Brief communication

Comparative studies on two culture methods for hookworm species identification

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Abstract: Ninety stool specimens obtained from patients referred to the National Research Institute of Health and found to reveal hookworm ova were cultured using the Test Tube Filter Paper and the Charcoal methods with a view to recover and identify the third stage larvae. Infective larvae were harvested in a total of 88(97.8%) specimens, 79(87.8%) of which were detected by both methods. Only 5(5.6%) and 4(4.4%) were
exclusively detected by the Test Tube and Charcoal methods, respectively. Except in one specimen where filariforms of both species (Necator americanus and Ancylostoma duodenale) were encountered, N.americanus was dominant. Both methods exhibited no significant difference (P > 0.05) in the development capacity of third stage larvae. The importance of culturing hookworm species in relation to the understanding of species dominance and the merits and demerits of both methods are discussed.[Ethiop. J. Health
Dev.1995;9(3):189-191].

Introduction

About one quarter of the world population is said to be affected by hookworm infection, one of the commonest of the soil transmitted helminthiasis(l). Insanitarily disposed faeces or usage of human faeces as soil fertilizer are the main sources of human infection in
countries where individuals are barefooted. It is therefore expected that hookwonn infection will have a higher prevalence in rural than in urban workers and that in many tropical countries it has become an occupational disease of farming communities(2).

In Ethiopia both human hookwonn species, Ancylostoma duodenale and Necator americanus , are known to exist at varying evels of prevalence in different geographical
locations(3-6). Armstrong and Chane(3) reported the existence of the two species by examining adult worms from treated patients. More detailed studies were carried out by
Shibiru Tedla(4) in 10 administrative regions, by Leykun Jemaneh(5) in 31 communities in Gojam and Gonder and also by Terefe Wondimagegnehu and his co-workers(6) among banana plantation residents in Middle Awash valley.

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The morbidity and mortality resulting from hookworm infection appears to depend much on
the worm load and type of species. As the morphological characteristics of parasitic
stages continue to offer the best form of practical diagnosis, diagnosis of hookworms depends largely on the microscopical examination of stool specimens. Several techniques such as the direct smear, salt floatation, and faecal concentration procedures are available. However, it is not possible to identify hookworms to species level on the basis of their egg morphology alone.

Differentiation at species level is necessary for the choice of therapeutic agents also. Therefore, culturing of the third stage larvae is of importance as they are readily dentifiable to species level. The purpose of this study was, therefore, to compare the Test Tube Filter Paper and Charcoal methods, in terms of their capacity to maintain development of the larval stages which may be of importance for clinicians and suggest a fairly feasible method adaptable in modest diagnostic laboratories in Ethiopia.

Materials

During January 30- June 24, 1992 fresh stool specimens from patients attending the National Research Institute of Health (NRIH) in Addis Ababa were obtained and examined for intestinal parasites using standard procedures(7). Specimens found to be positive for hookworm ova were subjected for culturing using the following two Methods(8-9).

1. The Harada-Mori Test Tube Filter Paper Culture Method A thin film of faeces was smeared on one side of the middle portion of a 13 x 120 mm filter paper strip. The strip was placed in a test tube with approximately 3 ml of tap water . The lower edge of the filter paper was submerged in the water but not the faeces. The culture tube was covered with cotton plug and kept at 28°C in an incubator for a maximum of thirteen days. The culture was examined under a low power magnification (l00) for emerging larvae each day starting on the third day to rule out the presence of strongyloides filariform larvae. Identification was done following the standard identification key(10).
2. Charcoal Culture Method A charcoal culture was made by mixing 1 part of softened (with water) faeces with 5 to 10 parts of fine, granular animal charcoal in a petri dish. The culture was kept at 28°C in an incubator. Water was added daily to moisten the material without getting it too wet. As filariform larvae would migrate to the surface of the mixture, it was checked each day starting on the third day with a dissecting microscope. a When a culture was found to reveal larvae, L thick 12 to 15 ply pad of absorbent gauze of Lppropriate size to fit the culture container was lampened with warm water and it was then pressed onto the culture surface, the cover was .eplaced and it was left for 30 minutes in order o stimulate the larvae to migrate onto the pad. The pad was then picked up at the center with concepts and was transferred upside down to a iedimentation flask filled to the brim with Narm water. The larvae were removed with a Jipette after 30 minutes as they fell to the 10t tom of the flask and observed under the IDicroscope for species identification as per key :10). In addition, the drops of moisture on the lid of the petri dish was washed, centrifuged md examined for the filariform larvae under a low power magnification (10X).

Results

Ninety stool specimens were cultured by the rest Tube and Charcoal methods. Third stage hookworm larvae were recovered from B8(97.8%) specimens while 79(89.8%) were successfully cultured in both methods, 5(5.6%) and 4(4.4%) were cultured only by the Test rube and Charcoal methods, respectively. Both cultivation methods were comparable in their culturing capacity. Only 2(2.2%) of the specimens failed to reveal third stage larvae in either methods despite being initially ova-positive. N. americanus was identified in all specimens except in one where filariforms of both species were recovered. While larvae were detected in 5(5.6%) of the specimens only by the Charcoal Method, they were also detected in 4(4.4%) other specimens by the Test Tube Method. The average number of days for hatching in the Test Tube and the Charcoal methods were seven and eight, respectively. The difference between the two methods in either culturing capacity or the number of days required for hatching was not statistically significant($P>0.05$). However, from handling and processing points of view, the Test Tube technique was considered simple and practically convenient.

Discussion

According to our findings, the two methods are quite comparable for the development of
the third stage larvae. Failure of the two specimen to allow larval development may perhaps be due to the light infections. The four specimens which revealed larvae by the Charcoal but not by the Test Tube method may be explained by the fact that the bigger sample usually mixed with charcoal compared with the thin

smear of the Test Tube may have a bearing on the chance of recovering larvae even in light infections. And the five
specimen where larvae were missed by the Charcoal but observed by the Test Tube method may be due to the fact that the larvae light have been covered within the charcoal
and thus possibly overlooked. Although our study design and study subjects are not comparable with that of other investigators(3-5), N. americanus as a dominant species in Ethiopia holds true in our observation as well.

Based on the relative simplicity, the Test Tube (Harada-Mori) method is more preferable than the Charcoal method. The former method could be useful for routine purposes specially for rural hospitals and health centers. However, if the idea of culturing is to harvest a large number of larvae for research purposes, the Charcoal method is recommended.

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