Soil-transmitted helminth infection and intestinal inflammation among the Shuar of Amazonian Ecuador

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Abstract

Objectives: Little research exists documenting levels of intestinal inflammation among indigenous populations where exposure to macroparasites, like soil-transmitted helminths (STHs), is common. Reduced STH exposure is hypothesized to contribute to increased prevalence of elevated intestinal inflammation in wealthy nations, likely due to coevolutionary histories between STHs and human immune systems that favored anti-inflammatory pathways. Here, we document levels of intestinal inflammation and test associations with STH infection among the Shuar of Ecuador, an indigenous population undergoing socioeconomic/lifestyle changes that influence their hygienic environment. We predict that fecal calprotectin (FC; a measure of intestinal inflammation) will be lower in STH infected individuals and that FC will be negatively associated with infection intensity.

Methods: Stool samples to analyze FC levels and STH infection were collected from 69 Shuar participants (ages 5–75 years). Children (<15 years) and adults (15+ years) were analyzed separately to understand the role of exposure in immune system development and the intestinal inflammatory response.

Results: Two species of STH were present: *Ascaris lumbricoides* and *Trichuris trichiura*. The relationships between infection and intestinal inflammation were age- and species-specific. While no significant relationships were found among adults, children who were singly infected with *T. trichiura* had lower FC levels than uninfected children. Infection intensity was not significantly associated with FC in children or adults.

Conclusions: These preliminary results provide limited support for our hypotheses, documenting tentative age- and species-specific associations between FC and infection status. Findings may point to the importance of species-specific STH exposure during immune system development.

Keywords
fecal calprotectin, hygiene hypothesis, inflammatory bowel disease, old friends hypothesis, soil-transmitted helminths
1 | INTRODUCTION

Prevalence of inflammatory bowel disease (IBD), a term used to describe inflammatory disorders of the digestive tract, is on the rise in wealthy countries (Cosnes, Gower-Rousseau, Soksik, & Cortot, 2011; Hanauer, 2006; Kaplan, 2015; Molodecky et al., 2012). For example, between 1999 and 2015 self-reported diagnoses of IBD among adults in the United States rose from 2 to 3 million (0.9–1.3% of the population, respectively; Dahlhamer, Zammitt, Ward, Wheaton, & Croft, 2016). At a somewhat slower and less predictable rate, this increase is also evident among immigrant populations and among populations in lower socioeconomic status countries and regions of the world (Cosnes et al., 2011). Increased prevalence of IBD has been linked to economic development via changes in sanitation, infectious disease exposure, physical activity, and diet (Hanauer, 2006). However, few epidemiological studies have examined populations as they make the transition to the more market integrated, hygienic lifestyles hypothesized to be linked with increases in IBD (Kaplan, 2015; Molodecky et al., 2012).

The Old Friends Hypothesis, also called the Hygiene Hypothesis, posits that an increase in immune-related inflammatory disorders, like IBD, in wealthy nations is associated with evolutionarily novel low levels of exposure to infectious disease agents such as soil-transmitted helminths (STHs; intestinal parasitic nematodes contracted through fecally contaminated soil; Bloomfield et al., 2016; Maizels, McSorley, & Smyth, 2014; Rook, 2010; Weinstock & Elliot, 2009). Chronic infections with STHs are generally asymptomatic, although acute and heavy infections can result in symptoms ranging from diarrhea to nutritional deficiencies to organ failure and even death (Ahmed, 2011; Bethony et al., 2006; Blackwell, Snodgrass, Madimeno, & Sugiyama, 2010; Dold & Holland, 2011; Francis, Kirunda, & Orach, 2012).

Our long coevolutionary history with STHs has shaped several aspects of human life, including behavior (Roulette et al., 2014; Roulette, Kazanj, Breurec, & Hagen, 2016), fertility (Blackwell et al., 2015), and immune function (Allen & Maizels, 2011; Geiger et al., 2002; McSorely & Maizels, 2012). With regard to immune function, coevolved mechanisms in both the STHs and hosts appear to favor light to moderate chronic infection by activating the T-helper 2 (Th2) branch of the adaptive immune system. This process downregulates inflammation and regulates/reduces the immune response while triggering self-repair (Allen & Maizels, 2011; Geiger et al., 2002; McSorely & Maizels, 2012). From the parasite’s perspective, this reflects selection on mechanisms to avoid detection and ejection. From the host’s perspective, it reflects selection for a response that reduces the cost of damage to host tissue that would occur with a more aggressive immune response (Allen & Maizels, 2011; McSorely & Maizels, 2012). Accordingly, the heightened Th2 response triggered by STH infection is thought to have the secondary effect of reducing inflammatory disorder risk (Allen & Maizels, 2011; Gurven et al., 2016; Maizels et al., 2014; McSorely & Maizels, 2012; Weinstock & Elliot, 2009).

Ulcerative Colitis and Crohn’s Disease, two of the diseases associated with IBD, are incurable but manageable disorders of the digestive tract. These diseases have both autoimmune and immune-mediated components, including general and disease-specific autoantibodies, hyper-reactivity against indigenous microflora, and irregular humoral and cell-mediated immune responses (Wen & Fiocchi, 2004). Inflammatory responses associated with IBD differ from regular, non-pathological immune responses in the intestines due to the body’s inability to decrease intestinal inflammation on its own through normal regulatory processes (Hanauer, 2006).

Clinical studies in wealthy nations have tested numerous helminth species as possible treatments for IBD, but results are mixed, inconclusive, or based on very small sample sizes (Briggs, Weatherhead, Sastry, & Hotez, 2016; Croese et al., 2006; Dige et al., 2016; Garg, Croft, & Bager, 2014; Summers, Elliott, Urban Jr., Thompson, & Weinstock, 2005a; Summers, Elliott, Urban Jr., Thompson, & Weinstock, 2005b). In some cases, helminths were even shown to increase inflammation and exacerbate IBD symptoms (Briggs et al., 2016; Weatherhead & Hotez, 2015). One possible reason for these inconsistent results is that timing of STH exposure may be critical (Maizels et al., 2014). Some argue that exposure during immune system development in childhood is crucial for stimulating an adaptive anti-inflammatory immune state that continues in adulthood (Blackwell et al., 2011; Djiardi, Wannmes, Supalli, Sartono, & Yazdanbakhsh, 2011; Maizels et al., 2014). Others argue that short-term adult exposure also has important anti-inflammatory effects (Maizels et al., 2014; McSorley et al., 2011; Weinstock & Elliott, 2013). Examination of differential effects of STH exposure on inflammation between adults and children is crucial for understanding the impact of exposure timing (i.e., during childhood, adulthood, or both) for immune system development.

Studies of the relationship between IBD and STH exposure in populations with moderate to high worm burden are difficult because the procedures used to diagnose IBD are invasive and expensive, and proper storage of whole blood and tissue samples in remote locales is limited or nonexistent (Gisbert & McNicholl, 2009; McDade, Williams, & Snodgrass, 2007; Tibble & Bjarnason, 2001). Fecal calprotectin (FC) has been shown to be a noninvasive, easily preserved and reliable biomarker for intestinal inflammation, suitable for use among people living in more remote regions of the world (Fagerhol, Andersson, Naes-Andersen, Brandtzaeg, & Dale, 1990; Gisbert & McNicholl, 2009; Tibble et al., 2000; Tibble & Bjarnason, 2001). Calprotectin is a protein found in key immune cells, like neutrophils, monocytes, and macrophages, critical to the inflammatory immune response (Fagerhol et al., 1990; Fagerhol, Dale, & Andersson, 1980). Fecal calprotectin provides a measure of localized intestinal inflammation (de Gier et al., 2018; Gisbert & McNicholl, 2009), with higher levels of calprotectin in fecal samples associated with more intestinal inflammation (Fagerhol et al., 1990; Gisbert & McNicholl, 2009; Joshi, Lewis, Creanor, & Aylng, 2010). This makes FC a useful biomarker for understanding relationships between intestinal infections and inflammation.

Fecal calprotectin levels have been shown to vary significantly by life-stage (Joshi et al., 2010; Poullis, Foster, Shetty, Fagerhol, & Mendall, 2004). In infants (2 years of age and younger), elevated and variable FC levels are common due to maturation and development of the intestinal mucosa (Campetott et al., 2003; Fagerberg, Lööf, Merzoug, Hansson, & Finkel, 2003; Olafsdottir, Aksnes, Fluge, & Berstad, 2002;
in the Morona Santiago province of Ecuador, where this study was conducted. Traditionally, Shuar subsistence consisted of foraging, hunting, fishing, and horticulture. However, Shuar are currently experiencing increasingly rapid but widely variable market integration (i.e., the degree of production for and consumption from market-based economies) within and across communities. Market integration among the Shuar has increased variation in several aspects of infrastructure and lifestyle, including in healthcare, house construction, sanitation, and exposure to pathogens (e.g., STHs; Cepon-Robins et al., 2014; Gildner et al., 2016; Liebert et al., 2013; Stagaman et al., 2018; Urlacher et al., 2016; Urlacher et al., 2018).

Research by the Shuar Health and Life History Project (SHLHP) has documented high prevalence of STH infection among the Shuar (Cepon-Robins et al., 2014; Gildner et al., 2016), with children having significantly higher STH infection rates and intensities than adults (Cepon-Robins et al., 2014; Gildner et al., 2016). Furthermore, biomarkers associated with the adaptive immune response to STH infection, like immunoglobulin E (IgE), peak mid-childhood (about 10 years of age) (Blackwell et al., 2011), indicating that childhood is an important time for macroparasite exposure and associated immune system development (Blackwell et al., 2011; Cepon-Robins et al., 2014; Gildner et al., 2016). Furthermore, Shuar who live in rural villages characteristic of most of the population, exhibit no evidence of chronic low-grade systemic inflammation measured via C-reactive protein in adulthood, suggesting that elevated systemic inflammation with age is not common in this population (McDade et al., 2012). Comparing relationships among STH infection and intestinal inflammation among children and adults separately can help elucidate when exposure to macroparasites may have the largest impact on immune regulation.

2.2 Participants and sampling

Cross-sectional data were collected by the SHLHP in 2016 from the remote Cross-Cutucú region of Ecuador. At the time of study, Shuar in this region were isolated from regional market centers, with travel to Sucúa (a local market center) taking about 2–3 hr by motorized canoe and an additional 5–8 hr by bus. Cross-Cutucú Shuar are therefore more dependent on traditional subsistence activities than Shuar living in more market integrated areas (Urlacher et al., 2016), though many still make occasional trips to Sucúa or other local centers to sell produce or engage in wage labor. Households sampled in this study were at relatively low to intermediate levels of market integration, especially related to household infrastructure. No household in this study had indoor or outdoor plumbing, with only 24% of participants reporting access to a latrine. Participants reported getting their water from rivers/streams (64%) or wells (36%), with the average participant traveling about 9 min for water access. Participant houses were made of wood (91%) or cement (9%) and had floors made of wood (87%) or earth (13%).

2 METHODS

2.1 Study population

This study was conducted among the Shuar, an indigenous Amazonian population of Southeastern Ecuador and Northeastern Peru, centered...
are all factors that contribute to fecal-oral contamination and STH exposure.

Data were collected from 69 Shuar participants (ages 5–75 years). To control for highly variable FC levels in infants and very young children (Campeotto et al., 2003; Fagerberg et al., 2003; Olafsdottir et al., 2002), only children aged 5 and older were included in this study. To account for variation in immune system development based on early-life exposure, as well as to explore the importance of timing of exposure, we analyzed children (ages 5 through 14 years; 13 boys, 13 girls) and adults (age ≥ 15 years; 21 men, 22 women) separately. This age range mirrors that used in previous studies of FC in children (Bunn et al., 2001; Fagerberg et al., 2003; Hestvik et al., 2011), and encapsulates, at least theoretically, the period of early immune system development (Blackwell et al., 2011). Because parasites mimic immune states common in pregnancy (Blackwell et al., 2015), pregnant women were excluded from this study.

Informed consent was obtained from all adult participants. For children, parental consent and child assent were obtained. All methods and procedures were approved by the University of Oregon’s Institutional Review Board. The Federación Interprovincial de Centros Shuar (FICSH) authorized the research in sample communities.

2.3 Soil-transmitted helminths

First-morning stool samples were collected and analyzed in the field for presence and intensity of species-specific STH infection based on methods reported previously by the SHLHP (Cepon-Robins et al., 2014; Gildner et al., 2016). Two species of STH were detected: *Ascaris lumbricoides* (large roundworm) and *Trichuris trichiura* (whipworm). Infection status and intensity, measured in eggs per gram (EPG) of feces, were recorded. Infection intensity levels (light, moderate, and heavy) were determined based on guidelines established by Montresor, Crompton, Hall, Bundy, and Savioli (1998).

2.4 Fecal calprotectin

Small portions of each stool sample were collected in a cryotube and stored in a portable freezer at −20°C until completion of the field season when they were shipped on dry ice to the Global Health Biomarker Laboratory (GHBL) at the University of Oregon. At the GHBL, they were stored at −30°C until analysis. Calprotectin was extracted using the CALEX cap device (B-CALEX-C; BUHLMANN Diagnostics Corp, Amherst, NH) and analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EK-CAL; BUHLMANN Diagnostics Corp, Amherst, NH). See Table S1 for assay reliability measures. Fecal calprotectin levels are considered elevated when values are greater than 50 μg/g, based on reference values provided by the manufacturer and used/validated in previous studies (Campeotto et al., 2003; Fagerberg et al., 2003; Gisbert & McNicholl, 2009; Hestvik et al., 2011; Michels, Van de Wiele, & De Henauw, 2017; Olafsdottir et al., 2002).

2.5 Data analyses

Data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL). Prior to analysis, variables were tested for normality. Fecal calprotectin, *Ascaris* EPG, and *Trichuris* EPG were natural log-transformed for all analyses due to non-normal distributions. One-way ANOVA and chi-square analyses were performed to compare infection and FC variables between adults and children. Curve estimates were used to investigate possible nonlinear relationships between age as a continuous variable and infection/FC variables.

To test Hypothesis 1, separate two-way ANOVAs for children and adults were performed to compare Ln FC based on overall infection status (0 = uninfected; 1 = infected with one or both STH species), specific infection type (0 = uninfected; 1 = *Ascaris* only; 2 = *Trichuris* only; 3 = coinfected), and then based solely on *T. trichiura* infection status (0 = uninfected; 1 = infected). Because of very small sample sizes, especially within specific infection types, bias-corrected and accelerated (BCa) bootstrapping with 1,000 replications was utilized in the ANOVA analyses to calculate estimated marginal means, p values, and standard errors. This type of bootstrapping has been utilized in past human biology studies to account for small sample size (Meehan, Quinlan, & Malcom, 2013). Follow-up simple main effect tests were used to examine the differences in means between specific infection types. Prior to analysis, Levene’s test of equality of error variances was used to confirm that the homogeneity of variance assumption was not violated across all ANOVA tests. ANCOVAs were originally run to control for age (as a continuous variable) and sex (0 = female; 1 = male), but these predictors did not contribute significantly (Tables S2–S4) and were removed to simplify the model.

To test Hypothesis 2, linear regression analyses utilizing BCa bootstrapping with 1,000 replications were conducted for children and adults separately to test relationships between Ln FC, Ln Trichuris EPG, and Ln Ascaris EPG. Regressions were originally run to control for age (as a continuous variable) and sex (0 = female; 1 = male), but these predictors did not contribute significantly (Table S5) and were removed to simplify the model.

3 RESULTS

Table 1 shows descriptive statistics for age, intestinal inflammation, and infection data for children and adults. Fecal calprotectin and STH infection variables were compared between the two age groups (Table 1). Children and adults did not differ significantly on any of the STH infection or FC variables. No nonlinear relationships were observed between age and any infection or FC variables.

Hypothesis 1 FC will be lower in STH infected individuals. Bias-corrected and accelerated (BCa) bootstrapped two-way ANOVAs showed no significant relationship between general STH infection status (uninfected vs. infected with at least one STH species; Table 2), although there was a nonsignificant trend with a medium effect size,
with infected children having lower Ln FC than uninfected children \((p = .07; \text{BcA CI}_{95\%} = −1.76, −0.06; \eta^2_p = 0.11)\). BCA bootstrapped two-way ANOVAs showed a significant relationship between specific infection type and Ln FC for children \((p = .03)\) but not adults (Table 3). A Partial Eta Squared \((\eta^2_p)\) of 0.33 suggests this was a large effect size. Follow-up simple main effect tests with BCA bootstrapping (Tables 4 and 5) showed that children who were singly infected with *T. trichiura* had significantly lower Ln FC levels than those who were uninfected with any STH species \((p = .04; \text{BcA CI}_{95\%} = −3.64, −0.16)\). When Ln FC was compared based only on *T. trichiura* infection status (infected vs. not; Table 6), there was a nonsignificant trend \((p = .06; \text{BcA CI}_{95\%} = −2.12, −0.13)\), suggesting lower Ln FC in children who were infected with *T. trichiura* \((M = 2.15; \text{SE} = 0.39)\) compared to children who were not infected with *T. trichiura* \((M = 3.21; \text{SE} = 0.31)\).

Although the association between child Ln FC and *T. trichiura* infection status did not reach significance, the effect size of this relationship was large \((\eta^2_p = 0.16)\). There were no significant relationships between infection status and Ln FC for adults.

**Hypothesis 2** FC will be negatively associated with infection intensity.

Linear regressions (Table 7) showed no significant relationships between infection intensity and Ln FC for children or adults.

### TABLE 1 Descriptive statistics for intestinal inflammation and STH infection variables for children and adults

<table>
<thead>
<tr>
<th></th>
<th>Children (N = 26)</th>
<th>Adults (N = 43)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>8.8 (3.2)</td>
<td>35.1 (15.7)</td>
</tr>
<tr>
<td><strong>Intestinal inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal calprotectin (FC; ug/g)**</td>
<td>17.7 (36.6)</td>
<td>22.0 (51.5)</td>
</tr>
<tr>
<td>Elevated FC (%)</td>
<td>23.1 (n = 6)</td>
<td>34.9 (n = 15)</td>
</tr>
<tr>
<td><strong>Infection status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only <em>Trichuris</em> infected (%)</td>
<td>15.4 (n = 4)</td>
<td>11.6 (n = 5)</td>
</tr>
<tr>
<td>Only <em>Ascaris</em> infected (%)</td>
<td>23.1 (n = 6)</td>
<td>14.0 (n = 6)</td>
</tr>
<tr>
<td>Coinfected (%)</td>
<td>23.1 (n = 6)</td>
<td>16.3 (n = 7)</td>
</tr>
<tr>
<td>Uninfected (%)</td>
<td>38.5 (n = 10)</td>
<td>58.1 (n = 25)</td>
</tr>
<tr>
<td><strong>Species-specific eggs per gram (EPG)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichuris</em> EPG</td>
<td>414.5 (1,341.1)</td>
<td>159.1 (801.7)</td>
</tr>
<tr>
<td><em>Ascaris</em> EPG</td>
<td>3,839.1 (7,588.7)</td>
<td>3,581.6 (12,014.3)</td>
</tr>
</tbody>
</table>

**Trichuris** infection intensities

- **Light** (1–999 EPG) 30.8 (n = 8) 23.3 (n = 10)
- **Moderate** (1,000–9,999 EPG) 7.7 (n = 2) 4.7 (n = 2)

**Ascaris** infection intensities

- **Light** (%: 1–4,999 EPG) 19.2 (n = 5) 11.6 (n = 5)
- **Moderate** (%: 5,000–49,000 EPG) 15.4 (n = 4) 16.4 (n = 7)
- **Heavy** (%: ≥50,000 EPG) 11.5 (n = 3) 2.3 (n = 1)

Values are presented as mean (SD) unless otherwise noted.

*Variables were compared between adults and children, but no significant differences were found.
*Denotes Median (IQR).

### TABLE 2 Bootstrapped two-way ANOVAs comparing Ln FC by STH infection status for children and adults

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>df</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>3.01</td>
<td>2.23</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>.07</td>
<td>.17</td>
</tr>
<tr>
<td><strong>\eta^2_p</strong></td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Mean difference</strong></td>
<td>−0.89</td>
<td>−0.66</td>
</tr>
<tr>
<td><strong>BCa CI_{95%}</strong></td>
<td>−1.76 to 2.12</td>
<td>−1.54 to 0.25</td>
</tr>
</tbody>
</table>

Infection status: 0, uninfected; 1, infected with one or more STH species.

*Comparing infected individuals to uninfected individuals.

### TABLE 3 Bootstrapped two-way ANOVAs comparing Ln FC by specific STH infection type for children and adults

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>df</strong></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>3.56</td>
<td>1.63</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>.03*</td>
<td>.52</td>
</tr>
<tr>
<td><strong>\eta^2_p</strong></td>
<td>0.33</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Infection Status: 0, uninfected; 1, *Ascaris* only; 2, *Trichuris* only; 3, coinfected. Results are significant at *p < .05.

### TABLE 4 Estimated marginal means and simple main effect tests with BCA bootstrapping comparing Ln FC in children infected with *T. trichiura* only to other infection types

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M (SE)</strong></td>
<td>3.31</td>
<td>2.22</td>
</tr>
<tr>
<td><strong>Mean difference</strong></td>
<td>0.89</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>.91</td>
<td>.86</td>
</tr>
<tr>
<td><strong>BCa CI_{95%}</strong></td>
<td>−3.37 to 0.35</td>
<td>−2.60 to 2.00</td>
</tr>
</tbody>
</table>

Results are significant at *p < .05.

*Represents estimated marginal mean for Ln FC and BCA Bootstrap standard error.

*Comparing Ln FC in *T. trichiura* infected individuals to other infection statuses.

### TABLE 5 Estimated marginal means and simple main effect tests with BCA bootstrapping comparing Ln FC in adults infected with *T. trichiura* only to other infection types

<table>
<thead>
<tr>
<th></th>
<th>Adults</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M (SE)</strong></td>
<td>3.64</td>
<td>1.75</td>
<td>2.48</td>
<td>2.60</td>
</tr>
<tr>
<td><strong>Mean difference</strong></td>
<td>0.98</td>
<td>0.22</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>.90</td>
<td>.86</td>
<td>.86</td>
<td>.79</td>
</tr>
<tr>
<td><strong>BCa CI_{95%}</strong></td>
<td>−2.37 to 0.35</td>
<td>−0.50 to 2.00</td>
<td>−0.25 to 2.00</td>
<td>−1.90 to 1.87</td>
</tr>
</tbody>
</table>

*Represents estimated marginal mean for Ln FC and BCA Bootstrap standard error.

*Comparing Ln FC in *T. trichiura* infected individuals to other infection statuses.
about sanitation and hygiene, and occasional wage-labor/market
and latrine infrastructure, exposure to domesticated animals, education
of market integration, including changes to housing, cooking, water,
more isolated regions of Shuar territory, but still experience the effects
\[\text{when} \text{begin to occur. Participants sampled in this study live in relatively}\]
\[\text{we should be able to document these changes to immune system}\]
\[\text{to alterations in immune system development and responsiveness, then}\]
\[\text{supported and lifestyle and hygiene-related changes are contributing}\]
\[\text{among indigenous populations as they transition from subsistence-}\]
\[\text{very little research exists examining levels of intestinal inflammation}\]
\[\text{children aged 8–16 years of age found that only 5% of participants}\]
\[\text{Shuar children appear to be higher in comparison to other populations.}\]
\[\text{Rates of elevated intestinal inflammation (FC > 50 } \mu\text{g/g) among}\]
\[\text{Our findings suggest that Shuar adults may have similar, or even}\]
\[\text{lower, rates of intestinal inflammation when compared to other adult}\]
\[\text{access. These factors affect infectious disease exposure (Campbell}\]
\[\text{TABLE 7 Bootstrapped linear regression analyses for}\]
\[\text{results may have several implications for understanding}\]
\[\text{intestinal inflammation and its importance for the health and well-}\]
\[\text{TABLE 6 Bootstrapped two-way}\]
\[\text{ANOVAs comparing ln FC by } T. \text{trichiura}\]
\[\text{T. \text{trichiura infection status: } 0, \text{uninfected; } 1, \text{infected.}\]
\[\text{Comparing } T. \text{trichiura infected individuals to uninfected individuals.}\]
\[\text{4.1 Intestinal inflammation among the Shuar}\]
\[\text{Very little research exists examining levels of intestinal inflammation}\]
\[\text{among indigenous populations as they transition from subsistence-}\]
\[\text{based lifestyles to those more dependent on regional and global mar-}\]
\[\text{innate response to the more regulated, anti-inflammatory adaptive}\]
\[\text{environment, poor sanitation, and high degree of fecal-oral contamination}\]
\[\text{highly variable nature of fecal calprotectin levels typically observed}\]
\[\text{inflammation compared to other children. This may be due to the}\]
\[\text{ Our findings suggest that Shuar adults may have similar, or even}\]
\[\text{lower, rates of intestinal inflammation when compared to other adult}\]
\[\text{populations, but Shuar children may have higher rates of intestinal}\]
\[\text{inflammation compared to other children. This may be due to the}\]
\[\text{highly variable nature of fecal calprotectin levels typically observed}\]
\[\text{among children (Rugtveit & Fagerhol, 2002). An alternative explana-}\]
\[\text{tion may be that the generally high bacterial and viral pathogen envi-}\]
\[\text{ronment, poor sanitation, and high degree of fecal-oral contamination}\]
\[\text{are elevating localized intestinal inflammation among Shuar children}\]
\[\text{as their primary immune response switches from the proinflammatory}\]
\[\text{innate response to the more regulated, anti-inflammatory adaptive}\]
\[\text{response (Blackwell et al., 2010, 2011). The result of this switch may}\]
\[\text{be environmental enteric dysfunction (EED), which is associated with}\]
\[\text{reduced growth rates and increased stunting in children from similar}\]
\[\text{regions (Crane et al., 2015; Syed et al., 2016). The Shuar, like other}\]
\[\text{Amazonian populations, experience high rates of stunting (~40%;}\]
\[\text{Blackwell, Pryor, Pozo, Tiwia, & Sugiyama, 2009), and our previous}\]
\[\text{research documented notable tradeoffs between growth and immune}\]
\[\text{function, with growth rates decreasing by up to 49% when the}\]
\[\text{immune response was even mildly elevated (Urlacher et al., 2018).}\]
The present study was conducted in the more remote Cross-Cutucú region of Ecuador. It is possible that we would see lower rates of elevated intestinal inflammation among Shuar children from more market-integrated regions with better sanitation and reduced bacterial and viral pathogen exposure. In fact, Shuar growth does appear to be improving in more market integrated areas (Urlacher et al., 2016), suggesting fewer tradeoffs between growth and immune function, likely associated with reduced fecal-oral contamination and pathogen exposure. In this case, we hypothesize that the high pathogen environment documented among the Shuar (Stagaman et al., 2018; Urlacher et al., 2018) may play a role in childhood rates of elevated inflammation in this sample. Soil-transmitted helminth infection, which triggers different immune pathways than bacteria and viruses, may ultimately work to counter this by favoring anti-inflammatory pathways (Allen & Maizels, 2011; Geiger et al., 2002; McSorely & Maizels, 2012).

4.2 | Soil-transmitted helminth infection and intestinal inflammation

This study found mixed and limited evidence supporting the Old Friends/Hygiene Hypothesis. There were no significant relationships between general infection status (infected with one or more STH species vs. not infected) or infection intensity and intestinal inflammation, although there was a trend toward lower levels of intestinal inflammation among STH infected individuals. The lack of significant data suggests that general, nonspecific STH infection may not be enough to reduce inflammation. Instead, species-specific infection may be important. Although based on a very small number of individuals infected with T. trichiura, we found that children singly infected with T. trichiura had significantly lower levels of intestinal inflammation than children who were uninfected with T. trichiura. Furthermore, T. trichiura infected children, including those who were coinfected with both species, had lower levels of intestinal inflammation than children who were not infected with T. trichiura, although this was a nonsignificant trend.

Because these results are based on a very small sample size and STH infection data is inherently noisy (i.e., various factors affect number of eggs shed throughout different points in the day), interpretation of these results must be conservative. If these species-specific relationships are valid, however, they may be related to how the parasites interact with and feed on their host. Trichuris trichiura has more immediate localized effect that triggers a greater immune response because adult worms directly attach to the intestine and injure host tissue (Bethony et al., 2006; Briggs et al., 2016; Bundy, 1986; Bundy & Cooper, 1989; Geiger et al., 2002). In contrast, A. lumbricoides never directly attaches and, instead, feeds passively (Bethony et al., 2006). The localized injury caused by T. trichiura may result in a more robust Th2 response to mask the presence of the parasite and avoid any further, more severe damage that would be caused by an immune response aimed at complete eradication (Allen & Maizels, 2011; McSorely & Maizels, 2012).

4.3 | Age, STH infection, and intestinal inflammation

Our preliminary findings documented relationships between species-specific STH infection and intestinal inflammation in children but not in adults. This may suggest that exposure to STHs and subsequent immune responses during childhood are important for shaping immune system development and intestinal health. Specifically, immune systems earlier in development, to varying degrees, may rely more heavily on innate immunity and inflammation to fight bacterial/viral infections, as well as STHs, in this high pathogen environment (Blackwell et al., 2010). Those exposed to STHs earlier in development and at a higher quantity may be developing adaptive immune responses more rapidly (Blackwell et al., 2010, 2011), thus turning down inflammation in the presence of T. trichiura.

Studies that examine specific immune markers, like immunoglobulin E (IgE), which is directly related to repeated and chronic macro-parasite exposure and the adaptive immune response, in relation to intestinal inflammation may be especially useful for understanding these patterns. After infection with STHs and other macro-parasites, IgE remains elevated for years, with high levels representing repeated infection over a long period of time (Iancovici et al., 2005; Urlacher et al., 2018). Immunoglobulin E binds to STH antigens during preliminary stimulation of the Th2 pathway in an adaptive immune response. Thus, IgE plays a crucial role in regulating immune function and turning down inflammation when STH infection occurs. Previous research among the Shuar documented a peak in IgE levels at 10 years of age (Blackwell et al., 2011). This suggests that around this age, children are successfully developing their immune systems to shift away from relying on innate immunity toward adaptive immunity for fighting macroparasites. These early findings, combined with the preliminary and tentative interpretation of data presented in this study, may provide support for the hypothesis that exposure to certain macroparasites during development is crucial for proper immune system development. Lack of exposure to specific STH species during childhood may be related to elevated levels of intestinal inflammation and associated disorders (e.g., IBD) seen in wealthy countries.

4.4 | Limitations

This study has several limitations. First, the preliminary nature of this study resulted in only a small sampling of individuals who provided stool samples for STH and FC analyses, making statistical analysis and interpretation difficult. In particular, subsamples representing species-specific infections and coinfections are particularly small. Bootstrapping methods were used in ANOVA and regression analyses to mitigate this limitation. For a conservative interpretation of the results, we use p values, confidence intervals, and effect sizes to interpret significance. A larger sample size would be useful for more robust hypothesis testing and would provide more interpretive value.

Second, only one stool sample per participant was analyzed for FC. Because of this limitation, we were unable to monitor change over time, which limits our ability to speak to the importance of timing of exposure for immune system development and intestinal inflammation. A longitudinal study would afford an opportunity to document
changes in inflammation and immune response throughout immune system development, which would allow for more thorough testing of the hypotheses discussed in this article. A single stool sample per participant was also used to determine infection intensity, which is a highly variable measure with variation in the number of eggs shed occurring throughout the day. By collecting the first-morning stool, we attempted to limit this variability, but measuring EPG from multiple stool samples would have made this variable more reliable.

Finally, because anthropometric measurements were only collected concomitantly (i.e., within a month of stool sample collection) in a small subsample of participants, we were not able to test relationships between body composition/nutrition status, intestinal inflammation, and infection. Inflammatory and infectious disease patterns are deeply interwoven with body composition and nutritional status (McDade, 2012; Urlacher et al., 2016, 2018) and, because of this small sample size, we cannot speak to their effects here.

5 | CONCLUSION

The present study provides mixed support, albeit based on a small sample size, for the Old Friends Hypothesis in IBD; however, more work needs to be done to understand the role of STHs in public health. This is difficult because, while STHs may have some anti-inflammatory and immune-regulatory effects, they also have serious consequences for naturally infected individuals, including negative health-related outcomes, poor childhood growth, and poverty promotion (Briggs et al., 2016). More research like the present study is important because it can pinpoint when exposure to STHs is most important, the degree to which infection intensity affects inflammatory response, which STH species have the greatest anti-inflammatory effects, and whether complete eradication of STHs in developing regions may eventually result in regionally novel health problems (e.g., IBD).

Results like the ones presented here can also increase understanding of the health of indigenous populations, especially those transitioning to increasingly market-based lifestyles. These populations are undergoing more rapid epidemiological transitions than previously documented populations, with many experiencing the double burden of both infectious and chronic diseases (Barrett, Kuzawa, McDade, & Armelagos, 1998; Gurven et al., 2009; Prentice, 2006; Valeggia & Snodgrass, 2015). Understanding the role STHs may play in preventing the development of chronic disease can shed light on the public health implications of lifestyle and economic change.

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DATA AVAILABILITY

All data used in these analyses are available upon request from the corresponding author.

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REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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