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STUDIES ON PREVALENCE OF *Strongyloides* INFECTION IN HOLAMBRA AND MACEIÓ, BRAZIL, BY THE AGAR PLATE FAECAL CULTURE METHOD

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SUMMARY

Prevalence of *Strongyloides stercoralis* infection in three areas of Brazil was surveyed by a recently developed faecal culture method (an agar plate culture). The *Strongyloides* infection was confirmed in 11.3% of 432 subjects examined. The diagnostic efficacy of the agar plate culture was as high as 93.9% compared to only 28.5% and 26.5% by the Harada-Mori filter paper culture and faecal concentration methods, when faecal samples were examined simultaneously by these three methods. Among the 49 positive samples, about 60% were confirmed to be positive only by the agar plate culture. These results indicate that the agar plate culture is a sensitive new tool for the correct diagnosis of chronic *Strongyloides* infection.

KEYWORDS: Strongyloidiasis; Prevalence; Brazil; Agar-plate culture.

INTRODUCTION

Strongyloidiasis, which is relatively common in tropical and subtropical areas, is a parasitic disease resulting from an infection with *Strongyloides stercoralis*. The parasite has several unique properties in its life cycle. One of the properties is its ability to propagate in a host by internal autoinfection. It is probable that the autoinfection commonly occurs in human intestine and that the phenomenon is responsible for pathogenicity, lifelong infection and a strong resistance to chemotherapy. The parasite is usually non-pathogenic in an immunocompetent host, but due to the autoinfection, the infection often progresses to the fatal hyperinfected state under immunosuppressed conditions (SCOWDEN et al., 1978). The parasitic infection, being opportunistically pathogenic, therefore, is one problem of medical importance, due to the increasing use of immunosuppressive therapy and the presence of many AIDS cases.

One of the current problems concerning strongyloidiasis is the difficulty to detect *S. stercoralis* larvae in faecal specimens. This is because the majority of recent cases involve chronic, low-level infection. In the present study, the authors tried to apply a newly developed faecal culture method on surveys of *Strongyloides* infection in three areas in Brazil.

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MATERIALS AND METHODS

Subjects

In the present study, we had opportunities of collaboration to survey the prevalence of intestinal helminthiases in two slums (Vila Brejal and Vila Aratu) in Maceió City, Alagoas State and in farms of Holambra, Santo Antonio de Posse, São Paulo State. The numbers of persons examined were 164 in Brejal, 46 in Aratu and 222 in Holambra, respectively. They were 200 males and 232 females. The subjects included 180 children less than 16 years-old.

Faecal examination

Faecal samples were collected within 4 hours after defecation in the morning. All stools were kept at about 18-25 °C until examination by faecal concentration (formalin-ether concentration), Harada-Mori faecal culture using a filter paper strip (HARADA & MORI, 1955) and an agar plate faecal culture. The last method is a recently developed method in Japan (ARAKAKI et al., 1988) in which about 3 grams of faeces were placed on a primary agar plate in a Petri dish for bacterial culture and incubated for 2 days. After incubation, the surface of the agar plate was examined under a microscope to find motile larvae. In many cases, characteristic alignments of bacterial colonies and tracks of wandering larvae could be observed on the agar surface (Fig. 1), suggesting the presence of larvae. In such cases, a careful search for larvae on the agar surface was performed. When found, larvae were transferred to a glass slide and a drop of iodine solution was added to immobilize them. The larvae recovered were identified morphologically from those of hookworm and *Rhabditis*. These faecal cultures were performed within the same day of stool sampling to avoid the death of larvae before examination.

RESULTS

The results of faecal examination on the 432 subjects are summarized in Table 1. *S. stercoralis* infection was confirmed in 11.3% of them. The rates were 10% or more in Holambra and Brejal but only 4% in Aratu. On the other hand, the mean infection rate of hookworm (*Necator americanus*) was more than 2-times higher than that of *S. stercoralis*; a rate as high as 36.6% was obtained in Brejal.

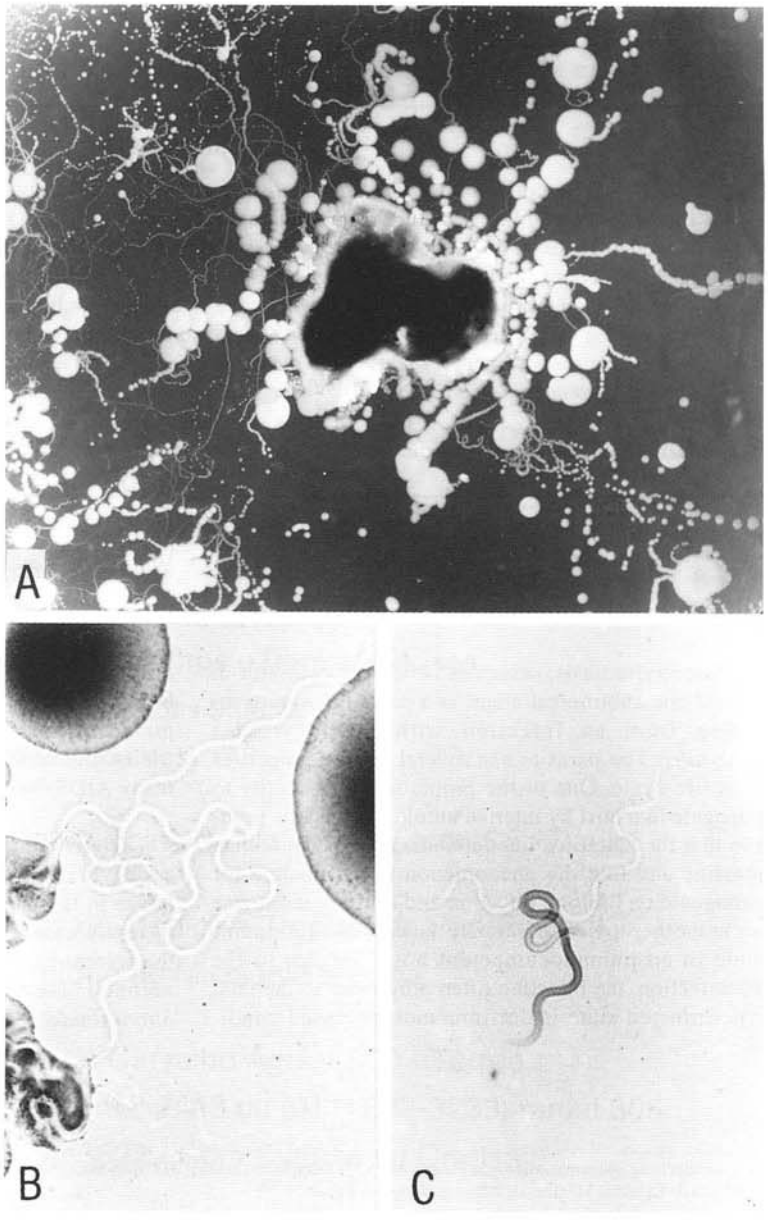

Fig. 1 - An agar plate culture of faeces positive for *Strongyloides larvae*. A) Linear bacterial colonies formed along the tracks of larvae which migrated from faecal mass on the plate. B) Furrows of wandering larvae on the surface of agar plate. C) A filariform larva and its furrows observed on the agar plate.

TABLE 1

Prevalence of intestinal helminthiases in three areas, Brazil, by faecal concentration, filter paper faecal culture and agar plate faecal culture methods.

Parasitic infection	Holambra (n = 222)	Brejal (n = 164)	Aratu (n = 46)	Total (n = 432)
S. stercoralis	23 (10.4)	24 (14.6)	2 (4.3)	49 (11.3)
N. americanus	44 (19.9)	60 (36.6)	8 (17.4)	112 (28.2)
A. lumbricoides	12 (5.4)	65 (39.6)	18 (29.1)	95 (22.0)
T. trichiura	19 (8.6)	66 (40.2)	29 (63.0)	114 (26.4)
E. vermicularis	3 (1.4)	1 (0.6)	0 (0)	4 (0.9)
H. nana	2 (0.9)	8 (4.9)	0 (0)	10 (2.3)
S. mansoni	0 (0)	26 (15.9)	10 (21.7)	36 (8.3)

Beside the *Strongyloides* and hookworm infections, five helminth species were also detected by the faecal concentration method. *Ascaris lumbricoides* and *Trichuris trichiura* infections were also prevalent parasitic infections in two areas of Maceió, although the infection rates of these two species were consistently low in Holambra. *Schistosoma mansoni* infection was observed in 15.9 and 21.7% of the subjects in Maceió but none of the subjects was found harboring the parasite in Holambra. The eggs of *Enterobius vermicularis* and *Hymenolepis nana* were detected in few specimens although the methods employed here are inadequate to estimate the prevalence of enterobiasis.

The efficacy of three methods on detection of *Strongyloides* and hookworm infections are shown in Table 2. An agar plate culture method was most effective for the detection of *Strongyloides* infection, indicating that more than 90% of the cases were diagnosed by the culture method. When either the Harada-Mori culture or faecal concentration method was applied, the infection rate in the subjects decreased to only 3%. In the case of hookworm infection, a high efficacy of over 75% was obtained by the Harada-Mori culture and faecal concentration methods, whereas the efficacy of the agar plate culture method was only 41%.

Tables 3 and 4 further represent the comparative efficacy of these three methods on detecting *Strongyloides* and hookworm infections. As shown in Table 3, about 60% of the *Strongyloides*-positive cases were diagnosed only by the agar plate culture. Contradictorily, the number

TABLE 2

Efficacy of agar plate culture, Harada-Mori filter paper culture and formalin-ether concentration on detection of *Strongyloides* and hookworm infections.

	Faecal culture		Faecal concentration	No. cases detected
	Agar plate	Harada-Mori		
S. stercoralis	46 (93.9)	14 (28.6)	13 (26.5)	49
Hookworm	46 (41.0)	96 (85.7)	85 (75.9)	112

( ): % efficacy

TABLE 3

Comparison of efficacy of three methods on detection of *Strongyloides* infection.

Agar plate culture	Harada-Mori culture	Formalin-ether concentration	No. of cases	%
+	+	+	6	12.2
+	+	–	6	12.2
+	–	+	5	10.2
+	–	–	29	59.2
–	+	+	1	2.0
–	+	–	1	2.0
–	–	+	1	2.0
Cases detected	46	14	13	49

TABLE 4
Comparison of efficacy of three methods on detection of hookworm infection.

	Agar plate culture	Harada-Mori culture	Formalin-ether concentration	No. of cases	%
	+	+	+	32	28.5
	+	+	–	10	8.9
	+	–	+	2	1.8
	+	–	–	2	1.8
	–	+	+	39	34.8
	–	+	–	15	13.4
	–	–	+	12	10.7
Cases detected	46	96	85	112	

of cases proven positive by the agar plate culture alone was only two (1.8%) in the case of hookworm infection.

DISCUSSION

The medical significance of strongyloidiasis is the opportunistic nature of the parasite which often produces a fatal hyperinfection under immunocompromised conditions (SCOWDEN et al., 1978). In Okinawa, Japan, the parasitic disease is frequently accompanied by human T-leukemia virus type-I (HTLV-I) infection which causes T-cell leukemia (NAKADA et al., 1984; FUJITA et al., 1985; SATO & SHIROMA, 1989). The parasitic infection often progresses to a fatal severe infection among patients who develop T-cell leukemia because the disease is manifested by severe depression of immunocompetence (YOSHIOKA et al., 1985; ASOU et al., 1986).

In Brazil, many severe cases of strongyloidiasis were also reported among patients with depressed immunopotency (HUGGINS, 1971; BATONI et al., 1976; PAES et al., 1979; HUGGINS, 1979). The prevalence of immunodeficiency virus (HIV) infection, as well as HTLV-I which was recently discovered to be endemic to Brazil (LEE et al., 1989; ANDRADA-SERPA et al., 1989; CORTES et al., 1989), may also present a great risk factor for fatal severe infection with this parasite in this country (LUCAS, 1990).

On the other hand, due to the opportunistic nature of the parasite, the majority of cases involve an asymptomatic chronic infection in an immunocompetent host. Diagnosis is sometimes difficult in such cases of chronic infection because very small numbers of larvae are intermittently excreted in faeces (JONES, 1950; GROVE, 1980). It was our experience that a single stool examination has a reconfirmation rate of only 15-24% by the 3 traditional methods, such as faecal smear, faecal concentration and Harada-Mori culture method, when 90 persons with proven *Strongyloides* infection were re-examined several months later without treatment. Recently, another method for faecal culture was developed in Japan in which a faecal mass as much as 3 g or more was cultured on a primary agar-plate commonly used for bacterial culture (ARAKAKI et al., 1988). In this method, unique alignments of bacterial colonies that outlined the tracks left by motile larvae on the agar surface prompted us to assume the presence of larvae. Using the new method, we are able to more effectively diagnose chronic infection. The positive rate of *S. stercoralis* among the inhabitants in Okinawa, Japan, is currently considered to be more than 10% by the agar plate culture, which is 3 to 5 times higher than those in recent studies by traditional methods (ASATO et al., 1992).

In the present study, the authors applied this new method to detect *Strongyloides* infection in Brazil. The mean infection rate obtained was 11.3%. Diagnostic efficacy for strongyloidiasis was highest in the agar plate culture, showing that about 60% of the cases would have been overlooked, if only the Harada-Mori culture and faecal concentration methods had been performed. On the other hand, the efficacy of the agar plate culture was only 40% for hookworm infection, which was less than half that of the Harada-Mori culture method. The number of cases found to be positive by the agar plate culture alone was only 2 in the case of hookworm infection. It seemed to be due to the short cultivation period in the present study. As well known, hookworm is excreted in fresh faeces during the early stages of egg development and the time for hatching is 1 to 2 days under favorable conditions. The cultivation time of 2 days in the present study might have been too short to detect hookworm larvae which migrated from faecal mass.

In Brazil, there were several reports on the prevalence of *Strongyloides* infection; the positive rates reported ranged from 6% to 67% (PEREIRA & CARNEIRO, 1955; COUTINHO et al., 1961; CAMPOS

et al., 1961; FERRIOLLI FILHO, 1961; LUMBRERAS, 1963; DIAS, 1968; ASAMI et al., 1970; FARIA, 1972; MARZOCHI & CARVALHEIRO, 1978; CAYMMI-GOMES, 1980). In these surveys, the Baermann method was effectively applied, indicating that the efficacy of this method was about 5 to 10 times that of the faecal concentration method (PEREIRA & CARNEIRO, 1955). In a comparative study in which the efficacy of direct smear, Baermann method and an agar plate culture were compared, it has been reported that the diagnostic efficacy of the agar plate culture was almost the same as the modified Baermann method, although the Baermann method has some advantages in terms of cost effectiveness and time in obtaining results. (KAMINSKY, 1993). Nevertheless, the agar plate culture is a sensitive new tool for the correct diagnosis of *Strongyloides* infection since approximately 40% of the cases were additionally diagnosed by the agar plate culture after examination by the Baermann method (KAMINSKY, 1993). Unfortunately, we could not compare, in the present study, the diagnostic efficacy of the agar plate culture and the Baermann method. The agar plate culture, as well as the Baermann method, should be applied for the diagnosis of strongyloidiasis in endemic areas to prevent severe infection in patients with an immunodepression risk factor.

RESUMO

Estudos sobre a prevalência da infecção por *Strongyloides* em Holambra e em Maceió, Brasil, pelo método de cultura de fezes em placa de ágar

Foi feito levantamento sobre a prevalência da infecção por *Strongyloides stercoralis* em três áreas do Brasil, através do desenvolvimento de método de cultura de fezes (cultura em placa de ágar). A infecção por *Strongyloides* foi confirmada em 11,3% de 432 pacientes examinados. A eficácia do diagnóstico pela cultura em placa de ágar foi de 93,9% comparado com apenas 28,5% e 26,5% pelo método de Harada-Mori de cultura em papel de filtro e método de concentração de fezes, quando amostras de fezes foram examinadas simultaneamente por estes três métodos. Entre as 49 amostras positivas, aproximadamente 60% foram confirmadas como positivas somente pela cultura em placa de ágar. Estes resultados indicam que a cultura em placa de ágar é um novo método sensível para o diagnóstico correto da infecção crônica pelo *Strongyloides*.

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