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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

INFECTION BY HOOKWORMS IN AN INDIGENOUS REMOTE REGION OF THE LEGAL AMAZON, BRAZIL

INFECCION POR ANQUILOSTOMAS EN INDIGENAS DE UNA REGION REMOTA DE LA AMAZONIA LEGAL, BRASIL

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Abstract

Hookworms infection has become an increasingly serious threat to public health in Brazilian indigenous. Detection and/or clinical cases of hookworms have been reported in the past; however, nothing is known about the prevalence among indigenous Tapirapé in Brazil. We studied the presence of hookworms in indigenous Tapirapé ethnic group from county of Confresa, State of Mato Grosso, Brazil. Stool samples were collected from 542 members of the Tapirapé ethnic group and analyzed using formalin-ethyl ether and formalin-ethyl acetate sedimentation, and zinc sulfate flotation. Hookworms were detected in 104 (19.24%) indigenous and the prevalence was similar in males (21.29%) and females (17.20%). Males aged under 16 years were more frequently infected. Symptoms such as diarrhea and abdominal pains were reported from 104 infected individuals . Other symptoms associated with hookworms infection were vomiting, cramps and weight loss. No significant association with diarrhea was noted in individuals infected with hookworms. Hookworms are frequent intestinal parasites in Tapirape indians and the results suggest a high level of environmental contamination.

Keywords: Brasil - diagnosis - hookworms - infection - indigenous.

Resumen

La infección por anquilostomas se ha convertido en una amenaza cada vez más grave para la salud pública en los indígenas de Brasil. La detección y casos clínicos de anquilostomas se han informado en el pasado; sin embargo, no se sabe nada acerca de la ocurrencia entre los indígenas Tapirapé en el Brasil. El objetivo de este estudio fue determinar la presencia de anquilostomas en indígenas del grupo étnico Tapirapé, municipio de Confresa, Estado de Mato Grosso, Brasil. Las muestras de heces fueron colectadas de 542 miembros de la etnia Tapirapé y analizadas por las técnicas de sedimentación de formalina-éter y con formalina-acetato de etilo, y flotación de sulfato de zinc, con el fin de obtener un diagnóstico de anquilostomas. Los anquilostomas se detectaron en 104 (19,24 %) indígenas y la prevalencia fue similar en los hombres (21,29 %) y en las mujeres (17,20 %). Los varones menores de 16 años se han asociado con positividad. Una parte de los 104 individuos infectados, reportaron síntomas como diarrea y dolores abdominales. Otros síntomas asociados con la infección por parásitos intestinales fueron vómitos, calambres y pérdida de peso. No se encontró asociación significativa con la diarrea en los individuos infectados. Los anquilostomas son parásitos intestinales frecuentes en los indios Tapirapé y los resultados sugieren una contaminación elevada del ambiente en el que viven estos indígenas.

Palabras clave: Anquilostomas – Brasil – diagnóstico – infección - indígenas.

INTRODUCTION

Soil-transmitted helminths are a major global cause of morbidity and malnutrition. The World Health Organization (WHO) estimates that approximately 740 million of the world's population is affected by hookworms, resulting in 65.000 deaths annually, which are closely correlated to poverty, poor environmental hygiene and impoverished health services. Two species of hookworms commonly infect humans, *Ancylostoma duodenale* (Dubini, 1843) and *Necator americanus* (Stiles, 1902). *A. duodenale* predominates in the Middle East, North Africa and India, while *N. americanus* predominates in the Americas, Sub-Saharan Africa, Southeast Asia, China, and Indonesia (Georgiev, 2000; Neves *et al*., 2005).

Several studies of the health conditions among indigenous groups in Brazil have proven that the issue represents a growing challenge. The processes of colonization and expansion of the economic frontiers has been accompanied by significant deterioration of the health conditions of the indigenous people, thereby leading to degrees of depopulation (Riveiro, 1956; Neves *et al*., 2005; Bóia *et al*., 2009). Within the epidemiological profile of these processes, strong presence of parasitic diseases has historically been denoted (Santos *et al*., 1995; Bóia *et al*., 2009; Palhano-Silva *et al*., 2009). The aim of this study was to determine the presence of hookworms in 542 indigenous Tapirapé ethnic group from Confresa, State of Mato Grosso (western Brazil), the region with the highest number of indigenous groups in Brazil.

MATERIALS AND METHODS

Study site and Population

The study was carried out in the Tapirapé community, situated 30 km from the Confresa municipality, State of Mato Grosso, Brazil. The indigenous reserve is located in Legal Amazonia and inhabited by 542 members of the Tapirapé ethnic group. The members of this tribe have many free roaming animals including dogs, cats, chickens, and pigs. The principal source of protein for this population is derived from fishing and hunting animals such as the paca (*Cuniculus paca* (Linnaeus, 1766)), agouti (*Dasyprocta punctata* Gray, 1842), anteater (*Tamandua tetradactyla* (Linnaeus, 1758)),

tortoise (*Chelonoidis denticulata* (Linnaeus, 1766)), capuchin monkey (*Cebus capucinus* (Linnaeus, 1758)), deer (*Mazama americana* (Erxleben, 1777)), armadillo (*Priodontes maximus* (Kerr, 1792)), tapir (*Tapirus terrestris* (Linnaeus, 1758)), mallard (*Anas platyrhynchos* Linnaeus, 1758), and curassow (*Crax globulosa* Spix, 1825). Piped water is drawn from nearby rivers and is delivered to every household, but it is neither filtered nor chlorinated. There is a general lack of adequate sanitation; only one public latrine is available for the entire village; however, this latrine is not used by all the villagers. The Tapirapé indigenous population lives in houses at ground level (Fig. 1). The people of this village speak their own native language and Portugues. Their children do not attend schools and do not wear shoes.

A total of 542 stool samples (279 from females and 263 from males) were collected during January 2009 to July 2011. These collection times thus occurred in each of the two main climatic seasons in the Amazon region (dry and raining season).

Laboratory Analyses

The fecal concentrates were obtained by techniques of formalin-ethyl ether and formalin-ethyl acetate sedimentation, and zinc sulfate flotation (Truant *et al*., 1981; Malheiros *et al*., 2011; Malheiros *et al*., 2014) and were examined by light microscopy (x400). The consistency (form, softness, loose, and watery) of all fecal samples was noted on collection. Demographic data and clinical information were recorded for all members of the tribe. At the end of each collection, the indigenous received medication by health workers (Mebendazole oral doses of 100 mg every 12 h during 3 days).

Statistical Analyses

The association of presence of hookworms eggs with age (young or adult), gender (male or female) and diarrhea was analyzed using Pearson's chi-square test and Fisher's exact tests. Differences were considered statistically significant when p? 0.05. Statistical analyses were performed using SPSS 15.0 (Statistical Package for Social Science (SPSS) Inc.).

Ethical considerations

The research was approved by the Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo, and consent was obtained from all human adult participants and from parents or legal guardians of minors, according to National Committee for Ethics in Research (CONEP-120/2008).

RESULTS

In the present study, 542 individuals from six indigenous villages were enrolled of whom 104 (19.24%) were positive for hookworms based on microscopy of fecal concentrates (Table 1, Fig. 2).

The relationship between gender of the host and hookworms infection was calculated and the prevalence was similar in males (21.29%, 56/263) and in females (17.20%, 48/279) (p=0.56) (table 1). Infection was more common in children (61.44%) than in adults (2.57%) and was often found within family. Males being under 16 years of age were associated with positivity for hookworms (p ? 0.001) (Table 1).

For a total of 104 individuals infected with hookworms, symptoms such as diarrhea and abdominal pains were reported. No significant association with diarrhea was noted in individuals infected with hookworms (p= 0.42). The mayority of hookworms infected indigenous were mono-infected (85.58% 89/104). Co-infection with other helminths was 14.42% (15/104) and included: *Ascaris lumbricoides* (n=8), *Trichuris trichiura* (n=6) and *Taenia* sp. (n=1).

From the collected samples, it was noticeable that hookworms were more prevalent in the dry season (67.31%), than in the raining season (32.69%). Seventy-one children (79.79%) acquired infection of hookworms at least once during the dry season; while in the raining season 19 were infected (20.21%). Hookworm was detected during each sampling throughout the study period.

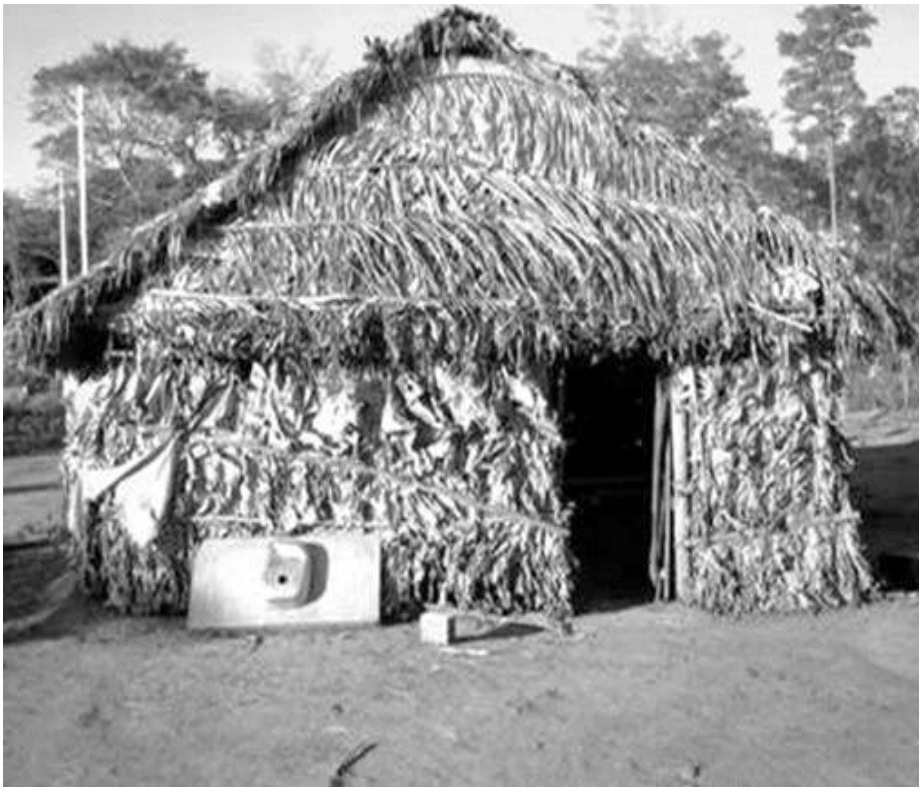

Figure 1. Raised dwelling of indigenous Tapirapé, Brazil.

Figure 2. Egg of hookworm examined in fecal concentrates observed by light microscopy (x400) (Scale bar spans 10µm).

Table 1. Prevalence of hookworms in members of the indigenous Tapirapé ethnic group from the Brazilian Amazon.

Variable	Individuals examined	hookworms		
		Positive	%	p value
Gender				
Females	279	48	17.20	NS
Males	263	56	21.29	
Age (years)				
3-16	153	94	61.44	?0.001[a]
?17	389	10	2.57	
Total	542	104	19.24	

NS: not significant.[a]p ? 0.001.

DISCUSSION

Understanding the epidemiology of hookworms infection in isolated indigenous communities is important in designing effective strategies to combat this neglected tropical disease. This study reports, for the first time, the presence of hookworms in members of the Tapirapé ethnic group. The diagnosis by microscopic examination used here is traditionally used for the diagnosis of parasitic infections. In the same manner, multiple sampling, as well as species-specific concentration and staining methods are all employed to enhance the sensitivity and specificity of the detection of different parasite species (Supali *et al*., 2010). Although, difficulties in differentiating to the species level using microscopy can result in an inaccurate picture of a disease, such as the differentiation between the hookworm species. Given this context, multiplex real-time PCR assay is highly recommended for the simultaneous detection and quantification of *A. duodenale* and *N. americanus* DNA in feacal samples (Haque *et al*., 1998; Verweij *et al*., 2007; Supali *et al*., 2010). This assay proved to be specific (100%) and sensitive (100%), for the detection of *A. duodenale* and *N. americanus* respectively. However, this tecnique has some disadventagens; it is technically complex, time consuming, and expensive (Haque *et al*., 1998).
The resulting prevalence in our study, was similar to other studies in Brazilian indigenous groups (Santos *et al*., 1995; Miranda *et al*., 1999; Bóia *et al*., 2009; Palhano-Silva *et al*., 2009) and similar to studies in other developing countries (French Guiana and Panamá) (Carne *et al*., 2002; Halpenny *et al*., 2013). The fact of the similarity in prevalence could be related to a number of factors such as poor hygiene, inadequate sanitation, water intake that was neither filtered nor chlorinated, lack of shoes, low education, low socioeconomic status which are prevalent in communities indigenous and in rural zones isolated (Bethony *et al*., 2006; Steinman *et al*., 2006; Ziegelbauer *et al*., 2012; Briones *et al*., 2013; Souza *et al*., 2013). Another important factor which affects the prevalence of hookworms is the habit of defecating in and around the habitation. The indigenous Tapirapé tends to live in houses at ground level and during the raining season eggs and larvas of helminths can be carried by rainwater into houses (Wongstitwilairoong *et al*., 2007; Briones *et al*., 2013). The eggs may remain in the houses for several days or months, reaching infective stages, thus children and adults indigenous can be constantly infected. According Briones *et al.* (2013) difference in housing design may contribute to the infection by helminths. Furthermore, The indigenous population that lives in houses on stilts would be less prone to infections by helminths when compared that live in houses at ground level (Briones *et al*., 2013).

In our study, the absence of statistical difference of infection between males and females is similar to other studies in Brazilian indigenous (Santos *et al*., 1995; Miranda *et al*., 1999; Bóia *et al*., 2009; Palhano-Silva *et al*., 2009). This fact is related to both gender are exposed to the same

impoverished conditions. However, we found that the Infection was more common in children than in adults. It is known that children are more susceptible to infection by parasites as compared to young and elderly humans. Indeed, studies in indigenous populations have highlighted a factor possibly involved in greater transmission of hookworms in children, that is the absence of the use of footwear for most of them allowing greater contact with soil containing infective forms (Carne *et al*., 2002; Briones *et al*., 2013).

Likewise, environmental factors must be considered. Indeed, hookworms are predominantly transmitted in tropical and subtropical regions of the world. The climate in the region is tropical humid with average annual temperature of 28.6°C, high humidity of 85%, and sandy soil of the region favor the development and maintenance of infective stages of these parasites. In the present study, the largest number of hookworms cases were observed bethween the months that corresponding the end of the rainy season (April) and early dry season (May to July). However, hookworm was detected during each sampling throughout the study period which is in accordance with previous studies that reported presence of hookworms and others intestinal parasites throughout all seasons (Siwila *et al*., 2011; Malheiros *et al*., 2011; Malheiros *et al*., 2014).

Hookworm infection is generally considered to be asymptomatic, silent and insidious (Georgiev, 2000; Neves *et al*., 2005). There are general some symptoms that an individual may experience soon after infection. Indeed, a high worm burden can lead to marked eosinophilia. In the present study, some symptoms such as diarrhea and abdominal pains were observed, however, not was measured the eosinophilia in the indigenous infected with hookworms, however, eosinophil count in the peripheral blood is highly recommended. Our study measured the presence or absence of hookworms infection in individuals. There was no attempt to quantify the burden of infection in individuals or assess individual propensity to infection. These issues will be addressed once deworming and follow-up monitoring programs be established in the ethnic group Tapirapé.

We found that the indigenous communities have a moderate prevalence of hookworms and is a frequent intestinal parasite in members of the indigenous Tapirapé. The transmission of the parasites is related with impoverished living conditions. Likewise, a lack of fecal hygiene and the habit of walking barefoot are widespread in the unchanging Amazonian environment and contribute to this phenomenon.

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