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Are intestinal parasitic infections associations with obesity in Mexican children and adolescents ?

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Running title: Parasitic infection and obesity

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1. INTRODUCTION

Obesity and associated morbidities are rapidly growing public health challenges that many low and middle income countries are facing [1, 2]. Often in these countries, infections are common as well, especially in children [3, 4]. Recent studies have shown that certain viral and bacterial infections are associated with obesity (described as “infectobesity”) [5-8]. However, studies on the association between parasite infections and obesity are still scarce [9-11].

We hypothesize that intestinal parasites may be associated with obesity by two plausible mechanisms. The first possibility is that both obesity and parasitic infections are positively associated with poverty, as reported previously in different low and middle income countries [12, 13]. However, a second alternative may be that intestinal parasites have an effect on the metabolism by the alteration of the gut microbiome composition.

It is assumed that “stress factors” or “insults” such as infectious diseases and under-nutrition presented during a “critical window” of development (i.e. childhood or puberty) can lead to changes in the gut microbiome composition [14-16]. These changes may be reflected as alterations in metabolism and may lead to fat deposition over time [17-20]. For instance, modified gut microbiota can increase caloric uptake from the diet and can modulate host genes that affect energy deposition in adipocytes and thereby increase the risk of diet-induced obesity [19].

According to the last health and nutrition survey of Mexico (ENSANUT 2012) Mexico has a combined prevalence of overweight and obesity of approximately 70% in adults and 30% in children [21, 22]. Approximately 50% of the total population is infected with one or more species of intestinal parasites [23, 24] with *A. lumbricoides* being the most common intestinal helminth infection, and *E. coli* the most prevalent intestinal protozoan infection [11, 25]. In a previous study, we found intestinal protozoa infection, particularly *Entamoeba coli* (*E. coli*) infection was associated with a higher percentage of body fat and food intake, while *Ascaris lumbricoides* (*A. lumbricoides*) infection was associated with a lower food intake in children [11]. The objective of this ecological study is to test if children living in states with high (reported) incidence of intestinal parasitic infection have higher BMIz and higher BMIz later in life.

2. Materials and methods

2.1 Datasets:

We used individual and state-wide data (32 states) collected by three different federal organizations of Mexico. Individual level data on height, weight and age was obtained from the 2012 National Health and Nutrition Survey (ENSANUT 2012). This survey of over 50,000 households is representative for the Mexican population at national and state level. The data is available at http://ensanut.insp.mx and the methodology is described elsewhere [26]. Statewide data on intestinal helminths and protozoa in 2012, 2006 and 2000 was obtained from the National System for Epidemiological Surveillance (SINAVE) [27]. The data is available at: http://www.epidemiologia.salud.gob.mx/anuario/html/anuarios.html Fina lly we used state-wide data on demographic and socioeconomic variables from the National Institute of Statistics and Geography (INEGI) 2012 report, available at http://www.inegi.org.mx/ [28].

2.2 Dependent Variable: BMI for age z-score

Using data from ENSANUT 2012, we calculated the BMI for age z-score (BMIz) for all individuals. BMIz for individuals above 19 years was calculated using the last available point of the WHO grow charts for children (228 months). The calculations were made using the World Health Organization (WHO) SPSS anthroplus macro for children 5-19 years (WHO, Geneva, Switzerland). The macro is publicly available at http://www.who.int/childgrowth/software/en/.

2.3 Independent variables: infection with intestinal parasites

Information on intestinal helminths and protozoa was extracted from SINAVE, which is the only publicly available data source on intestinal parasite infection across the county. In this system, data is reported annually as the incidence per 100 000 person-years in different age groups (i.e. less than 1y, 1-5y, 6-10y, 11-19y) following the same procedures in each of the 32 states of Mexico [27]. *A. lumbricoides* infection, the most common helminth infection in Mexico, is reported as the incidence of *A. lumbricoides* infection. For protozoa infection the only available data was on "intestinal protozoa infection": that is calculated by SINAVE as the grouped incidence of *Balantidium coli, Cryptosporidium and unspecified intestinal protozoa infection.*

We used the SINAVE incidence data as a proxy for the probability of infection, in function of the state and the age of the subject at a particular time point. For instance, the probability of infection in 2012 of an individual of 15 years old was approximated by the incidence of the intestinal parasite infection in his/her state of residence in 2012 for his/her corresponding age group. In addition, the same individual's probability of infection in 2006 and in 2000, was determined by the incidence of each intestinal parasitic infection in his/her state and for his/her age group at that given year.

2.4 Additional covariates

We included both individual-level covariates from the ENSANUT 2012 survey and state-level covariates from the INEGI survey in 2012. The individual-level covariates used in the analysis were: sex (male/female), age (years), place of residence (rural/urban) and "marginality" (marginalized/not-marginalized). Marginality indicates whether a child is from a marginalized socioeconomic status or not based on indicators such as parents education level, access to sanitation facilities, access to drinking water, income and population size of the community, as described in detail elsewhere [26]. State-level covariates were: population with health coverage (%), education level of adults (mean number of years in school), households without sanitation facilities (%) and the poverty rate (population living in poverty). The poverty rate was determined by the "poverty index", which indicates whether a household is poor or not. In addition to income, the poverty index also takes into consideration access to healthcare, social security, material of the roof, floor and walls of the household, access to basic services and food, according to standard procedures which are described elsewhere [29].

2.5 Statistical analysis

As shown in figure 1 the population was stratified into three age groups depending on the age of individuals in 2000, 2006 and 2012. For the cross sectional analysis on the association between infection and obesity in 2012 we extracted data on individuals aged 1-5y (n=8,927), 6-10y (n=16,347) and 11-19y (n=13,992) in 2012. For the analysis on the association between infection in 2006 and obesity in 2012 we selected those individuals from the ENSANUT survey of 2012 who were aged 1-5y (n=9,523), 6-10y (n=13,025), and 11-19y (n=7,845) in 2006. Likewise for the analysis concerning infection in 2000 we selected those individuals from the ENSANUT survey of 2012 who were aged 1-5y (n=6,625), 6-10y (n=7,580), and 11-19y (n=5,623) in 2000.

Linear regression models were used to determine the association between the probability of infection (2000, 2006 and 2012) with BMIz or in 2012. Associations were estimated for *A. lumbricoides* and intestinal protozoa for each of the three age groups separately. In order to facilitate interpretation of the results, the incidence of infection (proximate probability of infection) for the regression analysis was transformed from new cases in 100 000 person-years to new cases per 100 person-years. Findings bellow p value of 0.05 were considered significant.

Poverty rate and sex were explored as a possible effect modifiers [30]. For this purpose we performed the same analyses as described above, including an interaction term between sex or poverty rate and the probability of infection in each model. Models with statistically significant interaction terms ($p < 0.05$) were stratified in two groups, one above and one below the median of poverty rate.

In order to have a visual overview of the combined prevalence of overweight and obesity and the incidence of each parasitic infection in 2012, we mapped each variable stratified in quintiles. The unit of mapping was “the state” the largest administrative unit of Mexico, and the maps were generated using Arc GIS V10.1 (Redlands, CA).

3. Results

In 2012, the combined prevalence of overweight and obesity in Mexico was 10% for children aged 1-5y, 35% for children aged 6-10y, 36% for the age group of 11-19y (Figure 2). In total, 47% percent of the population lived in poverty. The health coverage was 62% and the rate of households without sanitation facilities was 12% (Table 1). The incidence of *A. lumbricoides* and intestinal protozoan infection decreased from 2000 to 2006 and from 2006 to 2012 for all age groups (Table 1).

3.1 Ascaris lumbricoides

Table 2 shows a positive association between the probability of *A. lumbricoides* infection in 2000 and 2006 with BMIz in 2012. In the adjusted model, an increase of 1% in the probability of infection in 2006 was associated with an increase of 0.13 in the BMIz in 2012 for age group 1-5y, 0.27 for age group 6-10y and 0.50 in BMIz for age group 11-19y. Furthermore, an increase of 1% in the probability of infection with *A. lumbricoides* in 2000 was associated with an increase of 0.10 in the BMIz in 2012 for age group 1-5y, 0.11 for age group 6-10y, and 0.25 for the 11-19y age group. In contrast table 3 shows that a higher probability of being infected with *A. lumbricoides* in 2012 was associated with a decrease of 0.32 in the BMIz for age group 1-5y and a decrease of 0.21 for age group 6-10y.

Table 4 shows the results stratified by poverty rate. In the states with low poverty rates in 2012, *A. lumbricoides* infection was associated with an increased BMIz in 2012 in all age groups. In states with high poverty rates *A. lumbricoides* infection differed between age strata; the probability of infection with *A. lumbricoides* was associated with a lower BMIz for age group 1-5y, no association in BMIz for age group 6-10y and a higher BMIz for age group 11-19y. Neither sex nor poverty rate were modifiers for the associations between the probability of *A. lumbricoides* infection in 2006 or 2000 with BMIz in 2012 for any of the age groups. Therefore no stratified analysis were performed for these years.

3.2 Intestinal Protozoa

Table 2 shows the associations between the probability of infection with intestinal protozoa in 2000 and 2006 and BMIz in 2012 across the three studied age groups. The probability of infection with protozoa in 2006 was associated with a higher BMIz in 2012 in the 6-10y and 11-19y age groups. An increase of 1% in the probability of infection in 2000 was associated with an increase in the BMIz in 2012 of 0.47 for age group 1-5y, 0.61 for age group 6-10y and 0.99 for age group 11-19y.

Table 3 shows the associations between the proximate probability of intestinal protozoan infection in 2012 and BMIz in 2012 for every age group. In the adjusted model an increase of 1% in the probability of protozoan infection was associated with an increase of 0.6 in the BMIz for age group 6-10y and an increase of 0.9 for age group 11-19y

Neither sex nor poverty rate were effect modifiers for the associations between the probability of protozoan infection in 2012, 2006 or 2000 with BMIz in 2012 for any of the age groups. Therefore no stratified analysis were performed.

4. Discussion

Our results indicate that children with a higher probability of *A. lumbricoides* or intestinal protozoan infection (*Balantidium coli, Cryptosporidium, unspecified intestinal protozoa infection*) are more likely to have a higher BMIz in the same year, 6, and 12 years later in life. This finding is consistent with other studies showing early child "insults" including infections to be associated to later overweight and obesity [31-33]. This finding could be related to changes in the gut microbiota and inflammatory reactions due to parasitic infection that may lead to changes in appetite, food intake and thereby BMIz [34-36]. In line with this hypothesis, we recently found that *E. coli* infection was associated with a higher percentage of body fat and food intake in children [11, 37]. Similarly Schilder et al., in a firefly model, observed that an intestinal protozoa common in insects caused fat deposition in the thorax which is comparable to obesity in mammals [9, 10]. Longitudinal studies are needed to assess the temporal association of intestinal parasites on obesity over time.

While a higher probability of intestinal protozoan infection in 2012 was associated with a higher BMIz in the same year, we found the opposite for *A. lumbricoides* infection. If the associations would have been explained purely by poverty, the same trend and direction on the associations would have been observed for both parasitic infection incidence, which was not the case either in the crude or adjusted model. We also found an association between BMIz and *A. lumbricoides* for the age groups 1-5y and 6-10y, and not for the oldest age group (11-19y). The difference between age groups might be explained by the fact that *A. lumbricoides* infection-related symptoms are more common in younger children [38]. Children infected with *A. lumbricoides* may experience abdominal pain, nausea and discomfort, which may lead to a lower food intake and therefore lower BMIz [39]. The results of our previous study in Mexican schoolchildren supports this hypothesis, as we found a negative association between *A. lumbricoides* infection and food intake[37].

We found opposite associations between BMIz and *A. lumbricoides* incidence in states with high and low poverty rates. These differences may be explained with previous studies, as shown in a review by Guerrant et. al [33] in which children living in poverty were more likely to be malnourished, but also more likely to have stronger symptoms when infected.

Our findings should be interpreted in the context of this being an ecological analysis and not an estimate on any causal effect of parasitic infection on obesity at individual level (ecological fallacy). However, we intended to minimize this issue using individual level data on BMIz and specific covariates. Although we adjusted for potential confounders, we cannot control for unknown or unmeasured factors such as food availability, diet, and physical activity, therefore the outcomes of this study should be interpreted with caution. In addition, it was not possible to take changes in the state of residence over the years in consideration, but according to INEGI, migration between states was relatively low. In the year 2000: 3,584 957 persons migrated between states, representing 3.6% of the population and in 2006: 2,406 454 persons migrated, corresponding to 2.3% of the population at that time [40]. We used incidence data of the studied parasites as a measure for the probability of infection [41], and the true prevalence of parasitic infection is most likely underestimated. A major strength of our study is that ENSANUT and INEGI surveys are representative of the Mexican population at national and state level. In addition the parasite infection data of the SINAVE is collected following the same procedures nationwide and is therefore a good measuring tool for comparison purposes.

5. Conclusions

Our results suggest that children living in states with a higher probability of infection with intestinal parasites have a higher BMIz later in life. The association between current intestinal parasite infection and BMIz is less straightforward, and seems to be opposite for *A. lumbricoides* and intestinal protozoa. Further research is needed to confirm these ecological associations and study possible mechanisms underlying the short-term and long-term consequences of intestinal parasite infections on health. These findings may have important implications for Mexico, given the context of a high prevalence of parasitic infection and obesity.

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Disclosure statement

the authors report no conflicts of interest

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Figure1. Diagram of the study design

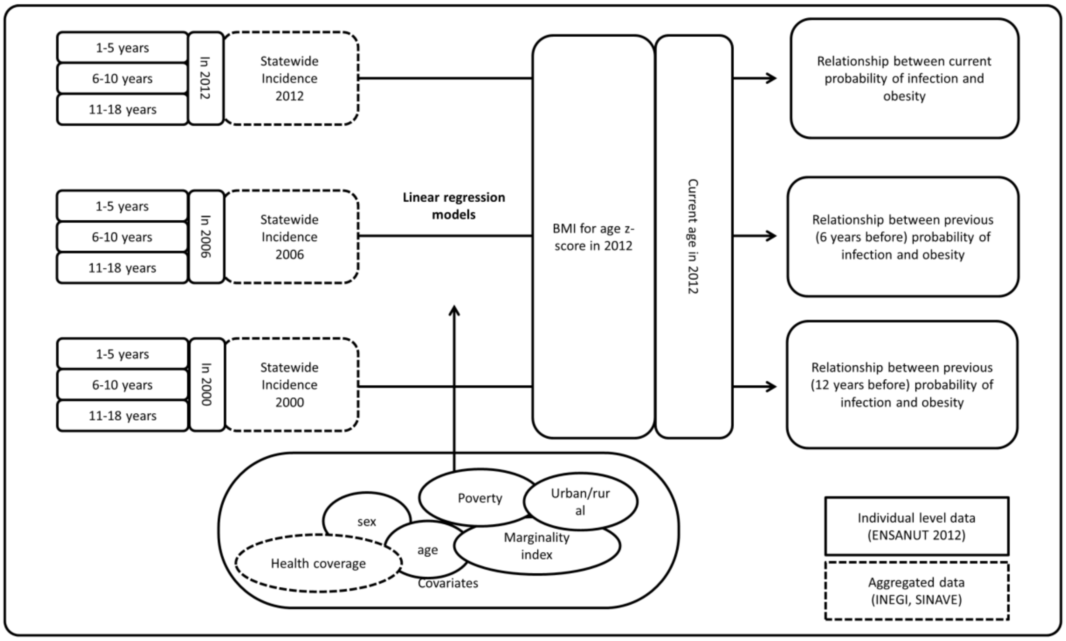

Figure 2. Map of combined prevalence of overweight and obesity and the incidence of *Ascaris lumbricoides* and protozoa in Mexico, 2012

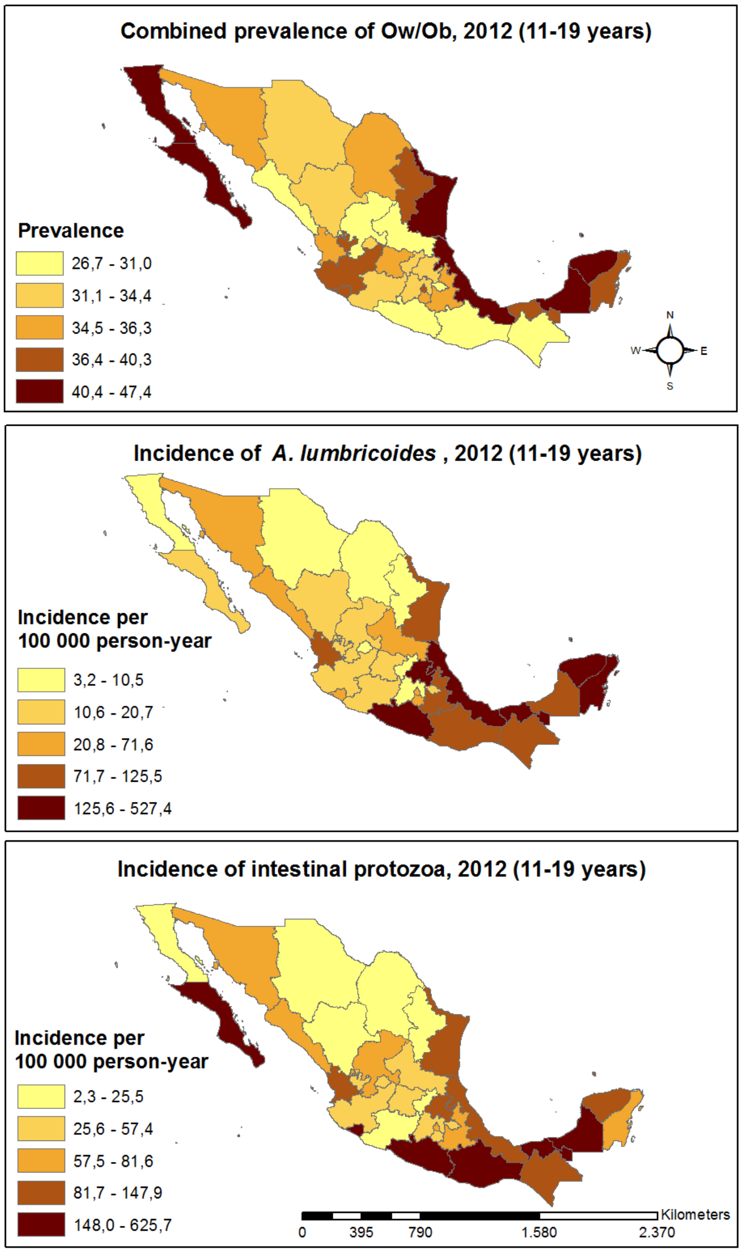

Table 1. General Characteristics of the population

	Mean		S.D.	Minimum	Maximum	Source
A. lumbricoides 1 to 5 years						
Incidence in 2012	94	±	129.3	3	625	SINAVE 2012
Incidence in 2006	404	±	461.1	5	1925	SINAVE 2006
Incidence in 2000	1177	±	1151.2	99	4559	SINAVE 2000
A. lumbricoides 6 to 10 years						
Incidence in 2012	67	±	108.2	0	526	SINAVE 2012
Incidence in 2006	212	±	301.8	3	1822	SINAVE 2006
Incidence in 2000	907	±	999.8	45	3990	SINAVE 2000
A. lumbricoides 11 to 18 years						
Incidence in 2012	27	±	42.7	0	219	SINAVE 2012
Incidence in 2006	90	±	140.0	1	757	SINAVE 2006
Incidence in 2000	367	±	421.5	18	1711	SINAVE 2000
Protozoa 1 to 5 years						
Incidence in 2012	93	±	104.0	2	545	SINAVE 2012
Incidence in 2006	239	±	275.2	9	1498	SINAVE 2006
Incidence in 2000	351	±	215.5	73	905	SINAVE 2000
Protozoa 6 to 10 years						
Incidence in 2012	78	±	93.7	1	568	SINAVE 2012
Incidence in 2006	150	±	200.7	4	1590	SINAVE 2006
Incidence in 2000	212	±	154.8	43	731	SINAVE 2000
Protozoa 11 to 18 years						
Incidence in 2012	48	±	65.4	0	354	SINAVE 2012
Incidence in 2006	78	±	97.5	2	902	SINAVE 2006
Incidence in 2000	113	±	88.0	23	420	SINAVE 2000
Prevalence of ow/ob (1-5 y) 2012	10.14	±	2.6	4.91	14.83	ENSANUT 2012
Prevalence of ow/ob (6-10 y) 2012	34.54	±	6.3	22.52	51.53	ENSANUT 2012
Prevalence of ow/obs (11-18 y) 2012	35.96	±	5.0	27.80	47.38	ENSANUT 2012
Females (%)	53.4		2.2	50.6	56	ENSANUT 2012
High marginality (%)	42.5		5.3	36.2	43.5	ENSANUT 2012
Poverty (%)	46.6	±	13.3	21.0	78.5	INEGI. 2012
Extreme Poverty (%)	11.2	±	8.7	1.8	38.3	INEGI. 2012
Years in school	8.6	±	0.9	6.3	10.6	INEGI. 2012
Health coverage (%)	62.4	±	10.9	39.9	81.0	INEGI. 2012

ow/ob: overweight/obesity

Table 2. Linear regression model between the proximate probability of infection in 2012 with BMI for age z-score in 2012

	Ascaris lumbricoides						Protozoa					
	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p
Incidence in 2012	Crude model			Adjusted model			Crude model			Adjusted model		
1 to 5 years (n=8927)	-0.17	(-0.18 - -0.16)	<0.01	-0.32	(-0.33 - - 0.31)	<0.01	0.02	(0.01 - 0.03)	0.74	0.08	(0.06 - 0.10)	0.34
6 to 10 years (n=16347)	-0.15	(-0.16 - -0.14)	<0.01	-0.21	(-0.22 - - 0.19)	0.01	0.19	(0.18 - 0.21)	0.02	0.61	(0.59 - 0.63)	<0.01
11 to 18 years (n=13992)	0.17	(0.16 - 0.19)	0.05	0.16	(0.13 - 0.18)	0.23	0.43	(0.42 - 0.45)	0.00	0.85	(0.83 - 0.88)	<0.01

Adjusted by: urban/rural strata, age, sex, marginality, poverty, health-coverage. Incidence per 100 person-year.

Table 3. Linear regression model between the proximate probability of infection in 2000 and 2006 with BMI for age z-score in 2012

	Ascaris lumbricoides						protozoa					
	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p	β	95% C.I.	p
Incidence in 2006	Crude model			Adjusted model			Crude model			Adjusted model		
1 to 5 years (n=9523)	-0.03	(-0.04 - -0.03)	0.16	0.13	(0.12 - 0.13)	<0.01	0.00	(0.00 - 0.01)	0.79	0.00	(0.00 - 0.01)	0.93
6 to 10 years (n=13025)	0.04	(0.04 - 0.05)	0.14	0.27	(0.26 - 0.28)	<0.01	0.07	(0.06 - 0.08)	0.14	0.13	(0.12 - 0.13)	0.01
11 to 18 years (n=7845)	0.33	(0.32 - 0.34)	<0.01	0.50	(0.49 - 0.52)	<0.01	0.24	(0.23 - 0.26)	0.00	0.16	(0.15 - 0.18)	0.03
Incidence in 2000												
1 to 5 years (n=6625)	0.04	(0.04 - 0.05)	<0.01	0.10	(0.09 - 0.10)	<0.01	0.29	(0.28 - 0.30)	0.00	0.47	(0.46 - 0.48)	<0.01
6 to 10 years (n=7580)	0.08	(0.08 - 0.09)	<0.01	0.11	(0.11 - 0.11)	<0.01	0.58	(0.56 - 0.60)	0.00	0.61	(0.59 - 0.63)	<0.01
11 to 18 years (n=5623)	0.19	(0.18 - 0.19)	<0.01	0.25	(0.24 - 0.26)	<0.01	0.88	(0.85 - 0.90)	0.00	0.99	(0.96 - 1.02)	<0.01

Adjusted by: urban/rural strata, age, sex, marginality, poverty, health-coverage. Incidence per 100 person-year.

Table 4. Linear regression model between the proximate probability of infection with *A. lumbricoides* in 2012 with BMI for age z-score in 2012 according to statewide poverty rates

	Low Poverty			High Poverty		
	B	95% C.I.	p	β	95% C.I.	p
***A. lumbricoides* Incidence in 2012**						
1 to 5 years	0.33	(0.31 - 0.35)	<0.01	-0.28	(-0.29 - -0.27)	<0.01
6 to 10 years	0.51	(0.48 - 0.53)	<0.01	-0.05	(-0.07 - -0.04)	0.42
11 to 18 years	1.14	(1.11 - 1.17)	<0.01	0.34	(0.31 - 0.36)	0.01

Adjusted by: urban/rural strata, age, sex, marginality, poverty and health-coverage. Incidence per 100 person-year.