lt; -2 SD</td>
<td>21 (11.9%)</td>
<td>9 (5.1%)</td>
</tr>
</tbody>
</table>

*Note.* HAZ = height-for-age z-scores; \( \leq \) = less than or equal to; \( > \) = greater than; \( < \) = less than; SD = standard deviation.
Table 3

*Anthropometric Characteristics by Ethnicity*

36 months

<table>
<thead>
<tr>
<th>Growth Measures</th>
<th>Afro-Ecuadorian</th>
<th>Mestizo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>93.47 (3.92), [92.31, 94.64]</td>
<td>92.10 (3.83), [91.41, 92.74]</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>13.96 (1.66), [13.47, 14.46]</td>
<td>13.34 (1.71), [13.04, 13.64]</td>
</tr>
<tr>
<td><strong>HAZ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>-0.68 (1.04), [-0.99, -0.37]</td>
<td>-1.07 (1.02), [-1.25, -0.90]</td>
</tr>
<tr>
<td><strong>WAZ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>-0.21 (0.95), [-0.50, 0.07]</td>
<td>-0.60 (1.01), [-0.78, -0.43]</td>
</tr>
</tbody>
</table>

60 months

<table>
<thead>
<tr>
<th>Growth Measures</th>
<th>Afro-Ecuadorian</th>
<th>Mestizo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>108.2 (4.81), [106.8, 109.6]</td>
<td>106.6 (4.49), [105.8, 107.3]</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>18.39 (2.52), [17.64, 19.14]</td>
<td>17.57 (2.80), [17.10, 18.10]</td>
</tr>
<tr>
<td><strong>HAZ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>-0.32 (1.03), [-0.62, -0.01]</td>
<td>-0.67 (0.96), [-0.83, -0.50]</td>
</tr>
<tr>
<td><strong>WAZ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ ($SD$), 95% CI</td>
<td>-0.32 (0.95), [-0.32, 0.24]</td>
<td>-0.37 (1.04), [-0.55, -0.19]</td>
</tr>
</tbody>
</table>

*Note.* HAZ = height-for-age $z$-scores; WAZ = weight-for-age $z$-scores; cm = centimeters; kg = kilograms; $M$ = mean; $SD$ = standard deviation
Table 4

Prevalence of Intestinal Parasitic Infections (n = 177)

<table>
<thead>
<tr>
<th>Infection Status</th>
<th>36 months n (%)</th>
<th>60 months n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>118 (66.7%)</td>
<td>103 (58.2%)</td>
<td>.077</td>
</tr>
<tr>
<td>Not Infected</td>
<td>59 (33.3%)</td>
<td>74 (41.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Prevalence of Intestinal Parasitic Infections by Parasite Status among Infected Children and Change in Prevalence Across Age.

<table>
<thead>
<tr>
<th>Parasite Status</th>
<th>36 mo. (n = 118)</th>
<th>60 mo. (n = 103)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>29 (24.6%)</td>
<td>24 (23.3%)</td>
<td>.014*</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>33 (28.0%)</td>
<td>41 (39.8%)</td>
<td>.701</td>
</tr>
<tr>
<td><em>Ancylostoma duodenale</em></td>
<td>1 (0.8%)</td>
<td>3 (2.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Necator americanus</em></td>
<td>2 (1.7%)</td>
<td>1 (1.0%)</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>10 (8.5%)</td>
<td>3 (2.9%)</td>
<td>.039*</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>92 (78.0%)</td>
<td>78 (75.7%)</td>
<td>.001*</td>
</tr>
<tr>
<td><em>Cryptosporidium spp.</em></td>
<td>4 (3.4%)</td>
<td>3 (2.9%)</td>
<td>.687</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>6 (5.1%)</td>
<td>3 (2.9%)</td>
<td>.125</td>
</tr>
<tr>
<td>Single parasite</td>
<td>76 (64.4%)</td>
<td>64 (62.1%)</td>
<td>.880</td>
</tr>
<tr>
<td>Polyparasite</td>
<td>42 (35.6%)</td>
<td>39 (37.9%)</td>
<td>.880</td>
</tr>
<tr>
<td>STH</td>
<td>23 (19.5%)</td>
<td>22 (21.4%)</td>
<td>.736</td>
</tr>
<tr>
<td>Protozoa</td>
<td>60 (50.8%)</td>
<td>48 (46.6%)</td>
<td>.672</td>
</tr>
<tr>
<td>Co-infection</td>
<td>36 (30.5%)</td>
<td>34 (33.0%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note. mo. = months; p = p value; p < .05*
Table 5

*Mann-Whitney U Test Results for Differences in Parasite Burden between Children with and without Growth Deficits Based on Anthropometric Indices at 36 months.*

Stunting (HAZ < -2 SD)

<table>
<thead>
<tr>
<th>Parasite Burden DNA fg/µL</th>
<th>U</th>
<th>z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em> (n = 29)</td>
<td>40.0</td>
<td>-2.147</td>
<td>.032</td>
<td>.40</td>
</tr>
<tr>
<td>Md = 81.05 (n = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = 4.11 (n = 21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris trichiura</em> (n = 33)</td>
<td>47.0</td>
<td>-1.938</td>
<td>.053</td>
<td>*</td>
</tr>
<tr>
<td>Md = .012 (n = 26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = .037 (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia lamblia</em> (n = 92)</td>
<td>430.0</td>
<td>-.580</td>
<td>.562</td>
<td>*</td>
</tr>
<tr>
<td>Md = 1.92 (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = .627 (n = 80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Underweight (WAZ < -2 SD)

<table>
<thead>
<tr>
<th>Parasite Burden DNA fg/µL</th>
<th>U</th>
<th>z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em> (n = 29)</td>
<td>20.00</td>
<td>-1.897</td>
<td>.058</td>
<td>*</td>
</tr>
<tr>
<td>Md = 147.49 (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = 5.35 (n = 25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris trichiura</em> (n = 33)</td>
<td>9.00</td>
<td>-2.254</td>
<td>.024</td>
<td>.40</td>
</tr>
<tr>
<td>Md = .202 (n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = .013 (n = 30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia lamblia</em> (n = 92)</td>
<td>199.00</td>
<td>-.933</td>
<td>.351</td>
<td>*</td>
</tr>
<tr>
<td>Md = 9.87 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = .627 (n = 86)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. HAZ = height-for-age z-scores; WAZ = weight-for-age z-scores; (< ) = less than; Md = median; DNA = deoxyribonucleic acid; fg/µL = femtograms per microliter; U = Mann-Whitney U test statistic; \( z \) =; \( p \) = p value; \( r \) = effect size.
Table 6
Mann-Whitney U test results for comparison of growth measures by parasite status at 36 months of age

<table>
<thead>
<tr>
<th>Parasite Infection Status</th>
<th>U</th>
<th>Z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Parasite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>3106.50</td>
<td>-1.165</td>
<td>.244</td>
<td>*</td>
</tr>
<tr>
<td>Md = -1.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>3227.50</td>
<td>-0.789</td>
<td>.430</td>
<td>*</td>
</tr>
<tr>
<td>Md = -0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Parasite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>3565.00</td>
<td>-0.809</td>
<td>.418</td>
<td>*</td>
</tr>
<tr>
<td>Md = -1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>3556.50</td>
<td>-0.834</td>
<td>.404</td>
<td>*</td>
</tr>
<tr>
<td>Md = -0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyparasite</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>2187.50</td>
<td>-2.233</td>
<td>.026</td>
<td>.20</td>
</tr>
<tr>
<td>Md = -1.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>2300.00</td>
<td>-1.845</td>
<td>.065</td>
<td>*</td>
</tr>
<tr>
<td>Md = -0.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>1963.50</td>
<td>-2.094</td>
<td>.036</td>
<td>.20</td>
</tr>
<tr>
<td>Md = -1.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>2064.00</td>
<td>-1.728</td>
<td>.084</td>
<td>*</td>
</tr>
<tr>
<td>Md = -0.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: U = Mann-Whitney U test statistic; z = z statistic; p = p value; r = effect size; HAZ = height-for-age z-score; WAZ = weight-for-age z-score; WHZ = weight-for-height z-score; cm = centimeters; kg = kilograms; Md = median.
Table 7

*Mann-Whitney U test results for comparison of growth measures by species-specific parasite status at 36 months of age*

<table>
<thead>
<tr>
<th>Parasite Status</th>
<th>U (z)</th>
<th>z</th>
<th>p</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>305.50</td>
<td>-2.048</td>
<td>.041</td>
<td>.20</td>
</tr>
<tr>
<td>Md = -0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.18</td>
<td>398.50</td>
<td>-1.326</td>
<td>.185</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>623.50</td>
<td>-0.123</td>
<td>.902</td>
<td></td>
</tr>
<tr>
<td>Md = -1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.17</td>
<td>585.00</td>
<td>-0.410</td>
<td>.682</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>3204.50</td>
<td>-0.118</td>
<td>.906</td>
<td></td>
</tr>
<tr>
<td>Md = -1.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.09</td>
<td>2931.50</td>
<td>-1.021</td>
<td>.307</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ascaris and Giardia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAZ</td>
<td>1032.50</td>
<td>-1.934</td>
<td>.053</td>
<td>.20</td>
</tr>
<tr>
<td>Md = -1.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -1.14</td>
<td>986.50</td>
<td>-2.157</td>
<td>.031</td>
<td>.20</td>
</tr>
<tr>
<td>WAZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md = -0.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note:* U = Mann-Whitney U test statistic; z = z statistic; p = p value; r = effect size; HAZ = height-for-age z-score; WAZ = weight-for-age z-score; WHZ = weight-for-height z-score; cm = centimeters; kg = kilograms; Md = median.
Table 8

Fisher’s Exact test results for risk of stunting with co-infection at 36 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giardia lamblia</td>
<td>1.23</td>
<td>[0.47, 3.20]</td>
<td>.804</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>1.06</td>
<td>[0.12, 9.10]</td>
<td>1.000</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>.953</td>
<td>[0.92, 0.99]</td>
<td>1.000</td>
</tr>
<tr>
<td>Trichuris/Giardia</td>
<td>1.804</td>
<td>[0.55, 5.96]</td>
<td>.303</td>
</tr>
<tr>
<td>Ascaris/Giardia</td>
<td>3.44</td>
<td>[1.10, 10.90]</td>
<td>.044</td>
</tr>
</tbody>
</table>

Note: OR = odds ratio; CI = confidence interval; p = p value
Table 9

**Multivariable Regression for Height-for-Age Z-scores (HAZ)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>36 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal helminth</td>
<td>-.36</td>
<td>.16</td>
<td>-2.32</td>
<td>.022</td>
<td>[-.607, -.676]</td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td>-.46</td>
<td>.17</td>
<td>-2.63</td>
<td>.009</td>
<td>[-.798, -.114]</td>
</tr>
<tr>
<td>Co-infection</td>
<td>-.54</td>
<td>.25</td>
<td>-2.14</td>
<td>.033</td>
<td>[-1.041, -.043]</td>
</tr>
<tr>
<td><em>Ascaris/Giardia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>60 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal helminth</td>
<td>-.39</td>
<td>.15</td>
<td>-2.57</td>
<td>.011</td>
<td>[-.681, -.090]</td>
</tr>
<tr>
<td>Maternal ethnicity</td>
<td>-.38</td>
<td>.17</td>
<td>-2.30</td>
<td>.023</td>
<td>[-.708, -.053]</td>
</tr>
<tr>
<td>Co-infection</td>
<td>.34</td>
<td>.27</td>
<td>1.23</td>
<td>.221</td>
<td>[-.204, .877]</td>
</tr>
<tr>
<td><em>Ascaris/Giardia</em></td>
<td></td>
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Note: Model = “Enter” method in SPSS; $B =$ unstandardized regression coefficient; CI = confidence interval; $SE =$ standard error of the coefficient; $t =$ t statistic; $p =$ p value. $p < .05$
Figure 1

*Change in parasite burden as a function of age*

![Graph showing parasite burden as a function of age for Ascaris lumbricoides.](image)

*Note.* *Ascaris lumbricoides* parasite burden (DNA fg/µL) was statistically significantly greater at 36 months (p = .045) compared to 60 months. Quantified in eggs per gram (epg), children more often had moderate-to-heavy parasite burden at 36 months of age.
Figure 2

Spearman’s correlation coefficient for parasite burden and sociodemographic factors

Note. Positive correlation between *Giardia lamblia* (DNA fg/µL) and crowding at 36 months ($r = .226, p = .030$) and at 60 months ($r = .257, p = .023$).
Figure 3

Association between parasite burden (DNA fg/µL) and growth

*Ascaris lumbricoides*

*Note.* *Ascaris lumbricoides* parasite burden (DNA fg/µL) was associated with stunting at 36 months of age ($p = .031$) but not at 60 months of age.
Figure 4

*Kruskal-Wallis results for comparison of HAZ across parasite status*

Note. At 36 months, children with *Ascaris* and *Giardia* co-infections had statistically significant lower height-for-age z-scores (HAZ) ($p = .025$) compared to children with *Ascaris* only and *Giardia* only infections. No associations were found at 60 months.
Appendix A

Soil-transmitted helminth lifecycles
Figure A1

Ascaris lumbricoides lifecycle

Figure A2

*Trichuris trichiura* lifecycle

Figure A3

*Hookworm lifecycle*

Figure A4

*Strongyloides stercoralis* lifecycle

Appendix B

Protozoa lifecycles
Figure B1

*Giardia lamblia lifecycle*

[Image of Giardia lamblia lifecycle diagram]

Figure B2

*Cryptosporidium spp. lifecycle*

Figure B3

*Entamoeba histolytica* lifecycle

[Image of the lifecycle of *Entamoeba histolytica*]

CURRICULUM VITAE

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Patricia.E.Bryan@uth.tmc.edu

**Education**

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<thead>
<tr>
<th>Degree</th>
<th>Institution</th>
<th>Completion Date</th>
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<tr>
<td>Doctor of Philosophy, Nursing</td>
<td>The University of Texas Health Science Center at Houston Cizik School of Nursing</td>
<td>Expected 08/2020</td>
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<tr>
<td>Master of Science, Nursing Education</td>
<td>The University of Texas at Tyler Tyler, TX</td>
<td>05/2008</td>
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<tr>
<td>Bachelor of Science, Nursing Magna Cum Laude</td>
<td>The University of Texas at El Paso El Paso, TX</td>
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<tr>
<td>Bachelor of Science, Biology Magna Cum Laude</td>
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**Licensure and Certifications**

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<tr>
<td>Registered Nurse - Texas</td>
<td>Texas Board of Nursing Active - 649522</td>
<td>01/1998-Present</td>
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<td>BLS for Healthcare Providers</td>
<td>American Heart Association</td>
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**Profession Positions**

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<tr>
<td>Houston Baptist University School of Nursing</td>
<td>Clinical Instructor Adjunct Faculty-Maternal/Child Health</td>
<td>2019-Present</td>
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<tr>
<td>University of Texas at Tyler School of Nursing</td>
<td>Lecturer and Clinical Instructor Full-time Faculty-Maternal/Child Health</td>
<td>2008-2013</td>
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<tr>
<td>University of Texas at Tyler School of Nursing</td>
<td>Graduate Teaching Assistant Health Assessment</td>
<td>2007-2009</td>
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<td>Institution/Position</td>
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<tr>
<td>Texarkana College</td>
<td>Adjunct Faculty Anatomy &amp; Physiology</td>
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<tr>
<td>University of Texas at El Paso</td>
<td>Graduate Research Assistant</td>
<td>2002-2004</td>
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<td>Providence Memorial Hospital, El Paso, TX</td>
<td>Staff Nurse: Pediatric Intensive Care/Neonatal Intensive Care</td>
<td>2000-2004</td>
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<tr>
<td>Providence Memorial Hospital, El Paso, TX</td>
<td>Research Nurse Coordinator: Pediatric Hematology/Oncology</td>
<td>2002-2003</td>
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<td>Thomason General Hospital, El Paso, TX</td>
<td>Pediatric Intensive Care/Trauma Intensive Care</td>
<td>1998-2000</td>
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**Honors & Awards**

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<td>Texas Children’s Hospital Scholar</td>
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<td>Sigma Theta Tau Nursing Honor Society</td>
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<td>Beta Beta Beta Biology Honor Society</td>
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**Professional Membership**

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<tr>
<td>Member</td>
<td>Southern Nursing Research Society</td>
<td>2013-Present</td>
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<tr>
<td>Member</td>
<td>American Nurses Association Texas Nurses Association</td>
<td>2004-Present</td>
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Grants

Health Resources and Services Administration (HRSA) Co-PI 2011
“Equipment to Promote Education in the Health Professions”
Award - $350,000
Grant award used for the development of a state-of-the-art simulation
laboratory with high-fidelity obstetric, neonatal and pediatric
simulators and audio/visual equipment for nursing students at the
University of Texas at Tyler and medical students from UT Health
Science Center at Tyler

Publications

Mejia, R., Damania, A., Jeun, R., Bryan, P. E., Vargas, P., Juarez, M., Cajal, P. S.,
Nasser, J., Krolewiecki, A., Lefoulon, E., Long, C., Drake, E., Cimino, R. O. & Slatko,
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parallel quantitative real-time polymerase chain reaction. Parasites & Vectors, 8(380),
https://doi.org/10.1186/s13071-015-0994-z
**Book Chapter**


**Posters**

Texas Children’s Hospital Pediatric Research Symposium 05/2017
“Detection of Gastrointestinal Parasites by Multi-Parallel Real-Time PCR and Associations with Growth Delays in Early Childhood: Findings from a Birth-Cohort in Rural Ecuador”
**Bryan, P. E., Aravelo, A., Sandoval, C., Chico, M. E., Cooper, P., Mejia, R.**

Baylor College of Medicine, Department of Medicine Research Symposium 03/2016
“Gastrointestinal Parasitic Infections in Colombian Children Living in Urban, Peri-urban, and Rural Environments”
**Bryan, P. E., Restrepo, A. & Mejia, R.**