Iranian J Parasitol: Vol.2, No.1, 2007, pp.29-34

Original Article

An Evaluation on the Efficacy of Agar Plate Culture for Detection of *Strongyloides stercoralis*

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(Received 21 Oct 2006; accepted 6 Feb 2007)

Abstract

Background: *Strongyloides stercoralis* is a common parasitic nematode in Iran, especially in north of the country. The early diagnosis and treatment of *S. stercoralis* is crucial to prevent complicated cases of strongyloidiasis. The value of the preference of agar plate culture, in detection of *S. stercoralis* compared to formalin ether concentration method, reported in different studies in the world is variable from 1.6 to 6 times. Therefore, the current study was performed to evaluate the efficacy of agar plate on some isolates from north of Iran.

Methods: Nine hundred stool samples were randomly collected from rural areas of Mazandaran Province, northern Iran. All samples were examined by agar plate culture, formalin ether concentration and direct smear.

Results: Agar plate was 2 times superior to formalin ether; however, it showed some false negative results, too. The direct method could only detect cases of hyperinfection strongyloidosis.

Conclusion: On the whole, combination of agar plate culture and formalin ether concentration is recommended to obtain higher efficacy.

Key words: Strongyloides stercoralis*, agar plate, Iran*

Introduction

Strongyloides stercoralis is an intestinal nematode of humans with a worldwide distribution, especially in tropical and subtropical countries, affecting probably 100 million humans (1, 2). Although most infected individuals are asymptomatic but all patients are at risk of developing severe complicated strongyloidosis, particularly if they become immunosupprssed (1, 2). Better approaches to identifying, screening and treating those at risk will likely decrease the morbidity and mortality associated with this infection.

During the last decade, nutrient agar plate culture has been shown to be better than other parasitological techniques in diagnosis of *S. stercoralis* (3- 5). However, its superiority to formalin-ether concentration in different studies is controversy (6, 7). This difference is thoughtful and can be affected by different factors especially effect of geographical strains. Therefore, in the present study, the efficacy of agar plate culture in detection of S. stercoralis,

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in comparison with formalin ether concentration and direct smear was evaluated on samples collected from Mazandaran Province, an endemic area in north of Iran.

Materials and Methods

Sampling

In this retrospective study, using random stratified sampling, first 30 villages from Mazandaran province were selected, then for every village, 30 people from the list of primary health care units selected. Therefore, overall a total of 900 stool samples, according to statistician advisor, were collected, during 2002-2004. Choosing Mazandaran Province as the study area was due to its endemicity for S. *stercoralis*. Mazandaran is an area of 46,645 Km^2 with temperate climate, in north of Iran, at the vicinity of Alborz mountain range and Caspian Sea. According to the census of 1996, 45.89% of population of this province was as urban dwellers and 54.1% villagers (8).

After the collection of samples, they were transported to "Babol Health Research Center", located in that province, for examination.

Stool examination

All samples were examined by agar plate culture, formalin-ether concentration and direct smear. For agar plate culture, 3-4 g of faeces was placed on the center of nutrient agar dish (10cm diameter). If the sample was too solid, a few drops of distilled water were added to the samples, before placing on the plate. The agar medium was prepared as used by Arakaki *et al.* (4). For the safety of examiners, each plate was sealed by paper adhesive tape, to prevent larvae crawling out of the petri dishes. Then the plates were incubated at 28 °C for 48-72 h. Afterwards, the dishes were screened under light microscope with a low magnification. During examination of plates, if any larva (Fig. 1) and/ or adult (Fig. 2) or their furrows (Fig. 3) were observed, surface of the plate was washed by warm phosphate buffered saline. Then the wash was collected and centrifuged at 1000×g for 2 min. Later, the supernatant containing larvae and/or adults were fixed in 10% formalin for identification. Differential diagnosis of *S. stercoralis* from other possible nematodes was fulfilled, especially from *Trichostrongylus* spp. based on the morphological characteristics of larvae (9) and from *Rhabditis* spp. by morphology of adults (9) and behaviour of nematodes in nutrient agar plate (4).

Results

In general, among 900 individuals examined, 44 people (4.9%) were found infected with *S. stercoralis* by at least one of the three methods, mentioned earlier.

Table 1 represents the efficacy of each of these methods. Accordingly the efficacy of agar plate culture was the most and it was 2 times superior to formalin ether concentration. The efficacy of direct method was too poor and only 2 cases could be found positive by this method. As Fig. 4 shows, these two positive cases were also detected by both agar plate culture and formalin ether concentration. Actually the positive cases detected by direct smear belonged to two patients with strongyloidosis hyperinfection syndrome and in every single view of microscopical filed intensive *S. stercoralis* larvae were observed. Although the agar plate culture was 2 times more effective than formalin ether concentration, however, there were 6 positive cases of *S. stercoralis* which were detected by formalin ether concentration but recorded negative by agar plate culture (Fig. 4).

Table 1: Efficacy of three different methods for detection of *Strongyloides stercoralis* among 900 inhabitants of rural areas of Mazandaran Province

Method	No. of positive cases	Percent of infectivity by each method
Agar plate culture	38	4.2
Formalin ether concentration	19	2.1
Direct smear	2	0.2
Total	44	4.9

Fig. 1: Filariform larva of *Strongyloides stercoralis* on the surface of agar plate

Fig. 2: A view of agar plate showing free living adults and larvae of *Strongyloides stercoralis*

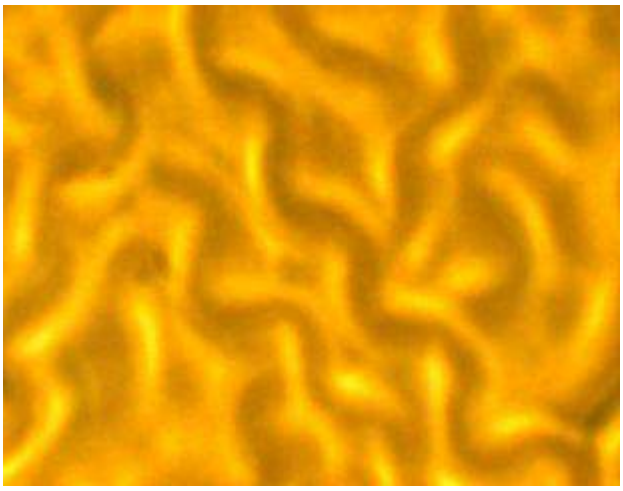

Fig. 3: Surface of agar plate showing furrows left by free living adults or moving larvae of *Strongyloides stercoralis*

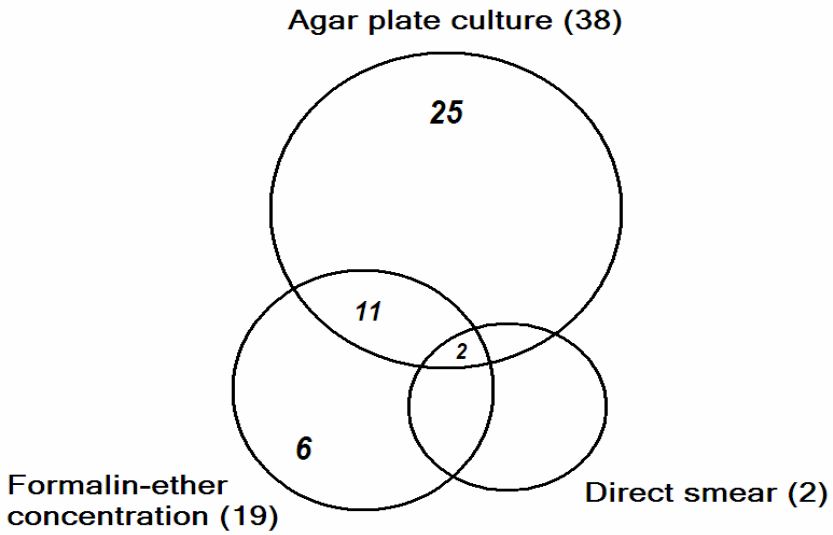

Fig. 4: Number of positive cases of *Strongyloides stercoralis* detected by three methods from 44 infected people among 900 individuals examined

Discussion

As with other parts throughout the world, the number of immunocompromised patients in Iran is increasing (10) and there is a risk of severe complicated strongyloidosis in infected individuals. During recent years, several cases of *S. stercoralis* hyperinfection syndrome in AIDS patients have been reported in Iran (11). Therefore, improving the diagnosis, screening and early treatment is necessary to prevent fatal consequences.

Since the introduction of agar plate culture as a method for detection of *S. stercoralis* by Arakaki *et al.* (12), several studies proceeded to evaluate the efficacy of this method with other methods, all indicating the superiority of agar plate culture to other coprological techniques. Considering the comparisons of agar plate culture with formalin ether concentration, although all studies point to the preference of agar plate culture, but its superiority is variable from 1.6 to 6 times more effectiveness (3-6, 13). Arakaki *et al.* mentioned that the differences in size of cover glasses used and the difference in skill of examiners were probably the cause of discrepancy in positive rate of *S. stercoralis* between the two facilities studied (4). Berezhnaia *et al.* observed considerable differences in the development of tropical *S. stercoralis* strains and strains from moderate climatic zone in faeces-soil mixture in the laboratory conditions (14). Although the 2 times superiority of agar plate culture to formalin ether concentration in the current study, which examined samples from temperate climate, is probably partly as an effect of geographical strain, however, manipulation and transportation of samples are also important in this issue. It should be considered that in this study agar plate culture was applied in filed condition, examining stool samples collected from rural residents, some in villages far distance from the laboratory. This fact may be an explanation for those samples which were found positive in formalin ether concentration, but negative by agar plate culture.

In contrast to the results of Koga *et al.* who found direct stool smear more efficient that formalin ether concentration, in the current study direct smear was found too poor in detection of *S. stercoralis* and only detected the two cases of strongyloidosis hyperinfection (3).

In conclusion, this study also stressed on the higher sensitivity of agar plate culture in coprological diagnosis of *S. stercoralis*, however, combination of agar plate culture and formalin ether concentration is recommended to obtain higher efficacy in detection of *S. stercoralis* in stool. Moreover, in Mazandaran Province discrimination of *S. stercoralis* from *Trichostrongylus* spp. and *Rhabditis* spp. needs especial consideration during screening for strongyloidiosis.

Acknowledgements

This study was financially supported by the Institute of Public Health Research, Academic pivot for education and research, Tehran University of Medical Sciences. Project No: 241/80/36. The authors would like to acknowledge the cooperation of the “Babol Health Research Center" staff especially Mr MA Baghbani. Also thanks to Mr I Gholami, Ms N Mirsepahi and Mr A Rahimi from the Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences for their kind assistance.

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