Factors influencing growth and intestinal parasitic infections in preschoolers attending philanthropic daycare centers in Salvador, Northeast Region of Brazil

Crescimento linear e infecções parasitárias intestinais em pré-escolares matriculados em creches filantrópicas de Salvador, Nordeste do Brasil

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Abstract

Poor growth and intestinal parasitic infections are widespread in disadvantaged urban children. This cross-sectional study assessed factors influencing poor growth and intestinal parasites in 376 children aged three to six years in daycare centers in Salvador, in the Northeast Region of Brazil. Data was obtained from seven daycare centers on child weight, height, socio-economic status, health and intestinal parasites in stool samples. Prevalence of moderate underweight (< -1SD > -2SD), wasting and stunting was 12%, 16% and 6% respectively. Socioeconomic status, birth order, and maternal weight were predictors of poor anthropometric status. Almost 30% of children were infected with more than one intestinal parasite. Helminths (17.8%), notably Trichuris trichiura *(12%) and* Ascaris lumbricoides *(10.5%), and protozoan* Giardia duodenalis *(13%) were the most common types of parasites detected. One percent of children had hookworm and* Cryptosporidium *sp. and 25% had non-pathogenic protozoan cysts. Boys from families with very low socio-economic status had lower linear growth and presented a greater risk of helminth infection. Deworming is considered an alternative for reducing the prevalence of intestinal parasitic infections in this age group.*

Preschool Child; Child Day Care Centers; Parasites; Parasitic Intestinal Diseases

Introduction

Gastro-intestinal parasitic infections, including soil-transmitted helminths are widespread in crowded urban environments with poor sanitation [1]. Therefore, it is not surprising that the risk of intestinal parasitic infection is particularly high in urban and periurban areas in the Northeast Region of Brazil, one of the poorest and most populous regions of this tropical country [2]. Preschool children living in such environments are especially vulnerable to helminth infections with *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm) because of the increased activity of these species in potentially infective environments and lack of appropriate sanitary behavior [3].

Pathogenic protozoan infections are also common in poor urban settings [3]. Children attending daycare centers are especially vulnerable to *Giardia duodenalis* and *Cryptosporidium* sp. because the primary mode of transmission of these organisms is fecal-oral [4]. Both helminth and protozoan infections have been linked with several adverse health consequences, notably impaired growth and deficiencies in vitamin A and iron, induced by anorexia, nausea, diarrhea and vomiting, reductions in digestion and absorption, and enhanced nutrient loss [5,6]. Our study therefore aimed to: (1) assess the prevalence of poor growth and gastro-intestinal parasites in a selected group of preschool children aged three

to six years attending urban and periurban daycare centers in Salvador; and (2) explore the factors that might influence poor growth and intestinal parasitic infections.

Methods

Study sites and participants

This cross-sectional study was conducted between August and November 2010 in the capital of the State of Bahia, Salvador, a densely populated city with 2.6 million inhabitants. Despite the implementation of a city-wide sanitation intervention in the last decade, a number of areas still lack appropriate sanitation coverage. Seven philanthropic preschool daycare centers located in the city center and in periurban areas were selected to participate in this study. The periurban daycare centers are located approximately 20 kilometers north and north-east of the city centre. One periurban daycare is located within a large gated complex with separate buildings for preschool daycare and primary and secondary education. The other five periurban daycares were located within two kilometer radius of a large *favela* (shantytown) community with a population of 65,000 [7], where residents live primarily in permanent houses with a concrete block or wooden structure and concrete, ceramic tile or earth flooring. All daycare centers are accessible by road, although transportation in the rainy season is hindered by heavy rains.

Children enrolled in the daycare centers (maximum class size of 25 children) were from low-income families and attended daycare five days per week, except during holidays, up until school age. All of the daycare centers provide breakfast, a mid-morning snack, lunch, an afternoon snack, and milk drink or soup before the children go home. Inclusion criteria for the study were apparently healthy children enrolled in three and four-year-old daycare classes for the 2010 school year (February to December). Of the 438 eligible children, parents/guardians authorized the participation of 376 children (86%). Of this total, approximately 40 children were recruited from each of the five periurban favela daycare centers, yielding a total of 202 children. The remainder were recruited from the city daycare centers (n = 79) and the gated complex (n = 95). Reasons for non-participation included children on the roll who had already moved or were moving during the year (n = 16), children not attending daycare because of chronic illness (n = 3) and children whose parent/guardian refused consent (n = 43). Data was not collected on the families who refused their child's participation in the study.

The study protocol was approved by the Ethics Research Committees of the Federal University of Bahia (Universidade Federal da Bahia) and the University of Otago. Written approval to carry out the study was obtained from the following participating philanthropic organizations: Santa Casa de Misericordia, responsible for the city centre and five periurban favela daycare centers and Mansão do Caminho, responsible for the periurban gated daycare center. Informed written permission to participate in the study was given by the children's parents or primary guardians.

Assessment of children's socio-demographic situation and health status

Trained Brazilian nutritionists administered a pre-tested questionnaire with mothers or guardians at the daycare center. Data on parental education and occupation, sanitation, household assets and other household characteristics were obtained. An overall socio-economic status score was determined for each participant based on a model designed to assess the poverty level of Brazilian families living in poor urban areas [8]. Points were assigned for family and house size and structure, parental education and occupation, marital status, house ownership and household assets, toilet and sewage facilities, type of drinking water, availability of electricity, and susceptibility to flooding during heavy rain. Socioeconomic status scores were divided into two categories: extremely low (≤ 34) and low (≥ 35).

Information on the child's ethnicity and health status was also collected based on maternal reports of vitamin and/or mineral supplement usage, vaccinations, parasite control (deworming), number of child hospitalizations and reasons, and exposure to tobacco smoke. Responses were verified where possible by reviewing children's health cards.

Assessment of growth

Weight and height were measured with children wearing light clothes and no shoes by a trained anthropometrist (R.L.L.) using standardized techniques and calibrated equipment [9]. Measurements were taken in duplicate and a third measurement was taken if the difference between the first two exceeded the allowable difference [9]. Z-scores were calculated for height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), and body-mass-index (BMIZ) using updated US Centers for Disease Control and Prevention (CDC) 2000 growth reference data [10]. Children

were classified as undernourished or moderately undernourished based on z-scores as an index of growth with a standard deviation (SD) of < -2 and < -1 ≥ -2, respectively. Possible risk of overweight or overweight in children was based on BMIZ of >1 ≤ 2SD and > 2SD, respectively, as defined by World Health Organization (WHO) [11].

Collection and microscopic assessment of parasites in stool samples

Labeled plastic stool collection containers and instructions were distributed by the daycare center coordinators to children's parents/guardians who were requested to bring the stool sample back to the daycare center the following morning. If the parent/guardian failed to return the child's stool sample, replacement containers were provided on up to three subsequent occasions. Single stool samples were obtained from 325 of the 376 participants (86%). Samples were transported to the laboratory in a chilled box, where an aliquot of each stool sample (two to four grams) was homogenized in a ~10mL sodium acetate-acetic acid formalin (SAF) solution (Ft. Richard Laboratories, Auckland, New Zealand) by manual stirring with a plastic spatula. Next, a fecal concentrate of the SAF mixture was prepared using the standard formalin-ethyl acetate sedimentation concentration procedure [12]. A standard amount of sediment (100µL) from each fecal concentrate was placed onto a slide and examined under the microscope by an experienced microbiologist (A.G.L.) for the presence of helminthic and protozoan intestinal parasites. Trichrome stain microscopy examination was used to confirm the presence of *Entamoeba histolytica/dispar* cysts but it was not possible to ascertain the presence of pathogenic *E. histolytica* or non-pathogenic *E. dispar* cysts. The presence of other non-pathogenic protozoan cysts, such as *Escherichia coli, Endolimax nana, Iodamoeba butschlii*, and *Chilomastix mesnili* was also noted. A semi-quantitative egg-burden estimate of positive helminth samples was also determined based on the number of eggs counted during the microscopy examination. Light, moderate, and heavy loads were defined as ≤ 1 egg, 2-9 eggs and ≥ 1 egg per 10 low power magnification fields (10x), respectively. The microscopic examination of fecal concentrates was repeated on a random sub-sample (10% of the total sample) by a microbiologist not involved with this study to determine reproducibility of the methods.

Qualitative detection of *Giardia* and *Cryptosporidium* antigens in feces

A separate SAF aliquot (~2mL) was used to detect the presence of *Giardia* and *Cryptosporidium* antigens using GIARDIA/CRYPTOSPORIDIUM CHEK, an *in vitro* enzyme-linked immunosorbent assay (ELISA) (TechLab Inc., Blacksburg, USA). This combined antigen assay uses monoclonal and polyclonal antibodies to cell-surface antigens of *Giardia* and an oocyst antigen of *Cryptosporidium* sp.; details of this method are given elsewhere [13]. Samples that tested positive were re-tested, once with a *Giardia*-specific ELISA test and once with a *Cryptosporidium*-specific ELISA test according to the manufacturer's instructions, to determine whether the stool sample tested positive for *Giardia*, *Cryptosporidium*, or both protozoa.

Statistical analysis

Selected sociodemographic and health characteristics of the children, parents and households are presented as a percentage for categorical variables and as a mean and SD for continuous variables. The mean (SD) of anthropometric variables for the children are summarized by sex.

Differences in age, anthropometric indices, sex, mother's education, socio-economic status, parasite control and use of vitamin A supplements between the participants who provided stool samples and those who did not were compared using a Student's t-test for the continuous variables and Fisher's exact test for the categorical variables.

Prevalence (95% confidence interval – 95%CI) was calculated using Poisson distribution for frequencies of < 10 for helminthic and protozoan intestinal parasites based on the results of the microscopic examination and the ELISA method used to detect *G. duodenalis* and *Cryptosporidium* sp. antigen. The daycare centers were divided into three groups: city centre, periurban *favela* and periurban gated, to analyze the association between prevalence of infection with helminths, selected protozoan intestinal parasites and non-pathogenic cysts with deworming treatment and use of vitamin A supplements using Fisher's exact test. Multiple linear regression analysis was used to examine the independent predictors of WAZ, WHZ, HAZ and BMIZ. Logistic regression models were used to examine the relationship between infection with helminths and *G. duodenalis* and the following factors: age 3.00 to 3.99 years and 4.00 to 5.65 years; sex, socioeconomic status (extremely low and low), deworming treatment and use of vitamin A supplements. The sandwich esti-

mator was used to obtain robust standard errors to account for the sampling procedure. Statistical analysis was carried out using Stata version 11 (Stata Corp., College Station, USA).

Results

Sociodemographic and health status

The overall response rate from the daycare centers was 86% (376/438) comprising 196 males and 180 females aged 3.00 to 5.65 years. The mean (SD) age of the children was 4.2 years (0.61) and 66.2% (249/376) of the sample were first or second-born children. Six percent of the participants were white, 42.2% were black, and 51.8% were mixed race. Fourteen percent (32/227) of children with known birth weight weighed less than 2,500g at birth.

Household sociodemographic characteristics are shown in Table 1. Forty-seven percent of fathers and 56% of mothers had primary school education, but only 3% of parents had concluded education beyond high school level. Mothers with low levels of education (no education or to primary school level) had significantly more children (three or more) ($p < 0.001$). Almost 50% of fathers were regularly employed, whereas one-third of mothers were unemployed, with another third working as casual wage earners. Nearly 40% of mothers/guardians were single or divorced, 16% did not know paternal education level and 12% did not know the father's occupation. Overall, 60.5% of households received a monthly income of one minimum monthly salary ($415 Brazilian Reais) or less. The following means (SD) were observed for the different variables in mothers: age 29.7 (7.0) years; height 159.1cm (6.7); weight 66.4kg (15.4); and BMI 26.2kg/m^2 (5.7). Fourteen percent of mothers/guardians had two or fewer meals per day according to the self reports.

The health characteristics of participants are shown in Table 2. Over half of the children in the sample (54.3%) had received dietary supplements at some time, 19% had received iron syrup in the previous six months and 53% had taken vitamin A capsules at some time in their life. The use of dietary supplements was greatest in children attending the city centre daycare center ($p = 0.008$); however the use of vitamin A supplements was most common among children attending the five periurban *favela* daycare centers ($p < 0.001$).

Most children (83.8%) received routine childhood vaccinations, and coverage was found to be highest ($p < 0.001$) among children attending the five periurban *favela* daycare centers. Fifty-one percent of children received parasite control (deworming) within the six months prior to the questionnaire, with treatment being more frequent among children enrolled at the periurban gated daycare center ($p = 0.004$). Fifty percent of children had been hospitalized at least once, and 25.7% had been hospitalized three times or more. Upper respiratory infections accounted for almost half (49.7%) of hospitalizations, whereas hospitalization due to diarrheal episodes was much less frequent (8.6%). The presence of a regular adult smoker was reported in almost a

Table 1

Household sociodemographic characteristics (n = 376).

	n	%
Father's education		
No schooling	15	4.0
Primary school	178	47.4
High school	111	29.5
> High school	11	2.9
Unknown	61	16.2
Mother's education		
No schooling	13	3.5
Primary school	212	56.4
High school	144	38.3
> High school	7	1.8
Father's occupation		
Regular wage earner	170	45.2
Casual wage earner	125	33.2
Business or trade owner	11	2.9
Unemployed	24	6.4
Unknown	46	12.2
Mother's occupation		
Regular wage earner	81	21.5
Casual wage earner	129	34.4
Business or trade owner	5	1.3
Domestic worker	35	9.3
Unemployed	126	33.5
Total income (≤ R$415)	227	60.5
Mother's marital status		
Married/Partner	228	60.6
Divorced	13	3.5
Single	135	35.9
	n	**Mean (SD)**
Maternal age (years)	368	29.7 (7.0)
Maternal height (cm)	312	159.1 (6.7)
Maternal weight (kg)	324	66.4 15.4)
Maternal BMI	312	26.2 (5.7)

BMI: body mass index.

Table 2

Health characteristics of preschoolers (n = 376).

	n	%
Dietary supplements	204	54.3
Vitamin A	200	53.2
Iron syrup in the previous 6 months	70	18.6
Vaccinations	315	83.8
BCG	281	74.7
Polio	291	77.4
DPT (Diphtheria, Pertussis, Tetanus)	288	76.8
Measles	272	72.3
Rotavirus	197	52.4
Pneumococcus	7	1.9
Influenza A (H1N1)	133	35.4
Deworming treatment	192	51.1
Hospitalizations	187	49.7
Once to twice	139/187	74.3
Three times or more	48/187	25.7
Reasons for hospitalizations		
Diarrhea	16/187	8.6
Upper respiratory infection	93/187	49.7
Other reasons	78/187	41.7
Smoking		
Mother smoking in the house	47	12.5
Adult smoking in the house	89	23.7
History of asthma in mother or sibling	68	18.1

quarter of households (23.7%), and 12.5% of mothers smoked at home.

Anthropometry

Table 3 presents the mean (SD) anthropometric z-scores for the children and differences by sex. Boys had significantly lower z-scores for height-for-age, weight-for-age, weight-for-height, and BMI than girls. No significant differences were observed by age group.

Although 5.5% (20/364) of children had a HAZ below -1 > -2SD, very few children (1.9%; 7/364) were stunted (HAZ < -2SD). Less than 2% (7/364) of the children were underweight (i.e., WAZ < -2SD), but 12.1% (44/364) were moderately underweight (i.e., WAZ < -1 ≥ -2SD). Although few children (5.2%; 19/363) were wasted (WHZ < -2SD), 15.7% (57/363) were moderately wasted (i.e, WHZ < -1 ≥ -2SD). Risk of overweight in children was 10.7% (39/364), based on a BMIZ > 1 ≤ 2SD; 2.5% (9/364) of children were overweight (i.e., BMIZ > 2SD).

Prevalence of parasitic infections

Of the 376 participants, 325 (86%) provided stool samples. No significant differences in age, anthropometric indices, sex, mother's education, socio-economic status, and de-worming treatment were found between participants providing a stool sample and those participants who did not (n = 51), with the exception of the use of vitamin A supplements. Prior use of vitamin A supplements in children who provided a stool sample was greater than in those who did not (p = 0.016).

The prevalence of helminthic and protozoan intestinal parasites (*Giardia* sp. and *Cryptosporidium* sp. antigens) is presented in Table 4. Almost 30% (95/325) of participants were infected with at least one intestinal parasite and 25% (80/325) had non-pathogenic protozoan cysts. Two or more parasites were present in 9.2% (95%CI: 6.3; 12.9) of children and 2.5% (95%CI: 1.1; 4.9) of children were infected with three or more parasites. No *Schistosoma* species were found in the stool samples.

Table 3

Mean (SD) growth measurements and z-scores by sex in children.

Variable	n	Females	n	Males	Difference (95%CI)	p-value
Birth weight (g)	104	3,055.5 (573.4)	123	3,215.4 (633.0)	159.89 (-38.81; 358.59)	0.099
Birth length (cm)	88	47.9 (3.6)	98	49.4 (2.5)	1.47 (-0.17; 3.12)	0.071
WAZ	171	0.21 (0.99)	193	0.07 (1.05)	-0.15 (-0.28; -0.02)	0.034
WHZ	170	-0.17 (1.01)	193	-0.29 (1.06)	-0.12 (-0.23; -0.001)	0.047
HAZ	171	0.60 (1.03)	193	0.37 (1.08)	-0.23 (-0.42; -0.05)	0.020
BMI	171	-0.11 (1.12)	193	-0.33 (1.17)	-0.23 (-0.37; -0.08)	0.007

BMI: body mass index; HAZ: height-for-age; SD: standard deviation; WAZ: weight-for-age; WHZ: weight-for-height; 95%CI: 95% confidence interval.

Table 4

Prevalence (% and 95%CI) of intestinal parasitic infection determined using microscopy and ELISA assay * (n = 325).

Variable	n	% (95%CI)
Presence of parasites	95	29.2 (24.3; 34.5)
Presence of ≥ 2 types of parasite	30	9.2 (6.3; 12.9)
Presence of ≥ 3 types of parasite	8	2.5 (1.1; 4.9)
Helminths	58	17.8 (13.8; 22.5)
Trichuris trichiura	39	12.0 (8.7; 16.0)
Ascaris lumbricoides	34	10.5 (7.4; 14.3)
Hookworm	3	0.9 (0.2; 2.7)
Giardia duodenalis	42	12.9 (9.5; 17.1)
Entamoeba histolytica/dispar	12	3.7 (1.9; 6.4)
Cryptosporidium sp.	1	0.3 (0.01; 1.7)
Hymenolepsis nana	1	0.3 (0.01; 1.7)
Strongyloides stercoralis	1	0.3 (0.01; 1.7)
Presence of non-pathogenic cysts **	80	24.6 (20.0; 29.7)

95%CI: 95% confidence interval.

* ELISA assay for *Giardia duodenalis* and *Cryptosporidium* sp.

** Does not include *Entamoeba dispar*.

The most common helminth species found were *T. trichiura* and *A. lumbricoides*. Prevalence of hookworm infestation was very low (0.9%; 95%CI: 0.2; 2.7). Prevalence of *T. trichiura* was highest in boys (p = 0.016). Almost half of *T. trichiura*-infected children (49%; 19/39) had a light egg burden, 36% (14/39) had a moderate egg load and 15% (6/39) had a heavy egg burden. The light, moderate and heavy egg burdens of children infected with *A. lumbricoides* was 50% (17/34), 26% (9/34) and 24% (8/34), respectively (results not shown). Fifteen children (5%) were infected with both *T. trichiura* and *A. lumbricoides*. Hookworm infestation occurred in conjunction with either *T. trichiura* (n = 2) or *A. lumbricoides* (n = 1).

Infection with *G. duodenalis* was found in 12.9% (95%CI: 9.5; 17.1) of stool samples. Fourteen children (4%) had both *G. duodenalis* and helminths, with the presence of either *T. trichiura* (n = 8), *A. lumbricoides* (n = 3), or all three parasites simultaneously (n = 3) (results not shown). *E. histolytica/dispar* cysts were found in 3.7% (95%CI: 1.9; 6.4) of the stool samples, of which approximately half (n = 5) were in conjunction with one or more helminths and/or *G. duodenalis*.

Frequency (%) of parasite infections, deworming treatment and use of vitamin A supplements by daycare group is shown in Table 5. Significant differences existed among the three daycare groups regarding frequency of infection with an intestinal parasite, specifically helminths and non-pathogenic cysts, and deworming and use of vitamin A supplements. In general,

Table 5

Frequency (%) of intestinal parasitic infection, deworming treatment and vitamin A supplementation by day care group (n = 325).

Variable	City centre (n = 60)	Periurban favela (n = 185)	Periurban gated (n = 80)	p-value
Presence of parasites	10 (16.7)	68 (36.8)	17 (21.3)	0.002
Helminth *	4 (6.7)	43 (23.2)	11 (13.8)	0.007
Giardia duodenalis	7 (11.7)	29 (15.7)	6 (7.5)	0.182
Entamoeba histolytica	0 (0.0)	10 (5.4)	2 (2.5)	0.162
Non-pathogenic protozoa cysts	6 (10.0)	56 (30.3)	18 (22.5)	0.004
Deworming treatment within 6 months	26 (43.3)	91 (49.2)	52 (65.0)	0.020
Vitamin A supplementation: whether ever used	35 (58.3)	121 (65.4)	25 (31.3)	< 0.001

* Helminth includes: *Trichuris trichiura*, *Ascaris lumbricoides* and hookworm.

prevalence of intestinal parasitic infections was greatest among children attending the periurban *favela* daycare center. Deworming treatment was more frequent among children attending the periurban gated daycare (65%), although only about a third of these children had taken vitamin A supplements.

Factors influencing poor growth and intestinal parasitic infection in preschoolers

Significant predictors of anthropometric z-scores identified by regression analysis differed by variable (Table 6). Helminth infection showed a significant inverse association with WAZ (p = 0.018) and HAZ (p = 0.003) using univariate analysis, but this association lost significance in multivariate analysis and was thus excluded. Infection with *G. duodenalis* was also excluded from the regression analysis because no significant negative association with growth indicators was found. A significant association was found between mother's weight, age and birth order and WAZ, between socio-economic status and WHZ and BMIZ, and between sex and HAZ. The association between socioeconomic status and WAZ and HAZ also showed a tendency to be significant. With regard to HAZ, sex and mother's height were significant predictors and socio-economic status and birth order tended to be significant. The logistic regression analysis (Table 7) showed that being male and from a family with extremely low socioeconomic status were significant risk factors for infection with helminths. Deworming was highly effective against helminth infection and the use of vitamin A supplements showed a modest inverse association with *G. duodenalis* infection.

Table 6

Multiple linear regression analysis using growth indices as dependent variables.

	β coefficient	95%CI	p-value
WAZ (n = 313)			
Sex (boys)	-0.11	-0.25; 0.03	0.100
Age (years)	-0.21	-0.40; -0.01	0.039
SES (extremely poor)	-0.22	-0.45; 0.02	0.069
Maternal weight (kg)	0.02	0.02; 0.03	< 0.001
Birth order (more than 2)	-0.30	-0.54; -0.07	0.019
WHZ (n = 312)			
Sex (boys)	-0.08	-0.21; 0.05	0.184
Age (years)	-0.15	-0.38; 0.09	0.182
SES (extremely low)	-0.31	-0.57; -0.05	0.028
Maternal weight (kg)	0.02	0.01; 0.03	0.002
Birth order (more than 2)	-0.09	-0.30; 0.13	0.359
HAZ (n = 302)			
Sex (boys)	-0.26	-0.35; -0.18	< 0.001
Age (years)	-0.08	-0.36;0.20	0.518
SES (extremely low)	-0.19	-0.40; 0.01	0.062
Maternal height (cm)	0.06	0.04; 0.08	< 0.001
Birth order (more than 2)	-0.26	-0.53; 0.01	0.055
BMI (n = 313)			
Sex (boys)	-0.18	-0.32; -0.05	0.015
Age (years)	-0.08	-0.34; 0.19	0.519
SES (extremely low)	-0.35	-0.64; -0.06	0.023
Maternal weight (kg)	0.02	0.01; 0.03	0.002
Birth order (more than 2)	-0.07	-0.29; 0.16	0.515

BMI: body mass index; HAZ: height-for-age; SES: socioeconomic status; WAZ: weight-for-age; WHZ: weight-for-height; 95%CI: 95% confidence interval.

Table 7

Logistic regression models of risk factors (by odds ratio and 95%CI) for infection with helminths (n = 58) and *Giardia duodenalis* (n = 42).

Risk factors	Helminths (n = 58)	p-value	*G. duodenalis* (n = 42)	p-value
Age group (older group)	1.09 (0.59; 2.03)	0.776	0.92 (0.37; 2.33)	0.866
Sex (male)	2.34 (1.14; 4.82)	0.021	1.15 (0.67; 1.97)	0.620
SES (extremely low)	2.04 (1.33, 3.13)	0.001	2.02 (0.87; 4.69)	0.101
Deworming treatment	0.60 (0.40; 0.91)	0.015	1.13 (0.51; 2.52)	0.764
Vitamin A supplementation	0.67 (0.29; 1.56)	0.355	0.46 (0.20; 1.03)	0.061

SES: socioeconomic status; 95%CI: 95% confidence internal.

Discussion

Our study highlights that there have been a number of improvements in the growth status of children from families with low socioeconomic status in Brazil over recent years. Prevalence rates for stunting, underweight and wasting among disadvantaged preschoolers were very low (≤ 5%) when compared to earlier studies carried out in Brazil (~16%) [8,14]. Prevalence of mild-to-moderate under nutrition was also low (~15%), and is similar to rates for toddlers from daycare centers in São Paulo in the Southeastern Region of Brazil, where families have a higher socioeconomic status [15]. Such improvements have been attributed to the expansion of healthcare and pro-poor social programs, together with increases in purchasing power and levels of maternal education among poor families [16,17]. Nevertheless, ~13% of the disadvantaged preschoolers that made up our sample were at risk of overweight or classified as overweight, emphasizing the importance of including nutrition education as a component of pro-poor social policies.

Despite improvements, poverty remains a major influencing factor affecting the growth status of these preschoolers. Anthropometric indices were lower in children living in households classified as extremely poor, indicating greater impairment of somatic and linear growth in these children than in their counterparts from better off families (Table 6). Such a marked affect was unexpected given that the weekly menus were the same for all children in the daycares studied. It is possible, however, that children from extremely impoverished households experience greater food quantity and/or quality deficits on weekends and during vacations compared to preschoolers from families with a higher socioeconomic status. These children's mothers reported eating fewer daily meals, had lower mean body weight and BMI ($p < 0.05$) and had more children to feed than their counterparts from families with a higher socioeconomic status. Moreover, both maternal weight and height had a significant impact on anthropometric indices ($p < 0.01$). These findings emphasize the importance of continuing poverty reduction efforts to improve the standard of living of families with extremely low socioeconomic status.

Standard of living was also a significant risk factor for certain parasitic infections in these children, particularly helminths. Several other studies have documented a positive association between risk of helminth infection and extremely low socioeconomic status [18]. Of the helminths identified, *A. lumbricoides* and *T. trichiura* were the most prevalent; infection with hookworm was negligible (< 1%). Infection intensity for these two helminths – *A. lumbricoides* and *T. trichiura* – was moderate to heavy in 50% of the infected children contributing to an increased risk of morbidity [19]. No significant association was found between age group and infection with helminths and other intestinal parasites, probably due to the narrow age range of the sample. The age range of the children and the setting may also explain the low prevalence of infection with hookworm and schistosomes as young children attending daycare centers in urban areas are less likely to be exposed to natural bodies of water than older children living in rural settings [20].

The direction and strength of the association between infection with *G. duodenalis* and standard of living was consistent with an earlier study in Salvador [21]; however, in contrast to helminths, no significant positive association was observed between infection with *G. duodenalis* and standard of living. Almost 13% of preschoolers were infected with these waterborne protozoan parasites which often cause infectious diarrhea and gastroenteritis, in contrast to other studies

which have reported much higher prevalence rates (61%) among children in public daycare centers in the State of São Paulo [23]. Although infection with this parasite is frequently acquired through drinking contaminated water [22], person-to-person transmission of *G. duodenalis* is common in daycare settings. Low income levels and a crowded living environment are probable risk factors influencing polyparasitism and the adverse health effects associated with infection with more than one parasite [24,25]. Of the preschoolers infected with more than one parasite (13%), almost two-thirds were from families with extremely low socioeconomic status.

After adjusting for sociodemographic factors and maternal weight or height, this study found that intestinal parasitic infections had no effect on growth (Table 6). These results differ from those of a previous study carried out in Salvador [26] as we used the growth index as the dependent variable rather than parasite type. We believe that this approach is more consistent with biological processes and other variables associated with growth were adjusted accordingly. We also observed a negative trend between *G. duodenalis* infection and growth which is consistent with the findings of Matos et al. [26]. However, this trend was not statistically significant, perhaps because of the small sample size. It is interesting to note that sex was a predictor of certain growth indices with boys having lower BMIZ and HAZ than girls, irrespective of standard of living. Several reasons may account for these differences. Boys are known to have lower total body fat levels and faster growth rates during early childhood than girls [10], and thus have higher energy and nutrient requirements [27,28]; hence, it is possible that deficits in intakes of energy and growth-limiting nutrients may have been greater in boys, resulting in impaired linear growth [29]. Additionally, risk of helminth infection was twice as high in boys (Table 7), which may be because boys are more likely to play outside barefoot than girls.

Other studies regarding intestinal parasitic infections in children attending public daycare centers in Brazil [30] have focused on the transmission of *Cryptosporidium* sp. since, like *G. duodenalis* and *E. histolytica*, this parasite is associated with diarrhoeal illness in preschool children. Although almost five percent of children had *E. histolytica/dispar* cysts (*E. histolytica* is the principle cause of amoebiasis) [12], only one stool sample tested positive for *Cryptosporidium* sp. Non-pathogenic protozoan cysts were found in a quarter of stools, which is indicative of fecal-oral transmission and the possible presence of pathogenic protozoan species not detectable by concentrate microscopy, for example an *E. histolytica* infection present in low numbers of cysts or high numbers of trophozoites [31].

The association between protection from helminth infection and deworming treatment reported here is not unexpected (Table 7), emphasizing the importance of providing this inexpensive treatment to preschoolers at appropriate intervals [19,32]. Both Albendazole and Mebendazole can be used and are reportedly effective in the treatment of *A. lumbricoides*, but are less efficacious against *T. trichiura*. Although a larger proportion of children in the periurban gated daycare center had received deworming treatment within the six months prior to the study (Table 2), the rate of helminth infection in this group was not the lowest. This is probably because *T. trichiura* was the predominant parasite in these children and not *A. lumbricoides*. Prevalence of parasitic infections and non-pathogenic cysts was greatest in the periurban *favela* daycare group, probably due to the close proximity to unpaved roads, use of contaminated water sources and poor drainage and sewage connections [33].

Unlike deworming treatment, the use of vitamin A supplements tended to provide a modest protection against *G. duodenalis* infection (Table 7). This is not the first study to report an inverse association between the use of vitamin A supplementation and *G. duodenalis* infection in children living in poor areas in the Northeast Region of Brazil. Lima et al. [34] also found that the rate of *G. duodenalis* infection was lower in children who had received vitamin A supplements. Associations between infection with *Giardia* and vitamin A deficiency based on low serum retinol concentrations in children have been reported in Mexico [35]. Vitamin A plays a critical role in both immune function and intestinal epithelial integrity [36] and hence may stimulate immune responses against *G. duodenalis*.

Our study has strengths and limitations. We used the fecal concentration test which has high reproducibility (i.e., > 95%) and is more sensitive than the commonly used Kato-Katz method [3]. Constraints imposed by the available laboratory facilities meant that it was not possible to use both the sedimentation and flotation procedures and therefore the sedimentation method for parasite examination was chosen based on standard recommendations [12]. Furthermore, a sensitive and specific ELISA antigen detection test was used in addition to microcopy examination to confirm the presence of *G. duodenalis* and *Cryptosporidium* sp. [13]. However, as it was possible to collect only one stool specimen per child, the results provide only a semi-quantitative assessment of the intensity of helminth infections.

Our study sample was restricted to philanthropically-funded preschool daycare centers, thus limiting the extrapolation of results to all preschool children attending daycare centers in urban and periurban slums in Salvador. Nevertheless, the mean BMI of the children in this sample (15.4) was comparable to that of preschool children (3.0 to 5.99 years of age) living in the State of Bahia (15.3) reported in the most recent *Brazilian National Nutrition Survey* [37]. Furthermore, given the observational nature of our study, it is not possible to establish a causal association between growth indicators and intestinal parasitic infections. Finally, future studies should collect information on the presence of domestic animals in and around the home as this is also a risk factor for transmission of *G. duodenalis* [21].

In conclusion, almost 20% of our preschoolers were classified as undernourished or moderately undernourished and nearly one-third presented with an intestinal parasitic infection, notably *A. lumbricoides*, *T. trichiura* and/or *G. duodenalis*. Certain variables were more pronounced in boys than in girls. Moreover, risk of morbidity was likely to be high in approximately 50% of infected children due to the level of intensity of infection with *A. lumbricoides* and *T. trichiura*. Deworming treatment and increased vitamin A supplementation coverage in children from this age group are two simple and cost-effective strategies for reducing the rate of infection with helminths and *G. duodenalis* and thus decrease the risk of polyparasitism among these daycare preschoolers. Our findings also emphasize that efforts to reduce urban poverty and provide access to high quality daycare centers for families with low socio-economic status must be continued to ensure the strong growth and healthy development of these children.

Resumo

Déficit de crescimento e parasitoses são comuns entre crianças residentes em periferias. Em estudo transversal com 376 pré-escolares (3-6 anos) de creches em Salvador, Nordeste do Brasil, avaliamos fatores predisponentes para déficit de crescimento e parasitose. Obtiveram-se dados em sete creches sobre peso da criança, altura, nível socioeconômico, estado de saúde e parasitos em amostras de fezes. Prevalência de baixo peso (-1 < DP > -2), desnutrição e baixa estatura foram 12%, 16%, e 6%, respectivamente; nível socioeconômico, ordem de nascimento e peso materno foram preditores da antropometria. Aproximadamente 30% estavam infectados com ≥ 1 parasita. Helmintos (17.8%), notavelmente Trichuris trichiura *(12%) e* Ascaris lumbricoides *(10.5%) e protozoário* Giardia duodenalis *(13%) foram os mais comuns; < 1% tinha ancilostomíase e* Cryptosporidium *sp.; 25% apresentaram protozoários cistos não patogênicos. Meninos de famílias muito pobres tiveram menor crescimento e maior risco de helmintose. A desparasitação pode ser considerada uma alternativa para a redução da prevalência de parasitoses intestinais nesse grupo etário.*

Pré-Escolar; Creches; Parasitos; Enteropatias Parasitárias

Contributors

R. L. Lander designed the research project, developed the overall research plan, conducted the research, analyzed the data, wrote the draft manuscript and approved the final manuscript. A. G. Lander assisted with data collection, carried out and interpreted the microscopic assessment of fecal samples, critically revised the manuscript and approved the final version of this paper. L. Houghton contributed to data interpretation, critically revised the manuscript and approved the final version of this paper. S. M. Williams supervised the statistical analysis and data interpretation, critically revised the manuscript and approved the final version of this paper. H. Costa-Ribeiro oversaw the study, critically revised the manuscript and approved the final version of this paper. D. L. Barreto and A. P. Mattos conducted the research, critically revised the manuscript and approved the final version of this paper. R. S. Gibson participated in the design of the research project and development of the overall research plan, assisted in writing the manuscript and approved the final manuscript.

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References

1. Sclar ED, Garau P, Carolini G. The 21st century health challenge of slums and cities. Lancet 2005; 365:901-3.
2. Utzinger J, Keiser J. Urbanization and tropical health: then and now. Ann Trop Med Parasitol 2006; 100:517-33.
3. Harhay MO, Horton M, Olliaro PL. Epidemiology and control of human gastrointestinal parasites in children. Expert Rev Anti Infect Ther 2010; 8: 219-34.
4. Thompson SC. Giardia lamblia in children and the child care setting: a review of the literature. J Paediatr Child Health 1994; 30:202-9.
5. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. Parasitology 2000; 121 Suppl:S23-38.
6. Koski KG, Scott ME. Gastrointestinal nematodes, nutrition and immunity: breaking the negative spiral. Annu Rev Nutr 2001; 21:297-321.
7. Centro de Mídia Independente. Bairro da Paz 25 anos de resistência, 2007. http://www.midiaindependente.org/pt/blue/2007/04/380475.shtml (accessed on 23/Feb/2011).
8. Issler RMS, Giugliani ERJ, Kreutz GT, Meneses CF, Justo EB, Kreutz VM, et al. Poverty levels and children's health status: study of risk factors in an urban population of low socioeconomic level. Rev Saúde Pública 1996; 30:506-11.
9. World Health Organization. Anthropometry training video: the WHO multicentre growth reference study [DVD-ROM]. Geneva: World Health Organization; 2004.

10. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Adv Data 2000; (314):1-27.
11. World Health Organization. Training course on child growth assessment, WHO child growth standards, module c: interpreting growth indicators. Geneva: World Health Organization; 2008.
12. Garcia LS. Practical guide to diagnostic parasitology. 2nd Ed. Washington DC: American Society of Microbiology Press; 2009.
13. Garcia LS, Shimizu RY. Evaluation of nine immunoassay kits (Enzyme Immunoassay and Direct Fluoresence) for detection of *Giardia* lamblia and *Cryptosporidium parvum* in human fecal specimens. J Clin Microbiol 1997; 35:1526-9.
14. Cesar JA, Mendoza-Sassi R, Horta BL, Ribeiro PR, D'Avila AC, Santos FN, et al. Basic indicators of child health in an urban area in southern Brazil: estimating prevalence rates and evaluating differentials. J Pediatr (Rio J.) 2006; 82:437-44.
15. Novaes Oliveira M, Martorell R, Phuong N. Risk factors associated with hemoglobin levels and nutrition status among Brazilian children attending daycare centers in São Paulo city, Brazil. Arch Latinoam Nutr 2010; 60:23-9.
16. Monteiro CA, Benicio MH, Konno SC, Silva AC, Lima AL, Conde WL. Causes of the decline in child under-nutrition in Brazil, 1996-2007. Rev Saúde Pública 2009; 43:1-8.
17. Barros FC, Matijasevich A, Requejo JH, Giugliani E, Maranhao AG, Monteiro CA, et al. Recent trends in maternal, newborn and child health in Brazil: progress toward Millennium Development Goals 4 and 5. Am J Public Health 2010; 100:1877-89.
18. Bethony J, Brooker S, Alonico M, Geiger SM, Loukas A, Diemert D, et al. Soil-transmitted helminth infections: acariasis, trichuriasis and hookworm. Lancet 2006; 367:1521-32.
19. Cromption DWT, Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr 2002; 22:35-59.
20. Hotez PJ. Forgotten people, forgotten diseases: the neglected tropical diseases and their impact on global health and development. Washington DC: American Society of Microbiology Press; 2008.
21. Prado MS, Strina A, Barreto ML, Oliveira-Assis AM, Paz LM, Cairncross S. Risk factors for infection with *Giardia duodenalis* in pre-school children in the city of Salvador, Brazil. Epidemiol Infect 2003; 131:899-906.
22. Lane S, Lloyd D. Current trends in research into the waterborne parasite *Giardia*. Crit Rev Microbiol 2002; 28:123-47.
23. Machado RC, Marcari EL, Cristante S, Carareto CMA. Giardiasis and helminthiasis in children of both public and private day-care centers and junior and high schools in the city of Mirassol, São Paulo State, Brazil. Rev Soc Bras Med Trop 1999; 32:697-704.
24. Saldiva SR, Silveira AS, Philippi ST, Torres DM, Mangini AC, Dias RM, et al. *Ascaris-Trichuris* association and malnutrition in Brazilian children. Paediatr Perinat Epidemiol 1999; 13:89-98.
25. Sawaya AL, Amigo H, Sigulem D. The risk approach in preschool children suffering malnutrition and intestinal parasitic infection in the city of São Paulo, Brazil. J Trop Pediatr 1990; 36:184-8.
26. Matos SMA, Assis AMO, Prado MS, Strina A, Santos LA, Jesus SR, et al. *Giardia duodenalis* infection and anthropometric status in preschoolers in Salvador, Bahia State, Brazil. Cad Saúde Pública 2008; 24:1527-35.
27. Butte NF, Wong WW, Hopkinson JM, Heinz CJ, Mea NR, Smith EOB. Energy requirements derived from total energy expenditure and energy deposition during the first two years of life. Am J Clin Nutr 2000; 72:1558-69.
28. World Health Organization/Food and Agriculture Organization. Vitamin and mineral requirements in human nutrition. 2nd Ed. Geneva: World Health Organization; 2004.
29. Ruz M, Castillo-Duran C, Lara X, Codoceo J, Rebolledo A, Atalah E. A 14-mo zinc-supplementation trial in apparently healthy Chilean preschool children. Am J Clin Nutr 1997; 66:1406-13.
30. Nascimento WRC, Calvalcanti IMF, Irmão JI, Rocha FJS. Presença de *Cryptosporidium* spp. em crianças com diarréia aguda em uma creche pública de Recife, Estado de Pernambuco. Rev Soc Bras Med Trop 2009; 42:175-8.
31. World Health Organization. Training manual on diagnosis of intestinal parasites, tutor's guide [CD-ROM]. Geneva: World Health Organization; 2004.
32. World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Geneva: World Health Organization; 2002. (WHO Technical Report Series, 912).
33. Moraes LR, Cancio JA, Cairncross SS. Impact of drainage and sewerage on intestinal nematode infections in poor urban areas in Salvador, Brazil. Trans R Soc Trop Med Hyg 2004; 98:197-204.
34. Lima AA, Soares AM, Lima NL, Mota RM, Maciel BL, Lvalsund MP, et al. Effects of vitamin A supplementation on intestinal barrier function, growth, total parasitic, and specific *Giardia* spp. infections in Brazilian children: a prospective randomized, double-blind, placebo-controlled trial. J Pediatr Gastroenterol Nutr 2010; 50:309-15.
35. Quihui-Cota L, Astiazaran-Garcia H, Valencia ME, Morales-Figueroa GG, Lopez-Mata MA, Ortiz FV. Impact of *Giardia intestinalis* on vitamin A status in schoolchildren from northwest Mexico. Int J Vit Nutr Res 2008; 78:51-6.
36. Semba RD. Vitamin A, immunity, and infection. Clin Infect Dis 1994; 19:489-99.
37. Instituto Brasileiro de Geografia e Estatística. Pesquisa de Orçamentos Familiares 2008-2009: antropométrica e estado nutricional de crianças, adolescentes e adultos no Brasil. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2010.

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