American Journal of PHYSICAL ANTHROPOLOGY
The Official Journal of the American Association of Physical Anthropologists

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Journal:	*American Journal of Physical Anthropology*
Manuscript ID	AJPA-2018-00378.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	07-Mar-2019
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Key Words:	Fecal calprotectin, soil-transmitted helminths, Old Friends Hypothesis, Hygiene Hypothesis, Inflammatory Bowel Disease
Subfield: Please select 2 subfields. Select the main subject first.:	Human biology [living humans; behavior, ecology, physiology, anatomy], Theory

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

Running Title: Helminths and intestinal inflammation among the Shuar

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Abstract

Objectives. Reduced exposure to macroparasites, especially soil-transmitted helminths (STHs), is hypothesized to contribute to increased prevalence of Inflammatory Bowel Disease in wealthy nations. This is likely due to coevolutionary histories between macroparasites and the human immune system that favored anti-inflammatory pathways. Here, we test whether STH infection is associated with reduced intestinal inflammation among the Shuar of Ecuador, an indigenous population undergoing socioeconomic/lifestyle changes that influence their hygienic environment. We predict that: 1) fecal calprotectin (FC; a measure of intestinal inflammation) will be lower in STH infected individuals; and 2) 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 to 75 years). Children (<15 years) and adults (15+ years) were analyzed separately to understand the role of exposure in immune system development and the resultant 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 those who were uninfected, singly infected with *A. lumbricoides,* or coinfected. There was also a significant negative relationship between FC and *T. trichiura* infection intensity among children.

Conclusions. These age- and species-specific associations between FC and STH infection demonstrate the importance of species-specific immune responses for immune system development and emphasize the need to consider timing and duration of exposure.

Keywords

Fecal calprotectin, soil-transmitted helminths, Old Friends Hypothesis, Hygiene Hypothesis,

Inflammatory Bowel Disease

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 et al., 2011; Hanauer, 2006; Kaplan et al., 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 million to 3 million (0.9% to 1.3% of the population respectively) (Dahlhamer et al. 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 et al., 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 et al., 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 et al., 2014; Rook, 2010; Weinstock & Elliott, 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 et al., 2011; Bethony et al., 2006; Blackwell et al., 2010; Dold & Holland, 2011; Francis et al., 2012).

Our long coevolutionary history with STHs has shaped several aspects of human life, including behavior (Roulette et al., 2014; 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 (T_H2) 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 T_H2 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 and Elliott, 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 et al., 2016; Croese et al., 2006; Dige et al., 2016; Garg et al., 2014; Summers et al., 2005a; Summers et al., 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; Djuardi et al., 2011; Maizels et al., 2014). Others argue that short-term adult exposure also has important anti-inflammatory effects (Maizels et al., 2014; McSoreley et al., 2011; Weinstock & Elliot, 2013). Thus, examination of how any relationships between STH exposure and inflammation differ in adults and children are crucial.

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 non-existent (Gisbert & McNicholl, 2009; McDade et al., 2007; Tibble & Bjarnason, 2001). Fecal calprotectin (FC) has been shown to be a non-invasive, easily preserved and reliable biomarker for intestinal inflammation, suitable for use among people living in more remote regions of the world (Fagerhol et al., 1990; Gisbert & McNicholl, 2009; Tibble & Bjarnason, 2001; Tibble et al., 2000). Calprotectin is a protein found in key immune cells, like neutrophils, monocytes, and macrophages, critical to the inflammatory immune response (Fagerhol et al., 1980; Fagerhol et al., 1990). 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; Joshi et al., 2010; Gisbert & McNicholl, 2009). 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 et al., 2004). In infants (2 years and younger), elevated and variable FC levels are common due to maturation and development of the intestinal mucosa (Campeotto et al., 2003; Fagerberg et al., 2003; Olafsdottir et al., 2002; Rugtveit & Fagerhol, 2002). These elevated levels are often considered normal (Campeotto et al., 2003; Fagerberg et al., 2003; Olafsdottir et al., 2002; Rugtveit & Fagerhol, 2002). However, they may be associated with environmental enteric dysfunction (EED), a poorly understood inflammatory disorder related to abnormal intestinal flora, undernutrition, and exposure to

environmental toxins (Crane et al., 2015). Many children living in conditions where fecal-oral contamination is common face stunted growth associated with EED, and it is possible that the highly variable and elevated levels of FC during childhood is pathogenic rather than normal (Crane et al., 2015; Syed et al., 2016). The role of STHs in reducing the likelihood of developing EED in these circumstances remains unclear and some studies have shown that helminth infections are associated with higher rates of stunting (Stoltzfus et al., 1997; Tanner et al., 2009).

A few studies have examined FC in populations living in high-pathogen environments. Studies testing whether different bacterial, protozoal, or helminthic intestinal infections increase intestinal inflammation have found no such evidence (Betson et al., 2010; de Gier et al., 2018; Hestvik et al., 2011). In a Ugandan sample, one study found no relationship between FC levels and *Schistosoma mansoni* infection (i.e., a parasitic intestinal trematode) among children, but a negative relationship in their mothers (Betson et al., 2010). Another found no evidence that *Helicobacter pylori*, *Giardia intestinalis*, and very low rates of other macro-parasitic infections (e.g., *Campylobacter jejuni*, *Hymenolepis nana*, *Entamoeba histolytica*, *Ancylostoma duodenale, Ascaris lumbricoides*) were associated with FC among Ugandan children (Hestvik et al., 2011). Most relevant to the present research, a study of Cuban and Cambodian children found no relationship between STH infection and FC levels (de Gier et al., 2018). As the authors note however, STH infection prevalence was low, and infection intensities very light, so the effects of higher helminth infection prevalence and intensity could not be assessed.

This study presents preliminary but novel data on relationships between STH infection and intestinal inflammation using FC among a small sample of Shuar children and adults. The Shuar are an indigenous population from Amazonian Ecuador with previously documented moderate to high rates of STH infection (Cepon-Robins et al., 2014; Gildner et al., 2016). If, as the Old Friends Hypothesis suggests,

STHs play a role in regulating immune responses and intestinal inflammation, *and* these relationships are associated with current STH infection, then amongst the Shuar we should see lower FC levels in STH infected individuals (Hypothesis 1). We should also see negative associations between FC and STH infection intensity (Hypothesis 2). We expect these relationships to be especially pronounced in children due to variation in immune system development, and the potential importance of early exposure in training the immune response (Blackwell et al., 2010; 2011; Djuardi et al., 2011; Maizels et al., 2014).

Methods

Study Population. This study was conducted among the Shuar, an indigenous Amazonian population of Southeastern Ecuador and Northeastern Peru, centered 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). Further, biomarkers associated with the adaptive immune response to STH infection peak mid-childhood (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). Further, Shuar who live in rural villages characteristic of the majority 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., 2012a). Comparing relationships among STH infection and intestinal inflammation among children and adults separately can help elucidate when exposure to macroparasites has the largest impact on immune regulation.

Participants and Sampling. Cross-sectional data were collected by the SHLHP in 2016 from the remote Cross-Cutucú region of Ecuador. Shuar in this region are fairly isolated from regional market centers, with travel to Sucúa (a local market center) taking about 2 to 3 hours by motorized canoe and an additional 5 to 8 hours 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 sampled in this study had indoor or outdoor plumbing, with all individuals using either "*campo libre*" (76%) or pit latrines (24%). Participants reported getting their water from rivers/streams (64%) or wells (36%), with the average participant traveling about nine minutes for water access. Participant houses were made of wood (91%) or cement (9%) and had floors made of wood (87%) or earth (13%). Further, most participants reported cooking on the ground over fire/firewood (87%), while some had gas stoves (13%). Many households owned animals, including dogs (91%), chickens (87%), ducks (62%), cows (55%), horses (36%), and pigs (36%). Many participants reported allowing these animals into their homes (42%). These are all factors that contribute to fecal-oral contamination and STH exposure.

Data were collected from 69 Shuar participants (ages 5 to 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 to 15 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.

Soil-transmitted helminths. 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, heavy) were determined based on guidelines established by Montresor and colleagues (1998).

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 immunoassay (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 ug/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 et al., 2017; Olafsdottir et al., 2002).

Data Analyses. Data were analyzed using SPSS version 25 (SPSS Inc., Chicago, IL). 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 (**Table 1**). Curve estimates were used to investigate possible non-linear relationships between age, infection, and FC variables. Controlling for continuous age and sex (0 = female; 1 = male), separate two-way ANCOVAs for children and adults were performed to compare Ln FC based on infection status (0 = uninfected; 1 = *Ascaris* only; 2 = *Trichuris* only; 3 = coinfected) (**Table 2**). The homogeneity-of-slopes assumption was evaluated to verify that any relationship between infection status and Ln FC did not differ significantly as a function of age and sex, and this assumption was not violated. Levene's Test of Equality of Error Variances was used to confirm that the homogeneity of variance assumption was not violated. Follow-up simple main effect tests were used to examine the differences in means between each category of infection status. Linear regression analyses were conducted for children and adults separately to test relationships between Ln FC and age, sex, Ln *Trichuris* EPG, and Ln *Ascaris* EPG.

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

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 non-linear relationships were observed between age and any infection or FC variables.

Hypothesis 1. FC will be lower in STH infected individuals. Two-way ANCOVAs controlling for age and sex showed a significant relationship between infection type and Ln FC for children, but not adults (**Table 2**). Simple main effect tests showed that children who were singly infected with *T. trichiura* (M = 1.13; SE = 0.58) had significantly lower Ln FC levels than those who were uninfected with any STH species (M = 3.30; SE = 0.38; p = 0.01), singly infected with *A. lumbricoides* (M = 2.83; SE = 0.49; p = 0.04) or coinfected (M = 3.05; SE = 0.50; p = 0.02). There were no significant relationships between age, sex, or infection status and Ln FC for adults.

Hypothesis 2. FC will be negatively associated with infection intensity. Linear regressions (**Table 3**) showed a significant negative relationship between Ln *Trichuris* EPG and Ln FC (β [SE] = -0.85 [0.14], p = 0.01) and a significant positive relationship between the interaction for species-specific Ln EPGs and Ln FC (β [SE] = 0.94 [0.02], p = 0.03) among children. There were no significant relationships between age, sex, or infection intensity and Ln FC for adults.

Discussion and Conclusion

In this study, we recorded levels of intestinal inflammation among the Shuar using FC, and tested the relationships between STH infection and intestinal inflammation among a small sample of Shuar children and adults. We found that children singly infected with *T. trichiura* had lower levels of intestinal inflammation than children who were uninfected with any STH species, singly infected with *A. lumbricoides*, or coinfected with both species. We also found a significant negative relationship between *T. trichiura* infection intensity and intestinal inflammation. Though preliminary and based on a small sample size, this research has several implications for understanding intestinal inflammation as it relates to the Old Friends/Hygiene Hypothesis.

Intestinal inflammation among the Shuar. Very little research exists examining levels of intestinal inflammation among indigenous populations as they transition from subsistence-based lifestyles to those more dependent on regional and global market economies. This is an important group for testing topics relevant to the Old Friends Hypothesis. If the Old Friends Hypothesis is correct, and lifestyle and hygiene-related changes are contributing to alterations in immune system development and responsiveness, then we should be able to see this process begin as relevant hypothesized features of this transition occur. Participants sampled in this study live in relatively more isolated regions of Shuar territory, but still experience the effects of market integration, including changes to housing, cooking, water, and latrine infrastructure, exposure to domesticated animals, education about sanitation and hygiene, and occasional wage-labor/market access. These factors affect infectious disease exposure (Campbell et al., 2014; Freeman et al., 2013; Godoy et al., 2005; Fitton, 2000; Scolari et al., 2000; Saker et al., 2004; Tanner et al., 2014), making this sample an ideal population for understanding the early effects of lifestyle change on intestinal inflammation.

Fecal calprotectin levels among this sample are within the range of those documented elsewhere. Studies from highly economically developed nations, where STH are non-existent or uncommon, like Sweden (Fagerberg et al., 2003), Norway (Olafsdottir et al., 2002), and the UK (Joshi et al., 2010; Poullis et al., 2004) have documented mean or median FC levels between 9.9 and 40 ug/g. Rates of elevated intestinal inflammation (FC > 50 ug/g) among Shuar children appear to be higher in comparison to other populations. In this sample, 23% of children and 35% of adults had FC above 50 ug/g, suggesting moderate intestinal inflammation. A study of Belgian children aged 8 to 16 years of age found that only 5% of participants had FC levels over 50 ug/g (Michels et al., 2017). When that same age range (8 to 10 years of age) is observed in this sample, we have elevated FC in 28% of participants. Another study among older adults ages 50 to 70 from the UK found that 24.7% had FC levels above

normal range; although, in this case, FC levels >65 ug/g were used as cutoffs. When 65 ug/g is used as a cutoff for the present sample, 19% of children and 19% of adults exhibited FC levels above the cutoff.

Our findings suggest that Shuar adults may have similar, or even lower, rates of intestinal inflammation when compared to other adult populations, but Shuar children may have higher rates of intestinal inflammation compared to other children. This may be due to the highly variable nature of fecal calprotectin levels typically observed among children (Rugtveit & Fagerhol, 2002). An alternative explanation may be that the generally high bacterial and viral pathogen environment, poor sanitation, and high degree of fecal-oral contamination is elevating localized intestinal inflammation among Shuar children as their primary immune response switches from the pro-inflammatory innate response to the more regulated, anti-inflammatory adaptive response (Blackwell et al., 2010; 2011). The result of this switch may be EED, which is associated with reduced growth rates and increased stunting in children from similar regions (Crane et al., 2015; Syed et al., 2016). The Shuar, like other Amazonian populations, experience high rates of stunting (~40%; Blackwell et al., 2009), and our previous research documented notable tradeoffs between growth and immune function, with growth rates decreasing by up to 49% when the 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).

Species-specific infection and intestinal inflammation. While we did find evidence supporting the Old Friends Hypothesis, the relationship appears to be age- and species-specific. In this study, we found that children who were singly infected with *T. trichiura* had lower levels of intestinal inflammation than those who were uninfected, singly infected with *A. lumbricoides*, or coinfected. We also found interesting significant relationships among children between intestinal inflammation and infection intensity, such that FC levels were lower with more intense *T. trichiura* infection but were higher among those with no infections, and more intense *A. lumbricoides* infections and coinfections.

These species-specific relationships may be related to how the parasites interact with and feed from their host. *Trichuris trichiura* has a 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 T_H2 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). Interestingly, those coinfected with both species also have significantly higher levels of intestinal inflammation that those singly infected with *T. trichuria*, and FC appears to increase with more intense coinfections. This may reflect a failed attempt to maintain moderate levels of STH infection. Overall, this may be indicative of children who are less protected by hygienic measures and exposed to more fecal-oral contamination, and thus more viral and bacterial pathogens as well. Because of the small sample size, however, these data are difficult to interpret statistically. A larger sample size is needed to fully understand these relationships.

Age, STH infection, and intestinal inflammation. We found evidence to suggest that exposure to STHs and current infection status during childhood may shape immune system development and intestinal health. The relationship between *T. trichiura* infection and reduced intestinal inflammation was significant only in children. Here, we may be observing the importance of early exposure for immune system development. 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, thus turning down inflammation in the presence of *T. trichiura* (Blackwell et al., 2010; 2011).

Studies that examine specific immune markers, like IgE, which is directly related to repeated and chronic macroparasite 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 T_H2 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 data presented in this study, provide important support for the hypothesis that exposure to macroparasites during development is crucial for proper immune system development. Lack of exposure to STHs during childhood may be related to elevated levels of intestinal inflammation and associated disorders (e.g. IBD) seen in wealthy countries.

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. This means that subsamples representing species-specific infections and coinfections are particularly low. A larger sample size would be useful for more robust hypothesis testing and would provide more interpretive value for results.

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 paper. 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, 2012b; Urlacher et al., 2016; 2018) and, because of this small sample size, we cannot speak to their effects here.

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 do appear to 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). 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 et al., 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.

Acknowledgements

The authors wish to thank the participants of this study. We declare no conflicts of interest. This research was conducted with support from the Wenner Gren Foundation, the National Science Foundation (NSF IBSS #1329091), the American Philosophical Society Lewis and Clark Fund, the Ryoichi Sasakawa Young Leaders Fellowship Fund, the University of Oregon Anthropology Department/Bray Fellowship, and the University of Colorado Colorado Springs.

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Table 1. Descriptive statistics for intestinal inflammation and STH infection variables for children and adults

	Children (N = 26)	Adults (N = 43)
Age	8.8 (3.2)	35.1 (15.7)
Intestinal Inflammation		
Fecal Calprotectin (FC; ug/g)†	17.7 (36.6)	22.0 (51.5)
Elevated FC (%)	23.1 (n = 6)	34.9 (n = 15)
Infection Status		
Only *Trichuris* Infected (%)	15.4 (n = 4)	11.6 (n = 5)
Only *Ascaris* Infected (%)	23.1 (n = 6)	14.0 (n = 6)
Coinfected (%)	23.1 (n = 6)	16.3 (n = 7)
Uninfected (%)	38.5 (n = 10)	58.1 (n = 25)
Species-specific Eggs Per Gram (EPG)		
Trichuris EPG	414.5 (1341.1)	159.1 (801.7)
Ascaris EPG	3839.1 (7588.7)	3581.6 (12014.3)
***Trichuris* Infection Intensities**		
Light (1 to 999 EPG)	30.8 (n = 8)	23.3 (n = 10)
Moderate (1,000 to 9,999 EPG)	7.7 (n = 2)	4.7 (n = 2)
***Ascaris* Infection Intensities**		
Light (1 to 4,999 EPG)	19.2 (n = 5)	11.6 (n = 5)
Moderate (5,000 to 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: †Denotes Median (IQR)

Table 2. Two-way ANCOVAs (controlling for age and sex) comparing Ln FC by STH infection status for children and adults

		df	*F*	*p*
Children				
	Age	1	1.43	0.25
	Sex	1	0.86	0.37
	Infection Type	3	3.53	0.03*
Adults				
	Age	1	0.54	0.47
	Sex	1	0.00	0.96
	Infection Type	3	0.86	0.47

Results are significant at: $^{*}p < 0.05$

Sex: 0 = female, 1 = male; Age is continuous; Infection Type: 0 = uninfected, 1 = *Ascaris* only, 2 = *Trichuris* only, 3 = Coinfected

Table 3. Linear regression analyses for relationships between Ln FC, age, sex, and STH infection intensity for children and adults

		Coefficients (SE)	*β*	*P*	Model r^2 and *p*
Children					0.35/0.11
	Constant	3.88 (0.91)		0.00	
	Age	-0.11 (0.08)	-0.27	0.17	
	Sex	0.66 (0.51)	0.25	0.21	
	Ln *Trichuris* EPG	-0.38 (0.14)	-0.85	0.01*	
	Ln *Ascaris* EPG	-0.10 (0.09)	-0.33	0.26	
	Ln *Ascaris* X Ln *Trichuris* EPG	0.05 (0.02)	0.94	0.03*	
Adults					0.08/0.64
	Constant	3.75 (0.72)		0.00	
	Age	-0.01 (0.02)	-0.12	0.49	
	Sex	-0.00 (0.47)	-0.00	0.99	
	Ln *Trichuris* EPG	-0.24 (0.20)	-0.37	0.21	
	Ln *Ascaris* EPG	-0.07 (0.11)	-0.20	0.50	
	Ln *Ascaris* X Ln *Trichuris* EPG	-0.02 (0.03)	0.21	0.61	

Results are significant at: $^{*}p < 0.05$

Sex: 0 = female, 1 = male; Age, Ln *Ascaris* EPG, and Ln *Trichuris* EPG are continuous