# Predictors and Nutritional Consequences of Intestinal Parasitic Infections in Rural Ecuadorian Children

by Mamie-Eleanor Sackey,<sup>a</sup> M. Margaret Weigel,<sup>a</sup> and Rodrigo X. Armijos<sup>b,c</sup>

<sup>a</sup> Community and International Nutrition and Health Section, Department of Human Nutrition, Foods, and Exercise, Virginia Polytechnic Institute and State University, Virginia, USA

<sup>b</sup> Laboratorio de Inmunologia, Division de Medicina Tropical y Nutricion, Universidad Central del Ecuador, Quito, Ecuador

<sup>c</sup> Dept de Microbiologia, Instituto Nacional de Higiene 'Leopoldo Izquieta Perez', Ministerio de Salud Publica, Quito, Ecuador

#### Summary

The study investigated the prevalence, risk factors, and nutritional consequences of intestinal parasitic infections (IPI) in rural Ecuadorian children. A total of 244 children aged 0.2-14 years were studied. The data were collected using a structured questionnaire, anthropometry, and laboratory analysis of blood and fecal samples. The results showed that 90 per cent of the subjects were infected with at least one pathogenic IPI: 51 per cent with helminths, 37.6 per cent with protozoa, and 21.4 per cent with both. Giardia-infected children had a risk for stunted growth that was twice that of other children (51.7 vs. 33.1 per cent; OR = 2.16, 95 per cent CI = 1.13-4.15; p = 0.01). They also had significantly reduced mean hemoglobin levels compared with their non-infected counterparts (11.8  $\pm$  1.5 vs. 12.2  $\pm$  1.4 g/dl; p = 0.023). However, the proportion diagnosed with iron-deficiency anemia was slightly, but not significantly, increased (29.4 vs. 24.3 per cent). The most consistent predictor of Giardia and other protozoal IPI risk was a high intra-/peri-domicilliary concentration of domestic animals. Children who lived in such households had an infection risk that was two to five times greater than that of their non-infected counterparts. The data indicate that Giardia intestinalis infection has an adverse impact on child linear growth and hemoglobin. They also suggest that domestic animals may be an important reservoir for *Giardia* and other intestinal protozoal infections observed in the Ecuadorian children studied.

#### Introduction

Intestinal parasitic infections (IPI) constitute a global health burden. They are estimated to affect approximately 3.5 billion persons worldwide and cause clinical morbidity in 450 million, many of

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Correspondence: Dr M. M. Weigel, Department of HNFE, 205 Wallace Annex, Virginia Tech, Blacksburg, Virginia, 24061-0430, USA. E-mail <mweigel@vt.edu>. these being children in developing countries.<sup>1</sup> Intestinal parasitic infections, mostly helminths, have been linked with poorer nutritional outcomes, including an increased risk for nutritional anemias, protein-energy malnutrition (PEM), and growth deficits in children.<sup>1–3</sup> However, the published evidence has not been entirely consistent regarding the impact of these on child growth and iron status.<sup>4</sup> This is especially true of protozoal infections such as *Giardia intestinalis*<sup>5,6</sup> about which, less is known.

Intestinal infections caused by parasites, bacteria, and viruses were ranked as the fifth leading cause of child mortality in Ecuador in 1998.<sup>7</sup> However, despite their assumed importance for public health, only a few studies have been published<sup>8-12</sup> documenting the prevalence of IPIs in Ecuadorian children and only one of these has identified any factors associated with IPI risk.<sup>8</sup> None have directly examined IPI impact on children's nutritional status. Thus, it was decided to investigate the prevalence, predictors and consequences of IPIs on growth and iron status in rural children living in a tropical rainforest area of northwest Ecuador.

# **Methods and Materials**

### Study protocol

The survey study was conducted during a 2.5 month period (June–August 2000) in five hamlets (Paraiso de Amigos, La Paz, Bosque de Oro I, Bosque de Oro II and Cristobal Colon) located within a rural tropical rainforest area in Pichincha Province, Ecuador. Institutional approval to conduct the study was given by the Virginia Tech Institutional Review Board and the local partner institution, Fundacion Internacional 'Biocencias'. Meetings were first held with local community leaders and the parents/guardians of prospective subjects to explain the study and request their participation. Children whose parents/guardians gave their informed written consent were enrolled in the study.

The study data were collected during house-tohouse visits except in instances where the parents/guardians requested another location, which was usually the local schoolhouse. Data were collected using a structured questionnaire, anthropometry, and laboratory analysis of blood and fecal samples. Additional qualitative data were obtained through the use of key informants and by direct observation. The parents/guardians of the subjects received oral and written notification of test results. Children diagnosed with IPIs and/or iron-deficiency anemia were given the appropriate anti-parasitic treatment or iron supplements. Those identified with PEM or other medical conditions were given written referrals to local health services. The parents/guardians of subject families received individual nutrition counseling performed by qualified nutritionists and when requested, had the fecal samples of other members analysed for IPIs. In addition, each participating child received vitamin supplements. These medications, supplements, and services were provided without cost to the families.

# Data collection

A structured questionnaire with open- and closedended questions collected information from the parents/guardians of subjects regarding sociodemographic, housing, water and sanitation characteristics. These included subject age, sex, and ethnicity, marital status, education and primary occupations of both parents, community residence site, family size and composition, house construction, water, sanitation, and garbage disposal, hygiene practices and the type and number of domestic animals living in and around the home.

Subject hemoglobin levels were determined using the HemoCue  $\beta$ -Hemoglobin photometer HemoCue (Angelholm, Sweden). The instrument was calibrated daily with the control cuvette supplied by the manufacturer. The cut-off points used to identify iron-deficiency anemia were based on CDC recommended values for child age and sex.<sup>13</sup>

Weight was measured to the nearest 0.5 kg using a portable scale (Detecto, USA). Standing height in children aged  $\geq 24$  months was measured to the nearest 0.1 cm using a portable stadiometer. For children under 24 months, recumbent length was measured to the nearest 0.1 cm with an infant measuring board that had a fixed headboard and a movable footboard. Subject weight-for-age, weightfor-height, and height-for-age measurements were calculated using the EPINUT program of Epi-Info, version 6.14 This compared subject measurements to an international National Center for Health Statistics growth reference curves.<sup>15</sup> The cut-off point used to identify malnourished children was less than -2 standard deviation units (SD) below the NCHS reference median. Children whose weight-forheight, height-for-age, and weight-for-age fell below -2 SDs were classified, respectively, with acute malnutrition (wasting), chronic malnutrition (growth stunting), or global malnutrition.

The subjects contributed three serial stool samples which were analysed for the presence of IPIs. The analyses were performed by certified laboratory technicians on the day of collection in the field laboratory of the Puerto Quito community clinic. After gross examination of fecal sample characteristics, a direct wet smear was prepared by emulsifying 2 mg of feces on a glass slide. A drop of Lugol's solution was applied to the first half of the split sample and isotonic solution to the second. The samples were covered with a coverslip and systematically observed at low (10  $\times$ ) and high magnifications  $(40 \times)$  with a light microscope in order to identify the presence of mature parasites, cysts or eggs. Children which had at least one stool sample in which an IPI was identified were classified as positive for that parasite.

# Data analysis

Descriptive data are reported as means with standard deviations, frequency counts and percentages. The bivariate analyses used contingency table analysis with  $\chi^2$  to investigate differences between proportions. Factors identified as significant (p <0.05) were subsequently analysed using multiple logistic regression analysis which adjusted for potential confounders. The crude and adjusted odds ratio obtained from the bivariate and multiple logistic regression analyses are presented in Tables 2-4 with their respective 95 per cent confidence intervals. Students t-test and one-way analysis of variance were used to test mean differences. The preliminary analyses examined the impact of: (1) parasite presence vs. absence, (2) parasite burden (low, medium, high), and (3) polyparasitism on child iron status and growth. However, only the results of those which considered the former are presented since the others yielded no significant associations with nutritional status or did not result in increased explanatory power over that of IPI presence vs. absence.

### Results

Ninety per cent of the child subjects were infected with at least one pathogenic IPI: 51 per cent with helminths, 37.6 per cent with protozoa, and 21.4 per cent with both. The most common pathogenic species identified were Ascaris lumbricoides (39.7 per cent), Giardia intestinalis (25.2 per cent), Trichuris trichiura (19.7 per cent), Entamoeba histolytica/dispar (18.5 per cent), and Ancylostoma duodenale (1.7 per cent). Table 1 shows the characteristics of the subjects and their households. Most household water was reported to come from the local river, open wells, springs, or other unprotected sources. Few parents/guardians reported that their households consistently boiled their drinking water for more than 5 min or treated it with chlorine. Fewer still said that they used treated water in preparing foods. Close to 95 per cent of subject households had chickens, pigs, dogs, cows, horses, and other domestic animals living inside or in close proximity to the family's living quarters. These averaged approximately 23 animals per household.

Table 2 indicates that children infected with *G. intestinalis* had significantly reduced mean serum hemoglobin levels compared with the others. Iron-deficiency anemia was detected in 26.4 per cent of the total sample. However, the proportion of *Giardia*-infected children with iron-deficiency anemia was slightly, but not significantly, increased over that of non-infected children (29 vs. 24 per cent). No significant associations were detected between infection with the other IPIs, serum hemoglobin, and the risk for anemia.

Nearly 40 per cent of the children in the sample had stunted growth, 21.5 per cent had global malnutrition and 4.1 per cent were wasted. Table 3 indicates that children with intestinal parasitic infections were twice as likely as non-infected children to suffer from growth stunting or chronic malnutrition. Likewise, those infected with *G. intestinalis* also had double the risk for stunted growth. However, no significant associations were identified between any of the intestinal parasitic infections identified in the study and the risk for either acute malnutrition or global malnutrition.

Table 4 shows the results of the bivariate analyses that investigated IPI risk. As indicated, residence (Paraiso de Amigos), close geographic proximity to neighbors, and high intra/peri-domilliciary domestic animal concentration (> 20 animals) were associated with a significantly increased risk for infection with *G. intestinalis.* Subjects whose parents were legally married were at reduced risk. No significant associations were identified between *Giardia* infection and child gender, age, other parental characteristics,

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 TABLE 1

 Subject and household characteristics

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	Mixed (treatment and non-treatment)	5 (2.5)
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Food preparation water treatment		
Boil 56 (29.0)		
No treatment 137 (71.0)		
Households with domestic animals living 190 (94.5)	6	190 (94.5)
inside or in close proximity to home (%) No. domestic animals living inside or in $22.7 \pm 22.3$ close proximity to home	No. domestic animals living inside or in	22.7 ± 22.3

Intestinal parasite species	Serum hem	oglobin (g/dl)	Iron-deficiency anemia <sup>a</sup>		
	$X \pm SD$	F-ratio	No. (%)	OR (95% CI)	
G. intestinalis present (51)	11.8 ± 1.5*	5.2	15 (29.4)	1.30 (0.6–2.8)	
G. intestinalis absent (144)	$12.2 \pm 1.4$		35 (24.3)	1.00	
E. histolytica/dispar present (42)	$12.4 \pm 1.5$	<1.0	11 (26.2)	1.00 (0.47-2.2)	
E. histolytica/dispar absent (152)	$12.0\pm1.3$		39 (25.7)		
A. lumbricoides present (84)	$12.3 \pm 1.3$	1.7	16 (19.0)	0.53 (0.27-1.1	
A. lumbricoides absent (111)	$12.0 \pm 1.5$		34 (30.6)	1.00	
T. trichiura present (43)	$12.3 \pm 1.1$	<1.0	10 (23.3)	0.85 (0.38-1.9	
T. trichiura absent (152)	$12.1\pm1.5$		40 (26.3)	1.00	
A. duodenale present (4)	$11.8 \pm 0.6$	<1.0	1 (25.0)	0.95 (1.0-9.3)	
A. duodenale absent (192)	$12.1 \pm 1.5$		50 (26.0)	1.00	

 TABLE 2

 Association between intestinal parasitic infection and child iron status

<sup>a</sup> Cut-off points based on CDC13 criteria according to age and sex.

\* p = 0.023.

Intestinal parasite species	Growth stunting		Wasting		Global malnutrition	
	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)
Any parasitic infection	66 (43.1)	2.10 (1.10–3.99)*	7 (5.0)	1.80 (0.37–0.92)	37 (24.0)	1.69 (0.82–3.46)
No parasitic infection	20 (27.0)	1.00	2 (2.9)	1.00	12 (15.8)	1.00
<i>G. intestinalis</i> present	30 (51.7)	2.16 (1.13–4.15)**	1 (2.0)	0.38 (0.50–3.1)	15 (25.9)	1.42 (0.71–2.84)
<i>G. intestinalis</i> absent	56 (33.1)	1.00	8 (5.1)	1.00	34 (19.8)	1.00
<i>E. histolytica/dispar</i> present <i>E. histolytica/dispar</i> absent	17 (41.5) 68 (36.8)	1.22 (0.61–2.43) 1.00	0 (0.0) 9 (5.1)	-	9 (21.4) 40 (21.4)	1.00 (0.44–2.27)
<i>A. lumbricoides</i> present	39 (42.9)	1.38 (0.80–2.38)	3 (3.7)	0.80 (0.2–3.15)	24 (26.1)	1.60 (0.85–3.01)
<i>A. lumbricoides</i> absent	47 (34.6)	1.00	6 (4.7)	1.00	25 (18.1)	1.00
<i>T. trichiura</i> present	19 (41.3)	1.20 (0.62–2.32)	1 (2.4)	0.49 (0.06–4.0)	11 (23.9)	1.20 (0.56–2.60)
<i>T. trichiura</i> absent	67 (37.0)	1.00	8 (4.8)	1.00	38 (20.6)	1.00
<i>A. duodenale</i> present	1 (25.0)	0.55 (0.60–5.30)	0 (0.0)	_	1 (25.0)	1.24 (0.13–1.22)
<i>A. duodenale</i> absent	85 (37.9)	1.00	9 (4.4)		48 (21.1)	1.00

TABLE 3
 Association between intestinal parasitic infection and child growth status

\* p = 0.023; \*\* p = 0.01

water, sanitation and other household indicators. The multiple logistic regression analysis (Table 4) investigated which factors made significant independent contributions to *Giardia* risk. The previously identified factors of residence and domestic animal concentration retained their associations with increased risk, and parental marital status with decreased risk. However, the contribution of close home proximity to neighbors was no longer apparent and was dropped from the model.

As Table 4 also indicates, residence (Paraiso de Amigos), high domestic animal concentration,

drinking water source (river), and paternal occupation (agriculturalist) were associated with an increased risk for *Amoeba histolytica/dispar* infection in the bivariate analyses, while young age (< 84 months) and adequate household garbage disposal were associated with a reduced risk. No significant associations were identified between the other factors measured and infection risk. As Table 4 indicates, the previously identified associations of high animal density, and adequate garbage disposal with infection risk remained significant in the multiple logistic regression analysis. However, the factors of

	No. (%)	OR (95% CI)	р	Adjusted OR (95% C.I.) p		
1. Giardia intestinalis infection						
Residence						
Paraiso de Amigos	13 (44.8)	2.81 (2.17-6.72)	0.02	3.53 (1.23-10.1)	0.018	
Other	46 (22.4)	1.00		1.00		
Maternal marital status						
Legally married	6 (10.9)	0.25 (0.10-0.61)	0.003	0.28 (0.11-0.74)	0.010	
Other	44 (33.3)	1.00		1.00		
No. of domestic animals living						
in/around home						
> 20	26 (36.1)	2.33 (1.20-4.57)	0.019	3.78 (1.68-8.49)	0.001	
≤20	22 (19.5)	1.00		1.00		
House proximity to nearest neighbor	. ,					
≤ 100 m	39 (33.6)	2.36 (1.14-4.92)	0.03	1.26 (0.54-2.91)	NS	
> 100 m	12 (17.6)	1.00		1.00		
2. Amoeba histolytica/dispar infection						
Residence						
Paraiso de Amigos	24 (40.7)	5.59 (2.61-12.0)	0.0001	1.68 (0.20-15.13)	NS	
Other	24 (40.7) 19 (10.9)	1.00	0.0001	1.08 (0.20–13.13)	183	
Subject age	19 (10.9)	1.00		1.00		
$\leq 84 \text{ months}$	24 (13.5)	0.08 (0.04-0.18)	0.0001	0.49 (0.22-1.1)	NS	
> 84 months	24 (13.3) 19 (35.2)	0.08 (0.04-0.18)	0.0001	1.00	183	
Paternal occupation	19 (33.2)			1.00		
Agriculturalist	27 (22.0)	4 10 (1 02 8 00)	0.047	2 78 (0 60 12 1)	NS	
Other	37 (23.9)	4.10 (1.02–8.00) 1.00	0.047	2.78 (0.60–12.1) 1.00	183	
	2 (7.1)	1.00		1.00		
No. of domestic animals living in/around home > 20	23 (31.9)	2.30 (1.14-4.62)	0.019	2.29 (1.1-4.98)	0.035	
≤ 20	· · · ·	2.30 (1.14–4.02) 1.00	0.019	2.29 (1.1–4.98) 1.00	0.055	
$\leq 20$ Source of household water	19 (17.0)	1.00		1.00		
	20(21.2)	2 22 (1 04 4 20)	0.020	1 72 (0 70 2 79)	NIC	
River water only	20 (31.3)	2.23 (1.04-4.80)	0.039	1.73 (0.79–3.78)	NS	
Other	21 (16.9)	1.00		1.00		
Household garbage	15 (14.0)	0.41 (0.10, 0.07)	0.010	0.2( (0.17, 0.00)	0.010	
Adequate disposal <sup>a</sup>	15 (14.9)	0.41 (0.19–0.87)	0.019	0.36 (0.17–0.80)	0.012	
Inadequate disposal	27 (30.0)	1.00		1.00		
3. Trichuris trichuria infection						
Residence						
La Paz	18 (30.5)	2.31 (1.16-4.58)	0.025	1.84 (0.87-3.87)	NS	
Other	28 (16.0)	1.00		1.00		
Subject age						
≤ 60 months	13 (10.9)	0.31 (0.15-0.67)	0.0016	0.36 (0.16-0.81)	0.013	
> 60 months	32 (28.1)	1.00		1.00		
Maternal education	. ,					
$\leq$ 3 years	26 (32.5)	2.62 (1.29-5.32)	0.011	1.96 (0.93-4.13)	NS	
> 3 years	16 (15.5)	1.00		1.00		

 TABLE 4

 Factors associated with the risk for intestinal parasitic infections

<sup>a</sup> Garbage disposed of by burning, burying and pickup.

household water source, paternal occupation, age and residence were no longer evident.

Table 4 reveals that residence in La Paz and a low maternal education ( $\leq$  3 years) were associated with an increased risk for *T. trichuria* infection. Younger subject age ( $\leq$  60 months) was associated with a decreased risk. No significant associations were observed between *Trichuris* infection and the other indicators investigated. The multiple logistic regression analysis results revealed that although subject age retained its respective identified contributions to

trichuriasis risk, that of residence and education were no longer evident.

The mothers of the children identified with ascariasis were slightly, but significantly, less well educated compared with those without the infection, i.e.,  $3.8 \pm$ 2.7 years vs.  $4.7 \pm 2.8$  years; p = 0.022). However, no significant associations were identified between *Ascaris* infection and the sociodemographic and other indicators measured in the study. None of the indicators measured in the study predicted the risk for hookworm infection. This may be the result of insufficient statistical contrast related to the low hookworm infection frequency.

## Discussion

The study investigated the prevalence, predictors and nutritional consequences of intestinal parasitic infections in rural Ecuadorian children. The pattern of a high prevalence and predominance of helminth infection concurs with that noted for other tropical child groups living in the Ecuadorian Amazon<sup>8,11</sup> but differs from that reported for children living in subtropical<sup>12</sup> and highland areas<sup>10</sup> of the same province.

The observed relationship between *Giardia* infection and hemoglobin is consistent with authors who linked the parasite with significantly reduced iron status in children living in Venezuela,<sup>16</sup> the Gaza<sup>17</sup> and Egypt<sup>18</sup> but differs from de Morais and associates<sup>6</sup> who reported that Brazilian children with asymptomatic infection showed no evidence of reduced hemoglobin levels compared to noninfected controls and did not benefit from oral iron therapy. On the other hand, Jimenez and associates<sup>16</sup> showed that *Giardia*-infected children who were given a single dose of secnidazole had significantly increased mean hemoglobin values by 15 days posttreatment.

Most of the risk for growth stunting found in children with IPI infection appeared to be attributable to the robust contribution of G. intestinalis infection rather than other species. Previously published studies have been inconsistent with respect to the association of giardiasis and growth stunting. For example, several authors have reported significant reductions in the linear growth of Giardiainfected children<sup>5,19</sup> and experimental mammals.<sup>20</sup> Others have demonstrated increased height and weight in children treated for giardiasis.<sup>21</sup> Furthermore, Fraser and colleagues<sup>5</sup> have reported finding that growth stunting followed rather than preceded Giardia infection in their prospective study of Bedouin infants. It has been suggested that *Giardiasis* may cause nutrient malabsorption and other adverse changes.<sup>2,22,23</sup> The resulting deficits in growth may be exacerbated where PEM is already present.24

In contrast to these findings, other authors have reported the lack of a significant association between giardiasis and growth stunting in young infants<sup>25</sup> or older children.<sup>26</sup> While differences in study design and methodology may be responsible for some of these between-study discrepancies, population differences in infection intensity and burden, nutritional status, breastfeeding, patterns of growth, and other attributes appear responsible for others. Evidence in support of the importance of population differences has been noted by Fraser and colleagues<sup>5</sup> who reported that the impact of giardiasis on child growth was different for two populations who lived in the same geographical area of Israel but who had distinct lifestyle and environmental characteristics.

The most consistent predictor of protozoal IPI risk was a high intro-/peri-domicilliary concentration of domestic animals. Children who lived in such house-holds had a risk for *Giardia, E. histolytica/diapar*, and *Blastocysis hominis* infection that was two to five times higher than that of those living in homes where fewer animals were present. Pigs, dogs, cats, chickens, cattle and horses have been identified as possible reservoirs for *Giardia* and other common protozoal IPIs.<sup>27–33</sup> In the Ecuadorian group studied, this may occur through contamination of the children's drinking water, their home environment, objects they play with, and via blowing dust which contains parasite cysts and mature forms.

Several caveats should be considered when evaluating the study results. First, self-reported data obtained by interview may be subject to significant under- or over-reporting of specific characteristics depending on whether the respondents believe these to be socially desirable. However, the use of direct observation during the home visits allowed for the verification of many of the reported household attributes. This information was also cross-checked with school teachers and other family members when possible. It is also possible that the detection methods used may not have been sensitive enough to detect all cases of G. intestinalis and other IPIs. Several newer techniques are reported to have higher sensitivity compared with the conventional methods used in the present study.<sup>34</sup> However, we attempted to offset this limitation by analysing three serial fecal samples per subject, an approach which is reported to increase the yield of protozoa, for example, by an additional 23 per cent for A. histolytica and 11.3 per cent for G. intestinalis.<sup>34</sup> Although one study has reported that growth faltering occurred subsequent to Giardia infection,<sup>5</sup> the crosssectional design of the present investigation did not allow for the definitive determination of whether infection itself augmented the risks for stunted growth and decreased hemoglobin or whether malnutrition increased infection susceptibility. Prospective cohort studies are needed to clarify this question and the mechanisms responsible. In addition, future studies should investigate the role of domestic animals in infection transmission since this risk factor is potentially modifiable.

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