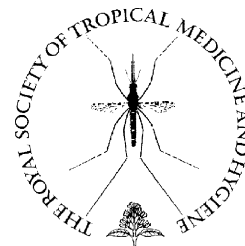




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Age-related changes in hookworm infection, anaemia and iron deficiency in an area of high *Necator americanus* hookworm transmission in south-eastern Brazil

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Summary Surprisingly few detailed age-stratified data exist on the epidemiology of hookworm and iron status, especially in Latin America. We present data from a cross-sectional survey examining 1332 individuals aged 0–86 years from a community in south-east Brazil for hookworm, anaemia and iron deficiency. Sixty-eight percent of individuals were infected with the human hookworm *Necator americanus*. The force of infection ($\lambda = 0.354$) was similar to estimates from other areas of high hookworm transmission. Individuals from poorer households had significantly higher prevalence and intensity of infection than individuals from better-off households. The prevalence of anaemia, iron deficiency and iron-deficiency anaemia was 11.8%, 12.7% and 4.3%, respectively. Anaemia was most prevalent among young children and the elderly. Univariate analysis showed that haemoglobin and serum ferritin were both significantly negatively associated with hookworm intensity among both school-aged children and adults. Multivariate analysis showed that, after controlling for socio-economic status, iron indicators were significantly associated with heavy hookworm infection. Our results indicate that, even in areas where

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there is a low overall prevalence of anaemia, hookworm can still have an important impact on host iron status, especially in school-aged children and the elderly.

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1. Introduction

Hookworm infection (with either *Ancylostoma duodenale* or *Necator americanus*) remains one of the most prevalent of chronic human infections in developing countries. It can result in intestinal blood loss leading to iron-deficiency anaemia (Crompton and Stephenson, 1990) and is associated with cognitive impairment (Sakti et al., 1999), probably mediated through the hookworm-induced anaemia (Drake et al., 2000). The extent of hookworm-associated anaemia depends, however, on the underlying iron status of the individual (Lwambo et al., 1992). In populations with inadequate iron stores, there is a significant association between haemoglobin (Hb) concentration and intensity of hookworm infection (Roche and Layrisse, 1966; Stoltzfus et al., 1997). Due to age-related differences in the intensity of infection (Bundy, 1990), as well as age-related differences in underlying iron status, it is expected that the association between hookworm infection and anaemia will differ by age group. However, surprisingly few quantitative, age-stratified studies on the association between hookworm and iron status have been undertaken, with most studies focusing on either school-aged children or adults (Olsen et al., 1998; Stoltzfus et al., 1997). Few studies on the association between hookworm infection and Hb have also been undertaken in Latin America. We present results from a cross-sectional survey of human hookworm infection and iron status in a rural community in Minas Gerais state of Brazil. The aim of the study was to investigate age-specific relationships between hookworm infection and iron status.

2. Materials and methods

2.1. Study area and population

The study was conducted from June to September 2004 in Americaninhas in Minas Gerais state of south-eastern Brazil. Full details of the study area and study design are provided in Fleming et al. (2006).

2.2. Ethical approval and consent

The study protocol (100310) was approved by the ethical review boards of George Washington University, USA, and the ethical committee of Centro de Pesquisas René Rachou-FIOCRUZ and the Federal Brazilian Ethical Review Board (CONEP). After the purpose and methodology of the study had been explained, consent was obtained from all adult subjects and from parents or guardians of minors by use of a written and oral consent form. The study protocol used the following inclusion criteria: (1) resident in the study area over the previous 24 months; and (2) willing and able to give informed consent and participate in the study

protocol. The following exclusion criteria were also applied: (1) attended school outside of study area; (2) worked full-time outside of study area; (3) tested positive on a urine pregnancy test or were already known to be pregnant; or (4) had received anthelmintic treatment within the last 24 months as determined by interview. All participants excluded from the study were offered a faecal examination and treated for all helminth infections, but were not considered part of the data set for analysis. Note that females were not excluded from the faecal examination if they tested positive on a urine pregnancy test or already knew they were pregnant, but received treatment for their helminth infection after delivery and breastfeeding. No blood was drawn from these female participants.

2.3. Parasitology

Collected stool samples were examined using the formalin-ether sedimentation technique for the presence of infection. Stools of individuals found positive for any helminth were then analysed by the Kato–Katz faecal thick smear technique for assessment of eggs per gram of faeces (epg). These same patients were asked for another faecal sample the next day. Two slides were taken from each day's faecal sample for a total of four slides from each individual and the average of the four slides presented as the mean egg count. Faecal samples from 27 albendazole-treated patients were searched on three consecutive days for expelled worms. Worms were washed in PBS and stored in 70% ethanol. For clarification and determination of the species, the worms were clarified in a phenol solution (70%) and the mouth parts were examined under the microscope (400× magnification). A total of 120 male and female worms were examined and were all identified as *N. americanus*.

2.4. Anthropometry

Weight was measured to the nearest 0.1 kg using a Soehnle electronic balance (Chasmors, London, UK) and height was measured to the nearest 0.1 cm using a portable fixed base stadiometer (Chasmors).

2.5. Assessment of iron status

Iron status was assessed by Hb and serum ferritin (SF), currently considered as the most efficient indicators of iron status (Mei et al., 2005). Hb can be used to assess anaemia, a specific condition where red blood cells are providing inadequate oxygen to body tissues, which can be caused by nutritional iron deficiency (anaemia-depleted iron stores) and anaemia arising from parasitic infections, certain other micronutrient deficiencies and haemoglobinopathies

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