A NEW METHOD TO DETECT STRONGYLOIDES STERCORALIS FROM HUMAN STOOL

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Abstract: A new method for the detection of Strongyloides larvae was established. A small amount of stool was placed in the center of an agar plate and was incubated at 37°C for 24 hr. Characteristically aligned bacterial colonies or furrows left by crawling Strongyloides larvae appeared on the agar surface are the positive findings. The larvae gathered in a well made on positive plate were identified. By using this method, Strongyloides was detected in 46 cases (4.5%) out of 1,017 healthy adults. Whereas, it was detected in 0 and 3 cases (0 and 0.3%) by direct stool smear method and filter paper technique, respectively. Examination of 246 cases by this agar plate method and formalin-ether method (MGL) revealed that 14 cases (5.7%) were positive by the former and 2 cases (0.8%) by the latter. Agar plate method is not laborious nor expensive, and recommendable for mass examination and for the detection of asymptomatic carriers.

INTRODUCTION

Strongyloides stercoralis is a well known parasitic nematode as the etiologic agent of human strongyloidiasis. This parasite is mainly distributed in the tropical and subtropical areas, and infection rate up to 20% or more among inhabitants has been reported (Beaver et al., 1984). In Japan, the endemic areas are located in southern part of Kagoshima and whole Okinawa Prefecture (Tanaka, 1966) where fatal cases are occasionally found. There has been no satisfactory method for detection of Strongyloides larvae from human stool (cf. Asato et al., 1984). For diagnosis of strongyloidiasis, the test tube culture method (Harada and Mori, 1955) has been recommended (Tanaka et al., 1958). However, recent works have claimed that this method is not so satisfactory as has been believed (Asato et al., 1984).
Although some immunological techniques have been developed (Neva et al., 1981; Sato et al., 1984), their value is still limited to screening test.

On bacteriological examination of the stools in Okinawa, the authors have occasionally noticed a network of furrows with bacterial colonies on the surface of agar plate media. Subsequently it was proved that these furrows were left by the crawling Strongyloides larvae. This phenomenon suggested a new diagnostic method for strongyloidiasis using agar plate. This paper describes the value of the agar plate method for the detection of Strongyloides in comparing with some traditional methods.

MATERIALS AND METHODS

Stool specimens: Stools collected from 1,017 adults visited Center for Preventive Medicine, Okinawa, for medical examination were used. The donors of stool specimens were healthy at the time of sampling without any remarkable diseases. The stools were examined within the day of sampling, but the stools for examination with MGL (formalin-ether sedimentation technique) were stocked in a refrigerator for 1 to 4 weeks before examination.

Detection of larvae: All specimens were examined by the agar plate method, traditional direct smear and test tube culture methods. Randomly selected 246 samples were also examined with MGL method.

Agar plate method: Meat extract agar plates media (E-MC01, Eiken Co., Tokyo) for bacteriological examination were used. Finger head-sized stool was placed at the center of a plate, and was incubated at 37°C for 24 hr or more. After incubation, the plates were examined. The plates with aligned bacterial colonies and/or furrows were searched for crawling larvae under low magnification microscope (40×).

Traditional methods: The examinations with direct smear, test tube culture and MGL method were carried out by routine procedures (Harada and Mori, 1955; Ritchie, 1948).

RESULTS

Forty-six (4.5%) out of 1,017 stool samples were found positive for Strongyloides by the agar plate method. Characteristic alignment of bacterial colonies and furrows left by crawling rhabditoid and/or filariform larvae were clearly observed on agar plates (Fig. 1). On the other hand, no larva was demonstrated by the direct smear method, and only 3 samples (0.3%) were positive for Strongyloides by the filter paper cultures. Among 246 samples examined with both agar plate and MGL method, only 2 (0.8%) were positive by MGL method, whereas Strongyloides was detected in 14 samples (5.7%) by the agar plate method (Table 1). The agar plate method detected Strongyloides larvae from all the samples which were positive by the other methods. When a well was made on the agar plate with positive findings (aligned bacterial colonies or furrows) and filled with water or physiological saline solution, the larvae gathered around the well and entered the water. The larvae moving in the water were easily collected by using a pipette to be identified. In several cases, adult free-living worms were observed on the agar plate. In such cases, oviposition and hatching were seen.

In 2 out of positive 46 cases, aligned bacterial colonies and/or furrows were observed at 48 hr although they were not discernible at 24 hr. Incubation more than 48 hr did not result
Figure 1. Positive findings found on agar plate culture of stools contaminated with *Strongyloides stercoralis* larvae.

A: Aligned bacterial colonies formed around the stool.
B: Enlarged view of the aligned bacterial colonies.
C, D: *Strongyloides* larvae and furrows left by them on the agar plate.
The results of the present study have clearly shown that the detection rate of Strongyloides stercoralis from human stools by the agar plate method was markedly higher than those by traditional methods. It is apparent that the detection of Strongyloides larvae depends on the number of worms in the stool specimens primarily. When the number of worms in the stool sample was very few (for example only one worm), it must be so difficult to find it out by the traditional methods (cf. Asato et al., 1984). But only one worm left a network of furrows by crawling on the agar surface and developed aligned bacterial colonies. Therefore, it is possible to find a very small number of worms by the agar plate method as long as the worms are alive. Considering this phenomenon, most of the stools examined in the present study were supposed to be only slightly contaminated with Strongyloides, because the detection rate by the traditional methods was very poor. If the number of worms in the stool was so many as easily detectable even by direct smear method, the detection rate by any methods is supposed to be similar. Therefore, the agar plate method is especially significant in the examination of asymptomatic carriers.

It has been well known that many soil and plant parasitic nematodes are readily cultured on agar plate (Yokoo, 1959). In this viewpoint, it is quite reasonable that Strongyloides, which is phylogenetically close to the free-living rhabditoids, is developed on agar plate. Panosian et al. (1986) found Strongyloides stercoralis on agar plate media in the routine laboratory work of bacteriological examination. They suspected the presence of Strongyloides because aligned bacterial colonies displaced from the streak marks were developed. However, as far as the authors are aware of, no attempt has been made to utilize agar plate for the diagnosis of strongyloidiasis.

In Okinawa Prefecture, the test tube culture method has been mainly used for Strongyloides detection, and the infection rate has been believed to be less than 2% (Center for Preventive Medicine, Okinawa, 1983). However, Asato et al. (1984, 1985) demonstrated, by using a combination of MGL and test tube culture methods, that the rate was much higher. Since the present results proved that the agar plate method is more efficient than MGL and test tube culture methods, it is suggested that the exact prevalence may be surprisingly higher than that believed so far. As agar plate method is not laborious nor expensive, its application

<table>
<thead>
<tr>
<th>Methods</th>
<th>Number of stools examined</th>
<th>Number of positive cases (%)</th>
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<tbody>
<tr>
<td>Direct smear</td>
<td>1,017</td>
<td>0</td>
</tr>
<tr>
<td>Filter paper</td>
<td>1,017</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>Agar plate</td>
<td>1,017</td>
<td>46 (4.5)</td>
</tr>
<tr>
<td>MGL</td>
<td>246</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Agar plate</td>
<td>246</td>
<td>14 (5.7)</td>
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in the increased detection rate. No parasite other than Strongyloides stercoralis was found by any of the methods employed in the present survey.
should be recommended in epidemiological survey.

Recently, strongyloidiasis has attracted special interests as opportunistic agent in immunocompromised condition such as AIDS (Ndayiragije and Matheron, 1985). Moreover, its special relationship with adult T-cell leukemia (ATL) has been stressed (Nakada et al., 1984, 1987). The unstable results of fecal examination with traditional methods might have disturbed these researches. The agar plate method may also contribute to laboratory research of strongyloidiasis.

Since living larva in the stool sample is indispensable for detection by agar plate method, stool samples should be stored in an appropriate condition until examination. It has been well documented that some free-living rhabditoid nematodes such as Rhabditis hominis are occasionally contaminated in human feces. Asato et al. (1985) found R. hominis in 2 cases (0.09%) of 2,176 inhabitants examined in Itoman–City, Okinawa. It is probable that such species may also developed on agar plate. Therefore, species identification is necessary to certify the diagnosis. Instead of making wells in agar plate to collect the worms, we usually used small amount of agar media (10 ml or less per plate). The Petri-dish was not completely covered with this amount of agar, and some agar-defect-area (hollow) appeared in the plate. The water was poured into that hollow of the plate with positive findings.

On proceeding the present study, some interesting facts were noted. When fungal colonies developed on the agar plate, the larvae of Strongyloides did not come close to the fungi. Although the fungus was not identified, there is a possibility to get some anti-Strongyloides agent from this fungus. Some cases with numerous furrows but without any bacterial colonies were occasionally seen. This is probably due to antibiotics taken prior to stool sampling.

REFERENCES
糞便中からの糞線虫の新しい検出法

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岩永 正明1

普通寒天平板培地を用いた糞線虫の新しい検出法を開発したので、その有用性を報告する。指頭大の糞便を寒天平板培地の中央に置き、37℃で24時間培養する。糞線虫陽性例では、寒天平板上に糞線虫が達った後に残された細や、その後に増殖した細菌コロニーの特徴的な線状配列を認めることができる。幼虫の同定に関しては、陽性所見のある平板に穴（well）を作製し、水を満たすと幼虫は水中に集まってくるので、それを吸い上げて鏡検し確かめた。検査結果の内訳は、人間ドック受診者1,017人中、陽性例が寒天平板法46人（4.5％）、直接塗抹法0人（0％）、濾紙培養法3人（0.3％）であった。そのうち、ランダムに抽出した246人についてMGL法を用いて検査したが、陽性率は寒天平板法14人（5.7％）、MGL法2人（0.8％）であった。以上の結果から、寒天平板法が従来の方法に比し、極めて高い検出率を示すことが知られた。本法は、手技が容易でかつ安価であり、特に健康保虫者のスクリーニングに有用であると思われる。