

Original Article

## Stunting and intestinal parasites in school children from high marginalized localities at the Mexican southeast

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### Abstract

**Introduction:** Children under five years of age from developing countries are in risk of not achieving an adequate human development due to stunting and extreme poverty. They were also affected by intestinal helminths. Inhabitants of the state of Chiapas, the poorest population in Mexico, register the highest prevalence of child malnutrition as well as intestinal parasitic infections. With the purpose of fight against poverty and hunger, the Mexican government launched a social program called “Prospera”. The aim of this work was to determine the prevalence of stunting and intestinal parasites in school children beneficiaries of that social program, from two marginalized municipalities of Chiapas, Mexico.

**Methodology:** A total of 106 school-age children were recruited for nutritional assessment as well parasitic load measures.

**Results:** Most children exhibited stunting (88.7%). In these children the prevalence of intestinal parasites was 32.1%, being *A. lumbricoides* the species with the highest prevalence (25.5%) with moderate parasitic load (15.1%). Positive associations were observed between the presence of intestinal parasites and the municipality where children lived, the type of footwear, or the educational level of the mother.

**Conclusions:** Extreme poverty conditions in these localities of Mexico are far from reaching the sustainable development goals.

**Key words:** Stunting; intestinal parasites; Chiapas.

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### Introduction

It has been estimated that 250 million children under five years of age, from countries with low and middle incomes, are in risk of not achieving a proper human development due to stunting and extreme poverty [1]. Some of the effects caused by stunting include cognitive development failure with concomitant scholastic retardation, which in the long run causes low incomes during adulthood. As a matter of fact, the second objective of the Sustainable Development Goals (SDG) aims a 40% decrease of such nutritional affliction by 2025 [2] through the boost of sustainable agriculture [3]. Indeed, the triad of impoverishment, malnutrition, and intestinal parasites is still observed as evidenced in a recent report in which the relationship among stunting and the presence of *Ascaris lumbricoides* infestation is documented in a poverty setting of Asia [4]. *A. lumbricoides* is the most common geohelminth or soil-transmitted helminth in

the world, with nearly 819 million people infected annually, followed by *Trichuris trichiura* and Hookworm (464.6 and 438.9 million people, respectively) [5]. Infestation with these parasites leads to a lagging linear growing of children, as well as negative effects in cognitive abilities [6]. In Latin America, Ecuador, Guatemala and Jamaica were the countries with highest prevalence of *A. lumbricoides*, Hookworm and *T. trichiura*, respectively [7]. Most *Ascaris* infestations are asymptomatic; however, infested individuals may experience acute lung inflammation, respiratory distress and fever. Other symptoms include abdominal pain, nausea, diarrhea, loss of appetite with the concomitant malnutrition, and eventually intestinal obstruction due to adult worms [6]. Individuals with a heavy burden of *T. trichiura* infestation, may suffer of the *Trichuris* dysentery syndrome (TDS) characterized of dysentery, rectal prolapse, and anemia [8,9]. In a similar manner, chronic

Hookworm infestations also cause anemia, because of these kind of worms have teeth to obtain blood as a food source [10]. *Entamoeba histolytica*, the causative agent of amebiasis, is also a leading parasitoses in developing countries. In general, affected individuals has not symptoms, however after a year, 4-10% may develop amebic colitis with abdominal pain and bloody and mucous diarrhea. Indeed, the main extraintestinal infection is amebic liver abscess or extraintestinal disease [11].

For more than half a century, the Mexican population has been immersed in an income inequality [12]. The poorest populations are located in the southern part of the country in the states of Chiapas, Guerrero, Oaxaca, Campeche, Quintana Roo, Veracruz and Yucatan. Since 2005, these states present the lowest rates of human development indexes allowing neglected diseases to become endemic [13]. Since 2000, mild protein-energy malnutrition has been one of the top twenty causes of morbidity, while neglected tropical diseases mainly intestinal parasitoses caused by *E. histolytica* and *A. lumbricoides* also have been the most prevalent etiologic agents from the onset of the twentieth century [14].

During 2010 and 2016, inhabitants of the state of Chiapas continued to head the list of the poorest populations of the country with 78.5 and 77.1%, respectively. Moreover, this state had a larger rural population than urban population during 2015 (2.6 and  $2.5 \times 10^6$  inhabitants, respectively [15]. Besides, this state registers the highest occurrences of child malnutrition [16] and other intestinal parasitic infections such as amebiasis and ascariasis [14]. Nowadays, this scenario still persists. According to updated records from the Mexican weekly epidemiological report (week number 37, 2018), Chiapas holds the first, fourth, and fifth places at the national level in cases of intestinal amebiasis (N = 17,678 cases), ascariasis (N = 3,613 cases) and malnutrition (N = 2,757 cases) respectively [17]. During 2015, the inhabitants of municipalities belonging to the regions known as “VII De los Bosques” as well as “V Highlands Tsotsil Tseltal” (formerly known as “The Chiapas Highlands”) have been immersed in extreme poverty as well as the concomitant consequences such as neglected diseases and malnutrition [18]. The first socioeconomic region is localized at north of the Chiapas state, next to “The Chiapas Highlands” region at south. It has 13 municipalities, with more than a half of those inhabited by population that considers itself indigenous (61.5%); three of them have been within the ten most extreme

poverty population in the state [18]. “The Chiapas Highlands” region has 17 municipalities, 15 of them inhabited by population that considers itself indigenous (88.2%) and is located at the center of the Chiapas state. Two of those municipalities held the first, and second place, with highest extreme poverty population in the Chiapas state [18]. In 1997, the “Progresa” government program (known as “Prospera” since 2014) was created with the aim of fighting against such unfavorable conditions faced mainly by Mexican infants from rural communities [19]. Thus, the purpose of this work was to determine the prevalence of stunting and intestinal parasites in school children of high marginalized localities from “De los Bosques” and “The Chiapas Highlands” regions and discuss the progress towards the SDG in regards of nutrition and intestinal parasitoses that have afflicted this population.

## Methodology

### *Population and study zone*

The current study was cross-sectional and took place from October to December 2016. In accordance to the available population census (2010) among 7-14 years old children living in Chiapas, the localities of Monte Grande and Ajiló, both from the municipality of Bochil, appertaining to the region “De los Bosques” registered 134 and 255 children, respectively. Canteal locality, from the municipality of Chalchihuitán and Takiukum locality, from the municipality of Chenalhó (both municipalities from the “The Chiapas Highlands” region) registered 21 and 49 individuals, respectively [15]. Thus, the calculated sample size for “De los Bosques” as well as “The Chiapas Highlands” regions were 254 and 64 school children, respectively. Calculations considered a finite population, a 95% of confidence level, a 50% level of expected and failure proportions, and a 5% level of precision [20]. In this preliminary study we were only able to recruit a total of 106 school children stratified as follows: 64 from “De los Bosques” region and 42 from “The Chiapas Highlands” region. This, however, was not a representative sample size. Recruitment was limited due to issues not controlled by investigators such as parents did not attend the meeting point due to poor accessibility, refusal to participate, or conditioned to a payment. All these localities are considered as rural, and because of their socioeconomic disadvantage results in highest levels of marginalization [21]. After obtaining the approval of the Committee on Ethics of the University of Sciences and Arts of the state of Chiapas (UNICACH, acronym in Spanish for this university) (approval ID number ICB/072016), the

informed consent of the parent or legal guardian and the authorization of the head teachers and school principals, the children from the “Gabino Barrera” (located in Canteal, Chalchihuitán, workplace ID number 07TAI0915R) and “Guadalupe Victoria” (located in Takiukum, Chenalhó, workplace ID number 07TAI0830K) indigenous child’s houses were invited to take part of the study. These indigenous child’s houses offers food, hosting and education support for indigenous scholars and are sponsored by the National Commission for the Development of Indigenous People. School children from “De los Bosques” region were chosen from the following indigenous primary schools: “Rafael Ramírez Castaneda” (workplace ID number 07DPB0523V) and “Guadalupe Victoria” (key workplace number 07DPB1273M) located in Monte Grande and Ajiló localities, Bochil, respectively. Parents and children were interviewed with the purpose of recording information including: personal hygiene habits, kind of consumed water, “Prospera” program membership, parent’s level of studies, parent’s kind of work, housing conditions, and excrement disposal.

#### Nutritional assessment

Weight was obtained with a scale (Taurus); height with a portable stadiometer (seca) and the age was checked against the birth certificate. These variables were entered into the WHO AnthroPlus software [22] in order to calculate the Body Mass Index (BMI) and *z* score of the height-for-age indicator (HAZ). The results were interpreted based on the criteria established by WHO for children and teenagers from 5 to 19 years of age. The BMI classifies a child with overweight when the *z* score is  $> +1$  SD (standard deviation), obesity when it is  $> +2$  SD, thinness when it is  $< -2$  SD and severe thinness when it is  $< -3$  SD. The HAZ identifies a child with high stature when the *Z* score is  $+2$  SD,

slightly high stature when it is  $+1$  SD, and stunting when it is  $-2$  SD [23].

#### Intestinal parasites and parasitic load determination

The fecal material sample (~5 g) was collected in polypropylene tubes (Sarstedt, Nümbrecht, Germany) and placed in wet ice. Specimens were immediately taken to the Genetics and Molecular Biology Laboratory of the Biological Science Institute, UNICACH. A qualitative analysis was performed using the formol/ ethyl acetate concentration method [24]. The parasitic load by helminthes was measured using the modified Stoll technique [24] and was interpreted in accordance to WHO criteria [25].

#### Statistical analysis

The mean and standard deviation were determined for the continuous variables. The normal distribution (according to age) of the populations was verified by means of the Shapiro-Wilk or Kolmogorov-Smirnov tests, while the mean comparison was made by the Student *t* test for independent samples. The categorical variables were analyzed with the Chi-squared or Fisher’s exact test. The Odds Ratio (OR) was calculated in order to know the risk of becoming infected with parasites in both regions, with a confidence interval (CI) of 95%. The relation between the presence of intestinal parasites and other categorical variables were analyzed through the bivariate logistic regression; all the analysis was performed with the SPSS program version 20 (Armonk, New York, IBM Corp.) establishing a statistical significance value where  $P \leq 0.05$ .

**Table 1.** Nutritional assessment in school children from two high marginalized rural localities from Chiapas, Mexico.

	Total N = 106	The Chiapas Highlands N = 42 (39.6)	De los Bosques N = 64 (60.4)	P value
Girls -N (%)	67 (63.2)	28 (66.7)	39 (60.9)	0.681
Boys -N (%)	39 (36.8)	14 (33.3)	25 (39.1)	
Age-mean (SD)	9.9 (1.8)	10.7(2.0)	9.4 (1.5)	< 0.001
Height mean (SD)	125.1 (10.0)	128.5 (11.1)	122.9 (8.6)	0.004
Weight mean (SD)	27.3 (6.2)	29.4 (6.6)	25.8 (5.6)	0.0032
BMI mean (SD)	17.2 (1.8)	17.6 (1.6)	16.9 (1.9)	0.063
BAZ mean (SD)	0.1 (0.7)	0.1 (0.8)	0.1 (0.7)	0.796
Normal -N (%)	85 (80.2)	34 (81)	51 (79.7)	1.000
Overweight -N (%)	21 (19.8)	8 (19)	13 (20.3)	
HAZ mean (SD)	-2.1 (0.8)	-2.0 (0.8)	-2.1 (0.8)	0.763
Normal -N (%)	12 (11.3)	4 (9.5)	8 (12.5)	0.760
Stunting -N (%)	94 (88.7)	38 (90.5)	56 (87.5)	

## Results

The mean age of children was 9.9 years  $\pm$  1.85, with a weight of 27.3 g  $\pm$  6.27, height of 125.1 cm  $\pm$  10.06, BMI of 17.2 kg m<sup>-2</sup>, and HAZ of -2.11  $\pm$  0.88. There were no significant differences by sex ( $p = 0.681$ ). The weight and height of school children from “The Chiapas Highlands” was significantly higher in comparison to the ones of “De los Bosques”, while there were no differences in the BMI or in the  $z$  scores of BMI-for-age (BAZ) and HAZ indicators. Nevertheless, it was worrisome to find out that almost all school children exhibited stunting with a score of less than -2 SD (Table 1).

The overall prevalence of intestinal parasites was 32.1%; nonetheless, this was significantly higher in “The Chiapas Highlands” in comparison to “De los Bosques” region. In both regions, parasitism caused by one parasite was the most frequent, while polyparasitism was only documented among school children from “The Chiapas Highlands” (Table 2).

Also, only school children from “The Chiapas Highlands” were infected with *A. lumbricoides* and *T. trichiura*; however, in both regions *Hymenolepis nana* was documented (Table 2). Most school children from “The Chiapas Highlands” had moderated ascariasis with an average of 11,305.3 eggs per gram of feces (epg) ( $\pm$  19,036.6) and ~5% presented heavy infection. *T. trichiura* only presented light infection with 84.5 epg ( $\pm$  161.7). The risk of school children from “The Chiapas Highlands” to be infected with parasites was ~15-fold higher in comparison to “De los Bosques” region (OR = 14.6; 95% CI: 5.4-40.1).

In “De los Bosques” region almost all households were made of cement, whilst more than half of the households of “The Chiapas Highlands” were built with wood (Table 3).

Another relevant difference was that nearly four-fold of households in “The Chiapas Highlands” presented dirt floor, compared to “De los Bosques”

region (6.3%) (Table 3). Given the fact that there were more homes with dirt floor in “The Chiapas Highlands”, school children from this region were at nearly five-fold increased risk to have parasites in comparison to school children from “De los Bosques” region (OR = 4.8; 95% CI: 1.5-15.8). Notwithstanding the fact that almost all households in “The Chiapas Highlands” have latrines, the risk of school children of having intestinal parasites was almost 15-fold greater when they were compared to school children from “De los Bosques” region (OR = 14.5; 95% CI: 1.9-112.9). On the other hand, school children from “De los Bosques” region were more likely to drink purified water compared to “The Chiapas Highlands”. A greater proportion of children from “De los Bosques” used hand soap, wear neat clothes and closed shoes, compared to children from “The Chiapas Highlands” (Table 3). However, from these three variables, there was only observed a positive association between the presence of intestinal parasites with walking with bare foot ( $p = 0.001$ ).

Moreover, almost half of the mothers from “The Chiapas Highlands” were illiterate. This implies that children from this region had a nine-fold increased risk of getting infected with parasites (OR = 9.0; 95% CI: 3.3-24.4). It was documented that there were more families affiliated to the “Prospera” program from “De los Bosques” region than “The Chiapas Highlands” (Table 3).

## Discussion

This study provides updated data about the prevalences of stunting and intestinal parasites among school children from two of the poorest regions at Mexican southeast. However, the results should be taken with caution, because of the non-representative sample size explained earlier. An alarming prevalence of stunting nearly 90% could be recorded in scholars from both regions. Although mild protein-energy

**Table 2.** Intestinal parasites in school children from two high marginalized rural localities from Chiapas, Mexico.

Parasitological profile N (%)	Total N = 106	The Chiapas Highlands N = 42 (39.6)	De los Bosques N = 64 (60.4)	P value
Intestinal parasites	34 (32.1)	27 (64.3)	7 (10.9)	< 0.001
Monoparasited	28 (26.4)	21 (50.0)	7 (10.9)	< 0.001
Polyparasited	9 (8.5)	9 (21.4)	0 (0.0)	
<i>A. lumbricoides</i>	27 (25.5)	27 (64.3)	0 (0.0)	< 0.001
<b>Type of ascariasis</b>				
Severe	2 (1.9)	2 (4.8)	0 (0.0)	< 0.001
Moderate	16 (15.1)	16 (38.1)	0 (0.0)	
Light	9 (8.5)	9 (21.4)	0 (0.0)	
<i>T. trichiura</i>	11 (10.4)	11 (26.2)	0 (0.0)	< 0.001
<i>H. nana</i>	9 (8.5)	2 (4.8)	7 (10.9)	0.313

**Table 3.** Profile of homes and hygienic habits of schoolchildren from two high-marginalized rural localities from Chiapas, Mexico.

Household characteristics N (%)	Total N = 106	The Chiapas Highlands N = 42 (39.6)	De los Bosques N = 64 (60.4)	P value
Cement	80 (75.5)	20 (47.6)	60 (93.8)	< 0.001
Wood	26 (24.5)	22 (52.4)	4 (6.3)	
Concrete floor	96 (86.8)	36 (76.2)	60 (93.8)	0.016
Earthen floor	14 (13.2)	10 (23.8)	4 (6.3)	
Latrine	83 (78.3)	41 (97.6)	42 (65.6)	< 0.001
Hygienic habits				
Purified water consumption	88 (83.0)	24 (57.1)	64 (100.0)	< 0.001
Use of hand soap	93 (87.7)	31 (73.8)	62 (96.9)	0.001
Closed shoes	37 (34.9)	4 (9.5)	33 (51.6)	< 0.001
Clean dress	95 (89.6)	34 (81.0)	65 (95.3)	0.024
Parents profile				
Number of children		5.7 (1.7)	3.9 (2.6)	< 0.001
Illiterate father	1 (0.9)	1 (2.4)	0 (0.0)	0.638
Illiterate mother	26 (24.5)	19 (45.2)	7 (10.9)	< 0.001
Farmer father	106 (96.2)	39 (92.9)	63 (98.4)	0.096
Housewife	102 (96.2)	38 (98.5)	64 (100.0)	0.023
Membership to Prospera program	95 (89.6)	33 (78.6)	62 (96.9)	0.006

malnutrition does not figures among 20 causes of morbidity in Mexico since 2014 [26], the Chiapas state occupied the fifth place at national level of severe protein-energy malnutrition accounting 208 cases until week number 37, 2018 [17]. Childhood stunting is a consequence of poor nutrition, repeated infection, and inadequate psychosocial stimulation, which are prevalent in Asia, followed by Africa. This condition is now considered the best indicator for both infant well-being and social inequality [27]. As demonstrated in 2010, but probably for decades, indigenous populations of the Mexican southeast has been immersed in extreme poverty, being the states of Chiapas, Guerrero and Oaxaca the ones registering the highest averages [18]. This poverty had had a profound effect on malnutrition of children, back in 2006, with stunting being the main nutritional disorder affecting children of less than 5 years of age [28]. The same trend has been maintained the last few years given that children from the states of Chiapas, Oaxaca, Guerrero and Yucatan recently showed high occurrences of malnutrition (>20%) [16].

Specifically in Chiapas, in 2006 the Mexico National Survey of Health and Nutrition revealed that children presented the highest prevalence of stunting among the school population (25.5%). Girls were the ones exhibiting the highest occurrence in comparison to boys (26 and 24.8%, respectively). It is worth mentioning that such prevalence was more accentuated in rural zones (32.7%) than in urban regions (15.2%). According to official reports, in Chiapas, children under 5 years of age presented the highest prevalence of stunting (27%) followed by the states of Oaxaca,

Guerrero and Yucatan, all appertaining to the southeastern part of Mexico [29].

During 2013, the WHO report pointed out in a similar trend, that children of 0-5 years from Chiapas ranked first in stunting (31.4% with -2 SD), followed by children from the states of Guerrero and Oaxaca (23.7 and 20.7%, respectively) [30]. A study conducted in 2012 in the rural regions of Chiapas showed that this same group of age exhibited a stunting prevalence of 44.2% [29]. The current follow-up study, conducted five years later, revealed that school students from these two regions located in the southeast part of Mexico still present high stunting prevalence.

A previous study performed in brothers of less than five years of age from “De los Bosques” region (Chiapas) showed stunting prevalences of 46.8% and 43.4% in the first and second generation, respectively [31]. These children were part of the “Prospera” government program (formerly named “Progres”) to eradicate malnutrition mainly in rural settings [19]. Another study conducted in the same region questioned the effectiveness of the aforementioned program as authors demonstrated 40.1% of stunting among 222 children under five years evaluated during 9 years (2002-2011). It was pointed out that even children presenting a normal diagnostic at the beginning of the program showed stunting after only two years [32]. We hypothesize that although children receive food supplements, they may not consume them in their diet, even though the food consumed in the home is of poor quality. The lack of consumption of the intervention may be favored by the poverty in which they live

immersed. This factor could explain why scholars from “De los Bosques” as well as “The Chiapas Highlands” region still exhibited a high prevalence of stunting.

It has been documented that the persistence of stunting among children from the rural zones of Mexico affects their cognitive development, increases morbimortality caused by infectious diseases and causes low economic development level in the adulthood [33,34]. Results shown in this study revealed that social marginalization and extreme poverty in which these children are still immersed was determinant for the high prevalence of stunting. Therefore, they are far from reaching the objectives from the SDG focused on reducing malnutrition.

Given the demonstrated outcomes, the limited effectiveness of this program to diminish child malnutrition is still questionable in Chiapas. In other words, even when 80% of the school children presented a normal BMI they exhibited almost 90% of stunting while most families had enrolled in the “Prospera” program (Table 3). In Chiapas, a change in public policies is required to fight against the problem. For example, the First of the Millennium Development Goals (MDG) issued in 2000 (eradicate hunger and extreme poverty) raised the use of underweight as an indicator for malnutrition, instead of stunting, which in turns underestimates such condition [34,35].

Regarding parasitic infections, there was a higher prevalence in “The Chiapas Highlands” compared to children from “De los Bosques” region. The most frequent parasites were *A. lumbricoides* and *T. trichiura*, which were only observed in school children from “The Chiapas Highlands” (Table 2). Other studies have documented a similar prevalence of intestinal parasites in this region of the state, for example in Chanal and Larráinzar, Chiapas it were of 47.3 and 11.9%, being *A. lumbricoides* the most prevalent (45 and 9%, respectively) [36]. In the border region of Guatemala, the prevalence of *A. lumbricoides* was also of 45%, followed by hookworm and *T. trichiura* (37 and 36%, respectively) [37]. As a matter of fact, in Latin America, ascariasis and trichuriasis lead the main infections caused by helminthes, being Brazil the country which holds the highest number of cases of ascariasis, followed by Mexico, Guatemala and Argentina (41.7, 9.3, 7.9 and 7.7 million, respectively). In the case of trichuriasis, Brazil, Mexico, Colombia and Guatemala are the countries registering the highest quantity of cases (18.9, 18.3, 15.4 and 8.6 million) [38]. The bivariate logistic regression revealed that the presence of intestinal parasites was associated to “The Chiapas Highlands” region ( $p = 0.010$ ), where a high

index of illiteracy in mothers of families is observed ( $p = 0.003$ ) as well as school children rarely wear closed shoes ( $p = 0.043$ ). This last variable locates the children from this region of Chiapas at risk of getting parasites six-fold higher in comparison to children from “De los Bosques” region (OR = 6.3, 95% CI: 2.0-19.9). Since *A. lumbricoides* eggs require moist and warm soil for their development [39], barefoot walking probably allows feet function as a vehicle for the presence of this parasite in households. Some other factors may help to explain these differences include (1) a lower proportion of school children from “The Chiapas Highlands” use hand soap, (2) that a great number of households had dirt floor and (3) that families had more members favoring overcrowding and the risk of transmitting parasites among them (Table 3). In Argentina it was reported that overcrowding, walking barefoot and housings with dirt floor were associated to an higher frequency of intestinal geohelminths. In the same way, the highest occurrence of moderate parasitic load by *A. lumbricoides* was mainly observed in the rural zone [40]. Also, in the rural zones of Venezuela the prevalence of *A. lumbricoides* and *T. trichiura* (27.4 and 3.7%, respectively) was associated to housings with dirt floors [41].

## Conclusion

The current health status of the school children studied in this work revealed that stunting and intestinal parasitoses, mainly caused by *A. lumbricoides*, remained highly prevalent. Eradicating hunger was planned in 2000 as part of the First of those MDG (rephrased as SDG). It is evident that a common component that frames such conditions, stunting and parasitic diseases, is poverty, a situation that has prevailed in Mexico and mainly in the state of Chiapas and that continues leading the first position besides extreme poverty. In addition, the implementation of the “Prospera” program has failed to reduce: 1) the high prevalence of stunting which is translated into a chronic malnutrition condition, 2) the high prevalence of intestinal parasitoses which may be prevented, controlled and eliminated reducing poverty and the concomitant health improvement and life quality of the people from Chiapas.

It is therefore necessary and crucial to reconsider the strategies and/or monitor the intervention actions of the “Prospera” program such as health promotion, basic sanitation, nutrition surveillance, as well as prevention and control of diseases. It is also important to include a greater coverage of the indigenous people, as well as to having community promoters who speak indigenous

languages. All of these actions will improve the human development in these and other neglected populations in Mexico and Latin America.

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### Author contributions

Carolina Cruz-Cruz: Performed parasitological studies, as well as field work; Dolores López-Hernández: Performed parasitological studies, and field work; Juan Antonio Hernández-Shilón: Performed parasitological studies, and field work; Lorena Mercedes Luna-Cazáres: Data analysis and wrote the manuscript; Jorge E. Vidal: Data analysis and wrote the manuscript; Javier Gutiérrez-Jiménez: Conceived the study, wrote the manuscript.

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