ORIGINAL ARTICLE

Speciation of Intestinal Hookworm among the Bangladeshi and the Indian Male Workers in Jeddah, Saudi Arabia

Majed H. Wakid, PhD

Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences
Diagnostic Parasitology Laboratory, Special Infectious Agents Unit, King Fahd Medical Research Center
King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence
Dr. Majed H. Wakid
P.O. Box 80324
Jeddah 21589, Saudi Arabia
e-M: mwakid@kau.edu.sa

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Abstract

Human hookworm infections occur worldwide and are caused by *Necator americanus* and *Ancylostoma duodenale*. Their clinical aspects, complications, and treatments depend on the species of the hookworm that had caused the infection. This study was conducted to determine for the first time the hookworm species that infect the Bangladeshi and the Indian workers in Jeddah. A total of 210 stool samples were included in this study. The microscopic examinations using direct and concentration techniques were used in the detection of the eggs of the hookworms. Fecal culture was used to obtain filariform infective larvae for species differentiation. This study revealed that the *Necator americanus* is the dominant hookworm species that was present among the investigated workers. The distribution of the parasites among the workers has no significant association with their age groups. The sizes of the *Ancylostoma duodenale* filariform larvae were found to be slightly longer than those of the *Necator americanus*. Based on the data of the workers, they had contracted the infection while they were in their home countries. Further thorough investigations about the infection should be undertaken using advanced molecular techniques. As a consequence of the hookworm infection, there is a need to set up or strengthen public health programs about soil-transmitted parasites, like hookworms, in Saudi Arabia.

Keywords

Hookworm, Parasites, Speciation, Necator americanus, *Ancylostoma duodenale*

Introduction

Human intestinal hookworm are helminths parasites, which are part of the soil-transmitted nematodes. The two main species of hookworms that affect humans are the *Necator americanus* and the *Ancylostoma duodenale*. The World Health Organization reported that 24% of the world's population, *i.e.*, more than 1.5 billion people, have been infected with soil-transmitted helminths. Hookworm infections are distributed worldwide, mainly in the tropical and subtropical areas of Africa and Asia[1].

The principal mode of transmission of the hookworm is via skin penetration, caused by walking barefoot on soils that are contaminated with filariform larvae (the infective stage). Adult worms attach themselves to the intestinal mucosa of their hosts, mainly in the duodenum, and they are fed by sucking blood of their hosts. Heavy infections lead to severe blood loss, causing iron deficiency anemia[2] in their hosts.

Previous studies reported that the acuteness of the disease and their attendant severity of anemia depend on the species of the hookworm and the worm burden. Therefore, in order for treatments and control measures to be effective in the long term, species identification of the infecting worm is important[3,4]. The main routine diagnosis for the *N. americanus* and the *A. duodenale* is microscopic examination of stool samples using direct and concentration techniques in the detection of the eggs and, rarely, the rhabditiform larvae[5]. These two growth stages appear to be similar morphologically in both species of the hookworm. However, their morphological differences can be observed in the filariform infective larvae that were reared in cultures. Although the adult stages of these two species have affirmed different morphological characteristics, these adults are not routinely detectable in stool samples. The morphological microscopic features of their L3 filariform larvae are traditionally used as the key characteristic used in the identification of their species.

Previous studies revealed that most hookworm infections had affected Asian workers, mainly those from Bangladesh and India[6,7], but the identification of the infecting hookworm species are lacking in these studies. Therefore, this study was mainly focused to culture the hookworm eggs and identify the species that are prevalent among the Bangladeshi and the Indian workers in Jeddah.

Materials and Methods

Sample Collection

This study was conducted using the stool samples of 210 male workers in Jeddah, broken down into a sample population of 110 Bangladeshis and 100 Indians, who were previously diagnosed to be infected with the eggs of the hookworms. Each worker was provided with a clean, wide-mouth, screw-capped stool container and instructions for the collection of fresh stool specimen. Nationality, age, gender, and other relevant data were obtained from each worker through a questionnaire.

Macroscopic Examination of the Stool

This examination included the color and consistency of the stools, as well as their gross blood or mucous contents, in addition to the detection of the presence of whole adult worms or tapeworm segments in the stools.

Microscopic analysis of the stool

Microscopic techniques in analyzing the stools included the use of direct smears, using saline and iodine, and the use of formal-ether sedimentation technique as previously described in the literature[7].

Culturing the hookworm

The hookworms from the stool samples were cultured using the Petri dish/slant culture method[5], with slight modification. About 4 grams of the stool was transferred onto a filter paper placed on a microscope slide. In a petri dish, one end of the slide was tilted on a piece of glass rod, and the other end was immersed in water at the bottom of the dish. The samples were incubated up to 10 days at 27°C. Daily microscopic examinations were performed for the detection of motile larvae using a stereomicroscope. For a positive case, a drop of water from the culture was mixed with a drop of iodine, and then, the mixture was examined using light microscopy at 10x and 40x magnifications. The morphological diagnostic features of the larvae, based on the criteria of the WHO, mainly the striations on their sheaths in the tail region and the esophago-intestinal junction, were used to identify the hookworm species[8].

Statistical Analysis

The data on this study were statistically analyzed using IBM SPSS Statistics for Windows (ver. 20, IBM Corp., Armonk, NY USA). P values that are <0.05 were considered as statistically significant values.

Results

All of the workers were males, working as food handlers in Jeddah, and with age range of 30–58 years old (Table 1). Most of these workers, i.e., 89% of the sample

Table 1. **Distribution of infected persons by nationality, based on their age groups**

Nationality	Age Group (Years)			Total	P value
	31–40	41–50	51–60	N (%)	
Bangladeshi N (%)	37 (33.6)	38 (34.6)	35 (31.8)	110 (100)	0.05
Indian N (%)	38 (38)	32 (32)	30 (30)	100 (100)	0.05
Total N (%)	75 (35.7)	70 (33.3)	65 (31)	210 (100)	0.05

Table 2. **Hookworm species that were identified in the positive culture samples**

Hookworm Species	No.	%	Length (µm)
Necator americanus	29	82.9	500–600
Ancylostoma duodenale	6	17.1	620–690
Total	35	100.0	

population, were asymptomatic and with normally formed stools. Five percent of them submitted loose stools, and the remaining 6% provided soft stools. There was no statistical difference in the hookworm infection among the age groups, with a *P* of <0.05.

Hookworm eggs that were detected through direct smears were found in 102 samples, i.e., in 48.6% of the total samples. Through the sedimentation formal ether, the eggs were detected in all of the 210 samples. Heavy infection was observed in 60 samples, which were cultured using the Petri dish. After 10 days, filariform larvae were detected in 35 samples or in 58.3% of the total samples. The microscopic examinations of the filariform larvae revealed that the size range of the *N. americanus* was determined to be 500–600 µm, which is slightly shorter than those measured for the *A. duodenale* (620-690 µm). Their prevalence levels were 82.9% and 17.1%, respectively (Table 2).

Discussion

Collecting more than one stool sample from each worker was difficult. To increase the chance of detecting the hookworm, using 2 grams of the stool for the formal-ether concentration technique was substantial[5]. Using direct stool smears, 51% of the samples were diagnosed to be false negative, while using the sedimentation concentration technique, all specimens were diagnosed as positive. The cases with light infection produced a limited number of eggs, which could be missed, when using just 1-2 mg of the sample through the direct smear technique.

In this study, the sizes of the *A. duodenale* that were found in the samples were slightly larger than those of the *N. americanus*, an observation that agrees with all of the previously reported observations. The dominant species was the *N. americanus*, found in 82.9% of the samples, which is likewise consistent with the findings of previous studies in Asia and Africa. Several studies in Bangladesh, India, Nigeria, and other countries reported the *N. americanus* with prevalences of 60-75%[9-12], 80-95%[13-17], and surprisingly, 100%[18-22]. Accordingly, the common names, New World hookworm for the *N. americanus* and Old World hookworm for the *A. duodenale*, are not related to their geographical distribution. The author hereby suggests further consideration for new common names.

The severity of infection, the rate of iron deficiency or anemia, and antihelminthic treatments are species dependent[1,3,4]. Routine microscopic examination of the eggs and the rhabditiform larvae cannot distinguish the different species of hookworms. Therefore, species identification requires using cultures from the stools or molecular techniques for species identification.

In this study, laboratory culture of the worms was performed for all of the heavily infected samples that contain 10 eggs or more, which were identified under a high power magnification field of 40x. All of the eggs were subjected to the same condition as those used in the cultures. The addition of a vital stain revealed the non-viable eggs in many samples. Most of the larvae hatched and were detected from the 7th day of cultivation. Only thirty-five samples produced larvae in the culture. This could be due to the presence of nonviable eggs, or some workers might have already received any therapy, which had influenced the presence of the parasites.

With the further assessment done on the data collected from the workers, the author assumes that most of the workers were infected in their home countries. Several factors provide the favorable conditions for the growth of the hookworm. Eighty-two percent of them mentioned that they live in farming communities, which are mainly flooded rice cultivation areas. In these areas, people defecate in their surroundings (70%), have a habit of walking bare foot (90%), and have poor hygiene and sanitation practices (86%). Previous studies also reported a

significant association between agricultural activities, low socioeconomic status, and hookworm infection[23].

Stool cultures are cheap and easy to perform in any laboratory with minimal resources. However, there is a risk of infection from exposures to filariform larvae, because the culture technique is time-consuming. Expertise is also essential in the differentiation of the diagnostic morphological characteristics between the hookworm species. Further, some studies also revealed the detection of false positive or negative results, compared to the results derived from molecular techniques[17,21,24,25]. Based on these findings, the study plan should investigate a large number of samples to differentiate the species, using the advanced molecular tools, which are very sensitive, specific, and reliable.

Conclusions

This study reports about the presence of hookworm species among the Bangladeshi and the Indian workers in Jeddah, Saudi Arabia. The studied samples confirm previous reports that *N. americanus* is the dominant species in the Old World.

There is a need to institute more public and educational programs and to improve the quality of environmental and personal hygiene among the agricultural communities. The epidemiological evaluation of hookworm infections should include the determination of the species of the parasites in order for the administration of therapies and the control of the infection to be successful.

Conflict of Interest

The author declared that there is no conflict of interest that is related to this study and this article.

Disclosure

The author did not receive any type of commercial support either in the form of compensation or financial support for this case report. The author has no financial interest in any of the products, devices, or drugs mentioned in this article.

Ethical Approval

The study was approved by the Ethics Committee of the KAUH in Jeddah, Kingdom of Saudi Arabia, also known as the Institutional Review Board of Hospitals.

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تحديد نوع الديدان الخطافية المعوية بين العمال البنجلادشيين والهنود الذكور في جدة ، المملكة العربية السعودية

ماجد حمدي مطر واكد[١،٢]

[١] *جامعة الملك عبد العزيز ، كلية العلوم الطبية التطبيقية ، قسم تقنية المختبرات الطبية.* [٢] *وحدة الكائنات المعدية الخاصة، مركز الملك فهد للبحوث الطبية. جدة، المملكة العربية السعودية.*

المستخلص. تنتشر الإصابة بالديدان الخطافية البشرية في جميع أنحاء العالم ويسببها نوعان هما (*Necator americanus*) و (*Ancylostoma duodenale*). الجوانب السريرية والمضاعفات والعلاج تعتمد على نوع الدودة الخطافية. أجريت هذه الدراسة في جدة لتحديد ولأول مرة أنواع الديدان الخطافية المصاب بها العمال الذكور من الجنسية البنغلادشية والهندية. تم تضمين ٢١٠ عينة براز في هذه الدراسة. تم استخدام الفحص المجهري للتقنيات المباشرة والتقنيات المركزة للكشف عن بيض الطفيلي. تم استخدام مزرعة براز بتري ديش للحصول على اليرقات المعدية الفيلارية وذلك للتمايز بين النوعين للديدان الخطافية. كشفت دراستنا أن (*N. americanus*) هو النوع السائد بين العمال الذين تم فحصهم في الدراسة. اتضح أن الإصابة بالطفيل بين العمال ليس له علاقة بالفئة العمرية. كان حجم اليرقة من نوع *A. duodenale* أطول بقليل من *N. americanus*. وفقًا للبيانات التي تم الحصول عليها من العمال، نعتقد أنهم أصيبوا بالعدوى من بلدانهم الأصلية. نقترح المزيد من الدراسات باستخدام التقنيات الجزيئية المتقدمة.

N. americanus هو النوع السائد بين العمال البنجلادشيين والهنود المشمولون في الدراسة وهنالك حاجة ماسة لبرامج صحية توعوية عامة حول الطفيليات التي تنتقل عن طريق التربة.

الكلمات المفتاحية: الدودة الخطافية ، الطفيليات ، الانتواع ، الفتاك الأمريكي ، الأنكلستوما الاثني عشر.