African Journal of Biotechnology Vol. 2 (11), pp. 470-473, November 2003
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315

Full Length Research Paper

Relative prevalence of the human hookworm species, *Necator americanus* and *Ancylostoma duodenale* in an urban community in Ogun State, Nigeria

AA Adenusi[1*] and EOA Ogunyomi[2†]

[1]Department of Biological Sciences, Olabisi Onabanjo University, P.M.B. 2002, Ago-Iwoye, Ogun State, Nigeria.
[2]Primary Health Care Department, Sagamu Local Government Secretariat, Ogun State. Nigeria.

Accepted 17 October 2003

In order to estimate the proportion of hookworm infections represented by *Necator americanus* and *Ancylostoma duodenale,* the 2 major species of human hookworms in Nigeria, stool samples from 1253 hookworm-positive schoolchildren were cultured to the third-stage (L_3), filariform larvae, using the Harada-Mori test-tube method. *N. americanus* larvae were recovered from a total of 1177 (93.9%) coprocultures while *A. duodenale* larvae were recovered from a total of 274 (21.9%) stool cultures. 58.2% of the hookworm infections were due solely to *N. americanus,* 6.1% solely to *A. duodenale* and 25.8% were mixed infections with both species. In all mixed infections, much higher number of *N. americanus* larvae were recorded compared with those of *A. duodenale*.

Key words: Hookworm species, *Necator americanus, Ancylostoma duodenale*, filariform larvae.

INTRODUCTION

Despite considerable advances in chemotherapy and control, hookworms rank amongst the most widespread of soil-transmitted intestinal helminth parasites and affect a significant proportion of the world population (approximately 900 million people) mainly in the tropics and sub-tropics (Bundy et al., 1991). Adult hookworms attach to the mucosa of the small intestine, feed on blood and are an important cause of anaemia in school-aged and adult populations in the tropics (Olsen et al., 1998).

Necator americanus and *Ancylostoma duodenale*, the 2 major species of human hookworms are sympatric over much of their distribution and people are often simultaneously infected with both species in endemic areas. Despite significant differences in their life histories, the 2 species have traditionally been considered to be identical for treatment and control strategies (Hoagland and Schad, 1978). Failure to consider these differences is probably responsible for reports of failed drug treatments and rapid reinfection rates following anthelmintic treatment. Report of different anthelmintic susceptibilities between the species (Rossignol, 1990)

*Corresponding author. E-mail: aaadenusi@yahoo.com.

†Recent address: Primary Health Care Centre, Ilishan, Ogun State. Nigeria.

indicates that administration of only 1 anthelmintic may not be sufficient to kill both species. However, the rational design of hookworm control strategies requires, among other things, knowledge of the species infecting a particular human population in order for treatment to be successful in the long term.

Most studies do not attempt to speciate hookworm infections and rely on past epidemiological data, which indicate the predominance of one species over the other (Brooker et al., 1999), because the eggs of the 2 species are similar and not readily distinguishable from one another by classical parasitological methods (Hawdon, 1996). Although, there are established morphological differences between the adult worms (Yoshida et al., 1974a,b; Pawloski et al., 1991), the adult stages are rarely available for routine parasitological examination. Thus, species identification has traditionally been done by using subtle morphological characteristics to differentiate the infective, third-stage filariform (L_3) larvae, reared from eggs in coprocultures (WHO, 1981; Pawloski et al., 1991).

Although, previous reports from some parts of Nigeria have shown that *N. americanus* is the ubiquitous and dominant hookworm species (Fisk, 1939; Cowper and Woodward, 1961; Oyerinde, 1978; Adenusi, 1997) and that infections with *A. duodenale* represent only a small proportion of the local hookworm infections, over the years however, the epidemiological situation may not be the same as previously reported. Moreover, the relative distribution of the 2 species may vary from one endemic locality to the other. Thus, similar studies need to be carried out in other parts of the country.

We report on a study to estimate the proportion of hookworm infections represented by *N. americanus* and *A. duodenale,* in schoolchildren in Sagamu, an urban community and the headquarters of Sagamu Local Government Area of Ogun State, Nigeria.

METHODS

The study was conducted on 1253 schoolchildren (655 males and 598 females), aged between 5 and 13 years (mean age 8 years), positive for hookworm and who were participants in a large study (to be described in detail elsewhere) on the epidemiology of intestinal helminth infections in Sagamu, the headquarters of Sagamu Local Government Area of Ogun State, Nigeria. Schoolchildren were chosen because they are easily accessible and also constitute the section of the population at greatest risk of parasitic infection.

Each subject was given a clean, plastic container and asked to bring freshly passed stool sample the following day. Instructions on how to avoid contamination were also given to each subject. A brief interview of each subject regarding the time of stool collection was done. Only stool samples collected within 2 hours were recruited in this study.

Hookworm eggs in about 4 g freshly passed and unfixed stool samples were cultured to the infective, third stage (L_3), filariform larvae at ambient temperature (26 to 28^o C), by the Harada-Mori test-tube method (Hsieh, 1961). The cultured larvae were concentrated by centrifugation and stained with Lugol's iodine. The first 150 larvae (if more than 150) were examined under the microscope and identified according to the criteria of WHO (1981).

RESULTS

Table 1 gives the relative prevalences of the 2 hookworm species, *N. americanus* and *A. duodenale* in the study population. Of the 1253 hookworm-positive coprocultures, *N. americanus* filariform larvae were recovered from a total of 1177, while *A. duodenale* filariform larvae were recovered from 274. 854 of the 1253 (68.2%) hookworm-positive stool samples had only *N. americanus* filariform larvae while 76 (6.1%) had only *A. duodenale* larvae. 323 (25.8%) of the coprocultures had double species infection as evidenced by the recovery of filariform larvae of both species (*N. americanus* and *A. duodenale*). However, in all double (mixed) species infections, higher numbers of *N. americanus* larvae were recovered compared to *A. duodenale* with the former ranging from 60 to 85% and the latter ranging from 15 to 40% in any mixed infection.

Table 1. Prevalence of *N. americanus* and *A. duodenale* in the hookworm positive subjects.

Hookworm species	No. of cases observed	Prevalence (% of total hookworm-positive study group (n=1253)
N. americanus	1177	93.9
A. duodenale	274	21.9

In the present study, filariform larvae of *N. americanus* varied in length from 490 to 620 μm, with majority measuring between 550 to 580 μm, while *A. duodenale* larvae varied in length from 670 to 760 μm (majority measured between 670 and 710 μm).

DISCUSSION

This study showed that *N. americanus* was the dominant hookworm species in the study population, as it solely accounted for 68.2% of all hookworm infections. This result is in agreement with previous ones from different parts of Nigeria (Fisk, 1939; Cowper and Woodward, 1961; Oyerinde, 1978; Adenusi, 1997) that *N. americanus* is the ubiquitous and dominant hookworm species.

In Lagos, Nigeria, Oyerinde (1978) showed that apparently all infections with *A. duodenale* occurred always in association with *N. americanus*, with the latter occurring independent of the former, and concluded that perhaps, less than 1% of hookworm infections from the Lagos population, were due solely to *A. duodenale*. This report (Oyerinde, 1978) would seem to suggest that *A. duodenale* seldom occurs solely in human hosts, at least in the studied Lagos population. A recent study in Lagos (Adenusi, 1997) however, found *A. duodenale* solely accounting for 4.5% of hookworm infections.

In the present study, although *A. duodenale* was solely responsible for 6.1% of all hookworm infections, it nevertheless occurred concurrently with *N. americanus* (mixed hookworm infection) in 25.8% of the subjects. These were probably more heavily exposed, as to have been infected with *A. duodenale*, whose overall prevalence was about a third that of *N. americanus*, the commoner of the two in this locality.

Inspite of the fact that a female *A. duodenale* lays an average of 30,000 eggs per day compared to about 9000 by an adult female *N. americanus* (Piekarski, 1989), much higher numbers of *N. americanus* infective larvae were recovered in coprocultures, compared to *A. duodenale* in all mixed infections. This would suggest that much higher number of adult female egg-laying *N. americanus* worms were present compared to *A. duodenale* in mixed infections, as all the eggs were subjected to the same culture conditions. Moreover, none of the patients had received any form of anthelmintic therapy in the 6 months preceding the study, which could have eliminated more *A. duodenale* than *N. americanus*. Evans et al. (1991) had reported that in mixed hookworm infections, a much higher percentage cure rate is obtained for *A. duodenale* than *N. americanus* by treatment with anthelmintics.

Epidemiological assessment of the public health significance of hookworm infections should not, as has been the case over the years, be focused only on estimation of the number of hookworm infections, which occur in a given population (prevalence). Rather, it should also include identification of the infecting hookworm species. This is vital to the evaluation of hookworm infection as a public health problem where therapy and control of the disease should be specific and targeted at the infecting hookworm species. It may be worth mentioning here, that the two hookworm species differ in susceptibility to the same anthelmintic and dosage regimen. Thus, efficacy of anthelmintic therapy is dependent on the infecting species of hookworm (Piekarski, 1989). Well-known anthelmintics such as Alcopar (Bephenium) and Pyrantel are known to be comparatively less effective against *N. americanus* than *A. duodenale* (Rajasekariah et al., 1986).

Also in hookworm infections, the degree of severity varies with the infecting hookworm species (WHO, 1998). *A. duodenale* is the more pathogenic of the two species as about 0.02 ml of blood is lost per worm per day with *Necator* compared to about 0.1 ml with *Ancylostoma* (Piekarski, 1989). Albonico et al. (1998) have recently shown that infection with *A. duodenale* in Zanzibari schoolchildren has a greater impact on anaemia than infection with *N. americanus*. Therefore, where *A. duodenale* is more prevalent, the effect on anaemia might be greater. This is of great public health significance.

It is concluded that although, *N. americanus* was the predominant hookworm species in the present study, *A. duodenale* also occurred. The results of the present study are reliably guaranteed by the Harada-Mori culture method, which is commonly used for the culture of hookworm eggs and the WHO (1981) identification scheme. Identification of infecting hookworm species based on the above is tedious and time consuming as it requires culture of eggs and microscopic examination of numerous larvae. A rapid and simple method that represents a considerable savings in time over current methods used for differentiating between the major species of human hookworms is urgently required.

Further studies are underway to estimate the total number of worms in each subject (including ratio of male to female worms, as well as the ratio of adult worms of the 2 species in mixed infections) following purgation of infected subjects and identification of passed (dislodged) adult hookworms.

ACKNOWLEDGEMENT

We are grateful to all subjects in this study for their excellent cooperation.

REFERENCES

Adenusi AA (1997). The distribution of *Necator americanus* and *Ancylostoma duodenale* among school children in Lagos, Nigeria. Trans. Roy. Soc. Trop. Med. Hyg. 91(3): 270.

Albonico M, Stoltzfus RJ, Savioli L, Tielsch JM, Chaway H M, Ercole E, Cancrini, G (1998). Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* and *Necator americanus*, on iron status of schoolchildren. Int. J. Epidemiol. 27: 530-537.

Brooker S, Peshu N, Warn PA, Mosobo M, Guyatt HL, Marsh K, Snow RW (1999). The epidemiology of hookworm infection and its contribution to anaemia among pre-school children on the Kenyan Coast. Trans. Roy. Soc. Trop. Med. Hyg. 93: 240-246.

Bundy DAP, Chandiwana SK, Homeida MMA, Yoon S, Mott KE (1991). The epidemiological implications of a multiple-infection approach to the control of human helminth infections. Trans. Roy. Soc. Trop. Med. Hyg. 85: 274-276.

Cowper SG, Woodward SF (1961). Parasitic infections recorded at University College Hospital, Ibadan, Nigeria, over a three-year period (1957-1960). W. Afr. Med. J. 10: 366-383.

Evans AC, Daly TJM, Markus MB (1991). Identification of human hookworm in failed-treatment cases, using Chinese hamsters (*Cricetulus griseus*) and scanning electron microscopy. J. Helminthol. 65: 67-72.

Fisk GH (1939). Helminthiasis in Lagos, Nigeria. Trans. Roy. Soc. Trop. Med. Hyg. 32: 645-652.

Hawdon JM (1996). Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. J. Parasitol. 82 (4): 642-647.

Hoagland KE, Schad GA (1978). *Necator americanus* and *Ancylostoma duodenale*: Life history parameters and epidemiological implications of 2 sympatric hookworms of humans. Exp. Parasitol. 44: 36-49.

Hsieh HC (1961). Employment of a test-tube filter-paper method for the diagnosis of *Ancylostoma duodenale, Necator americanus and Strongyloides stercoralis.* Geneva: World Health Organization, mimeograph AFR/ANCYL/CONF/16. Annex VI, 37-41.

Olsen A, Magnussen P, Ouma JH, Andreassen J, Friis H (1998). The contribution of hookworm and other parasitic infection to haemoglobin and iron status among children and adults in western Kenya. Tran. R. Soc. Trop. Med. Hyg. 92: 643-649.

Oyerinde JPO (1978). Human *Ancylostoma* infections in Nigeria. Ann. Trop. Med. Parasitol. 72 (4): 363-367.

Pawlowski ZS, Schad GA, Stott GJ (1991). Hookworm infection and anaemia. Approaches to prevention and control. World Health Organization, Geneva. 96p.

Piekarski G (1989). Medical Parasitology. Springer-Verlag, Berlin, New York. 363p.

Rajasekariah GR, Deb BN, Dhage KR, Bose S (1986). Response of laboratory-adapted human hookworm and other nematodes to ivermectin. Ann. Trop. Med. Parasitol. 80 (6): 615-621.

Rossignol JF (1990). Chemotherapy: Present status. In: Hookworm disease: Current status and new directions, Schad GA, Warren KS (eds.). Taylor and Francis, London. pp. 281-290.

WHO (1981). Intestinal protozoan and helminthic infections. World Health Organization, Technical Report Series 666, Geneva. World Health Organization.

WHO (1998). Guidelines for the evaluation of soil transmitted helminthiasis and schistosomiasis at the community level. Geneva. Schistosomiasis an Intestinal Parasite Unit, Division of Control of Tropical Diseases, World Health Organization, (WHO/CTD/SIP/98.1).

Yoshida Y, Matsuo K, Kondo K, Arizona N, Ogino K (1974a). Scanning electron microscopy of hookworms. 1. Adults and infective stage larvae of *Necator americanus* (Stiles, 1902). S. East Asian J. Trop. Med. Pub. Health. 5: 510-514.

Yoshida Y, Matsuo K, Kondo K, Arizona N, Ogino K (1974b). Scanning electron microscopy of hookworms. 2. Adults and infective stage larvae of *Ancylostoma duodenale* (Dubini, 1843). S. East Asian J. Trop. Med. Pub. Health. 5: 515-519.