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PARASITOLOGICAL AND SEROLOGICAL STUDIES ON AMOEBIASIS AND OTHER INTESTINAL PARASITIC INFECTIONS IN THE RURAL SECTOR AROUND RECIFE, NORTHEAST BRAZIL

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SUMMARY

Parasitological examinations were carried out during July to December, 1989, on 485 inhabitants of four villages in São Lourenço da Mata, 25 km northwest of Recife, Pernambuco, Brazil. Approximately 99.6% of the inhabitants were infected with at least one species of intestinal parasites. A high prevalence of **Schistosoma mansoni** (82.1%), hookworm (80.2%) **Trichuris trichiura** (69.9%), **Ascaris lumbricoides** (61.9%) and **Entamoeba coli** (36.7%) infections were demonstrated. Test tube cultivation revealed that the most common species of hookworm in this region was **Necator americanus** (88.4%), and also that the prevalence of **Strongyloides stercoralis** was 5.8%.

Three hundred and thirty-four sera were serologically examined for amoebiasis by the gel diffusion precipitation test (GDP) and enzyme-linked immunosorbent assay (ELISA). No positive reaction was observed in all sera as examined by GDP, while 24 sera were positive by ELISA.

KEY WORDS: Parasitic helminths; Parasitic protozoa; Amoebiasis; Entamoeba histolytica; Schistosoma mansoni; Stool examination; Sero-epidemiology; Northeast Brazil.

INTRODUCTION

Prevalence of parasitic infections was considered to be one of the major parameter for evaluation of local public health status in developing countries. In our previous study[10], a high prevalence of intestinal parasitic infections was demonstrated in the inhabitants of a suburban area around Recife (Cabo), Pernambuco. Moreover, because a high level of infantile mortality has also been demonstrated in the northeast Brazil[22], public health condition of the rural sectors around Recife seemed to be low.

Although approximately 10% of the Cabo inhabitants were positive for **Entamoeba histo-**

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lytica cysts by stool examination, no positive reaction was observed by the gel diffusion precipitin test (GDP)[10]. GDP is generally considered to be one of the most reliable serological tests for diagnosis of amoebiasis[7, 11, 20], because it well reflects the clinical course of invasive amoebiasis. Therefore, our previous finding may be explained as follows; non-pathogenic strains of **E. histolytica**, according to the concept established by SARGEAUNT and his colleagues[14, 15, 16], was primarily distributed in the suburban area around Recife.

In the present study, parasitological and serological examinations were conducted to define a reliable prevalence of intestinal helminths and protozoa in a rural sector around Recife (São Lourenço), and compared with our previous data on the suburban area of Recife to obtain further sero-epidemiological characteristics of amoebiasis in the circumferential areas of Recife.

MATERIALS AND METHODS

From July to December, 1989, the inhabitants in four villages of São Lourenço da Mata; Ceramica Bicopeba (refered to as Field-I), Engenho Pitangueira (Field-II), Engenho Camurim (Field-III) and Engenho General (Field-IV), located 25 km northwest of Recife, were examined in this study. They primarily received general medical examination, and their age, sex, medical history as well as socio-economic state were recorded. Stools were also collected on this occasion. After stool examinations were completed, blood specimens were collected and sera were separated by centrifugation within 4 hours after bleeding.

A single stool specimen was examined by the modified formalin-ether centrifugation technique[6] and by test tube cultivation method[5] to identify filariform larva of hookworms and **Strongyloides.**

The antigen used for serologic tests of amoebiasis was prepared by the method of TAKEUCHI & KOBAYASHI[19] from axenic strain of **E. histolytica** (HM-1:IMSS), grown in BI-S-33 medium[4]. Further manipulations for extraction and preparation of the soluble amoebic antigen were done according to the methods described previously[10].

All sera were tested for antibody to **E. histolytica** by the gel diffusion precipitin test (GDP)[7] and enzyme-linked immunosorbent assay (ELISA)[10] as described previously.

RESULTS

Sex, age distribution and socio-economic condition of the inhabitants in four villages of São Lourenço da Mata were summarized in Table 1. Approximately one-third of the inhabitants were younger than 10 years old, another one-third were 11 to 30 years old, and the remainder were over 31 years old. Virtually all of their family members were employed by sugar-cane plantations as a farm hand, and their social condition was estimated to be low judging from their socio-economic characteristics.

Prevalence of intestinal parasites of 485 inhabitants of São Lourenço were summarized in Table 2. Approximately 99.6% of them were infected with at least one species of intestinal parasites. No significant difference was observed in the prevalence of the parasitic infections between males and females or among four fields.

The prevalence of intestinal helminths was summarized in Table 3. **Schistosoma mansoni** infection, with a rate of 82.1%, was most prevalent among the inhabitants of São Lourenço, and followed by hookworm (80.2%), **Trichuris trichiura** (69.9%), **Ascaris lumbricoides** (61.9%) and **Hymenolepis nana** (3.3%). One case with **Enterobius vermicularis**, one case with **Hymenolepis diminuta** and 3 cases with **Heterophyes** sp. infections were also found in this population. Moreover, **Toxocara canis** eggs were demonstrated in four stool specimens. Furthermore, as also shown in this Table, the prevalence of all helminthous infections except for **S. mansoni** in the field-II were significantly lower than those of three other fields.

Test tube cultivation revealed that **Necator americanus** infection was much more common than that of **Ancylostoma duodenale** in the inhabitants of São Lourenço. Among 389 inhabitants,

TABLE 1

Sex, age distribution and socio-economic feature of the study population of São Lourenço

	Field				
	I	II	III	IV	Total
Sex					
Male	16	13	79	137	245
Female	25	21	76	118	240
Age					
1-10	17	11	46	96	170
11-20	12	8	41	67	128
21-30	4	6	18	34	62
31-40	3	1	10	17	31
41-60	5	5	27	32	69
60 +	0	3	13	9	25
Total	41	34	155	255	485
Occupation	← Farm hand →				
Income of family (%)					
0-500 US$/year 501 +	100	100	100	100	100
Toilet (%)					
absent	100	100	97	83	91
present	0	0	3	17	9
Number of family members (%)					
1-3	0	14	41	27	28
4-7	67	43	38	60	53
8 +	33	43	21	13	19

who discharged hookworm eggs in their stools, 334 subjects (88.4%) were positive for **N. americanus**, whereas **A. duodenale** infection was demonstrated in only 10 subjects of them. This procedure also demonstrated the prevalence of **Strongyloides stercoralis** infection was 5.8%.

The prevalence of intestinal protozoan infection was summarized in Table 4. **Entamoeba coli** infection (36.7%) was most prevalent among the inhabitants of São Lourenço, and followed by **Giardia lamblia** (7.8%), **Endolimax nana** (4.3%), **E. histolytica** (4.1%) and **Iodamoeba buetschlii** (3.7%). Two cases with **Entamoeba hartmanni** and 2 cases with **Chilomastix mesnili** infections were also found in this population. Moreover, the present examination showed that the prevalence of **E. histolytica** and **G. lamblia** in the field-II were much higher than those of three other fields, respectively.

As mentioned above, stool examination demonstrated the overall prevalence rate of **E. histolytica** cysts in the inhabitants of São Lourenço was 4.1%. Therefore, as shown in Table 5, 334 serum specimens were collected and examined serologically by GDP. No positive reaction was observed in the sera examined, although 5 positive control sera, obtained from Japanese pa-

TABLE 2
Prevalence of intestinal protozoan and helminthous infections in the study population of São Lourenço

	Field				
	I	II	III	IV	Total
Total number of stools examined	41	34	155	255	485
Number of positive cases for parasitic infections	41 (100)	34 (100)	154 (99.4)	253 (99.2)	482 (99.4)
Male	16 (100)	13 (100)	79 (100)	136 (99.3)	244 (99.6)
Female	25 (100)	21 (100)	75 (98.7)	117 (99.2)	238 (99.2)
Number of positive cases for protozoan infections	20 (48.8)	22 (64.7)	74 (47.7)	104 (40.8)	220 (45.4)
Number of positive cases for helminthous infections	41 (100)	34 (100)	154 (99.4)	251 (98.4)	480 (99.0)

Values in parentheses represent the prevalence rate (%) of each region.

TABLE 3
Prevalence of helminthous infections in the inhabitants of São Lourenço

	Field				
	I	II	III	IV	Total
Ascaris lumbricoides	28 (68.3)	10 (29.4)	92 (59.4)	170 (66.7)	300 (61.9)
Trichuris trichiura	20 (48.8)	9 (26.5)	114 (73.5)	196 (76.9)	339 (69.9)
Hookworm	35 (85.4)	17 (50.0)	125 (80.6)	212 (83.1)	389 (80.2)
Ancylostoma duodenale	2 (4.9)	0	2 (1.3)	6 (2.4)	10 (2.1)
Necator americanus	31 (75.6)	14 (41.2)	112 (72.3)	187 (73.3)	344 (70.9)
Unknown	2 (4.9)	3 (8.8)	11 (7.1)	19 (7.5)	35 (7.2)
Strongyloides stercoralis	1 (2.4)	1 (2.9)	6 (3.9)	20 (7.8)	28 (5.8)
Enterobius vermicularis	0	0	1 (0.6)	0	1 (0.2)
Toxocara canis	0	0	2 (1.3)	2 (0.8)	4 (0.8)
Hymenolepis nana	0	1 (2.9)	0	15 (5.9)	16 (3.3)
Hymenolepis diminuta	0	0	0	1 (0.4)	1 (0.2)
Heterophyes sp.	0	1 (2.9)	1 (0.6)	1 (0.4)	3 (0.6)
Schistosoma mansoni	31 (75.6)	34 (100.0)	140 (90.3)	193 (75.7)	398 (82.1)

Values in parentheses represent the prevalence rate (%) of each region.

tients with confirmed amoebic liver abscess, formed 3 to 5 clear precipitation lines.

As also shown in Table 5, however, the positive reaction by ELISA was observed on 24 out of 334 sera examined. Two (15.4%) out of 13 sera isolated from the subjects, who discharged **E. histolytica** cysts in their stools, exhibited positive reaction of ELISA, while twenty-two (6.9%) out of 321 cyst-negative subjects were also judged positive by ELISA.

TABLE 4
Prevalence of intestinal protozoan infections in the inhabitants of São Lourenço

	Field				
	I	II	III	IV	Total
Giardia lamblia	7 (17.0)	9 (26.5)	12 (7.7)	10 (3.9)	38 (7.8)
Entamoeba histolytica	2 (4.9)	5 (14.7)	6 (3.9)	7 (2.7)	20 (4.1)
Entamoeba coli	16 (39.0)	11 (32.4)	61 (39.4)	90 (35.3)	178 (36.7)
Entamoeba hartmanni	0	0	1 (0.6)	1 (0.4)	2 (0.4)
Endolimax nana	1 (2.4)	2 (5.9)	3 (1.9)	15 (5.9)	21 (4.3)
Iodamoeba buetschlii	3 (7.3)	3 (8.8)	7 (4.5)	5 (2.0)	18 (3.7)
Chilomastix mesnili	0	0	1 (0.6)	1 (0.4)	2 (0.4)

Values in parentheses represent the prevalence rate (%) of each region.

TABLE 5
Result of serological examinations for amoebiasis by GDP and ELISA on the inhabitants of São Lourenço.

Field	Cyst	GDP		ELISA		TOTAL
		+	−	+	−	
I	+	0	2	0	2	2
	−	0	25	2	23	25
	Total	0	27	2	25	27
II	+	0	5	1	4	5
	−	0	26	2	24	26
	Total	0	31	3	28	31
III	+	0	3	0	3	3
	−	0	103	8	95	103
	Total	0	106	8	98	106
IV	+	0	3	1	2	3
	−	0	167	10	157	167
	Total	0	170	11	159	170
Total	+	0	13	2	11	13
	−	0	321	22	299	321
	Total	0	334	24	310	334

DISCUSSION

Our present investigation indicates that the inhabitants of São Lourenço were heavily infected with intestinal parasites. The overall prevalence of this population were 99.4%, which seems to be compatible with that of Cabo area[10]. The high prevalence of these parasites in the rural

sector around Recife suggests that these parasitic infections are essential in the public health of agricultural zones with aboundat water resource, which play an important role in transmission of these parasites.

Test tube cultivation revealed that approximately 88.4% of the inhabitants in São Lourenço who discharged hookworm eggs in their stools were infected with **N. americanus**, which agrees with previous findings by us[10] and others[1]. Therefore, the most common species of hookworm in northeast Brazil seems to be **N. americanus**. This technique also demonstrated that the prevalence of **S. stercoralis** infection was 5.8% in the inhabitants of São Lourenço. As reported previously, test tube cultivation method showed that 9.6% of Cabo inhabitants[10] and 5.1% of out-patients of UFPE hospital[1] were infected with **S. stercoralis**. Therefore, **S. stercoralis** infection appears common in the suburban and rural regions around Recife.

Stool examination also revealed four positive cases for **T. canis** eggs in the stools of inhabitants in São Lourenço. This finding probably indicates that ingested **T. canis** eggs pass through human alimentary tract. Therefore, it is likely that the environment such as a playground may be heavily contaminated with **T. canis** eggs. Although a high incidence of toxocaral visceral larva migrans[17, 18] is expected in the rural sector around Recife, parasitological and epidemiological characteristics of this disease in the northeast Brazil remain obscure. Therefore, seroepidemiological study on **T. canis** infection will be carried out with the inhabitants of the rural sector around Recife.

The present study also demonstrated that three subjects were positive for the eggs of the genus of **Metagonimus** or **Heterophyes** in their stools. Classification of **Heterophydae** according to the morphological characteristics of the eggs *is generally* difficult[8] so that the term "**Heterophyes** sp." was used in the present study. Moreover, because a human case of **Heterophyes** infection has not been reported in Brazil[12, 13], it seems interesting to identify and classify this parasite. The morphological characterization of the adult worm which obtained by chemotherapy with bithionol is under investigation.

Approximately 82% of the inhabitants in São Lourenço were found positive for **S. mansoni** eggs by stool examination. Since some epidemiological studies[2, 3] have already demonstrated a high prevalence of this parasite in primary school pupils in São Lourenço, schistosomiasis is obviously of the most important parasitic diseases in this region.

A high prevalence of intestinal protozoan infections was also demonstrated in the present study. However, the prevalence of all intestinal protozoa except for **E. coli** reported here were apparently lower than those of the Cabo inhabitants[10]. For example, the prevalence of **E. histolytica** and **G. lamblia** were approximately one-half of those of Cabo inhabitants, respectively.

Twenty out of 485 subjects were positive for **E. histolytica** cyst by stool examination. It, therefore, indicates that **E. histolytica** infection is also distributed widely in São Lourenço. To estimate the reliable morbidity of invasive amoebiasis in this region, 334 serum specimens were examined by two serological tests. As shown in Table 5, none of the sera was positive by GDP, although these specimens included 13 sera isolated from the **E. histolytica** cyst-positive subjects. The present finding, therefore, suggests that there may be few case of invasive amoebiasis in the rural sector around Recife, as suggested in our previous study[10]. In other words, non-pathogenic strains of **E. histolytica**, according to the concept established by SARGEAUNT and his colleagues[14, 15, 16], is primarily distributed in the rural sector around Recife. This speculation is supported by recent evidence[9] that **E. histolytica** isolated from asymptomatic school children in Recife and its suburban area showed only non-pathogenic zymodeme patterns. Thus, it seems likely that **E. histolytica** is less important than generally expected as a causative agent of diarrheal disease in Recife and its circumferential areas.

On the other hand, as also shown in Table 5, 24 out of 334 sera were positive by ELISA. It is noteworthy that 15.4% of the cyst carriers exhibited positive reaction by ELISA in the present study, which is much higher than that of stool-negative subjects (6.9%). Recently, TA-

KEUCHI et al[21] reported that ELISA could demonstrate an anti-amoebic antibody over 80% of sera isolated from the asymptomatic cyst carriers, who were all negative by GDP. Although there is much difference in the positive rate by ELISA between these two observations, these findings may suggest that even asymptomatic cyst carriers with negative serology by the currently standard serodiagnostic methods are often associated with the production of low-levels of anti-amoebic antibody which can be detected only by a sensitive method like ELISA. To further characterize the epidemiological feature of amoebiasis in northeast Brazil, an extensive epidemiological survey utilizing serological and parasitological techniques seems to be necessary.

RESUMO

Estudos sorológicos e parasitológicos na amebíase e em outras infecções parasito-intestinais no setor rural dos arredores de Recife, nordeste do Brasil.

Foram realizados exames parasitológicos em 485 habitantes de quatro vilarejos da cidade de São Lourenço da Mata, distante 25 km à noroeste de Recife-PE, Brasil, no período de julho à dezembro de 1989. Aproximadamente 99,6% dos examinados mostraram-se infectados com pelo menos uma espécie de parasita intestinal. Observou-se ainda uma alta prevalência de **Schistosoma mansoni** (82,1%), ancilostomídeos (80,2%), **Trichuris trichiura** (69,9%), **Ascaris lumbricoides** (61,9%) e **Entamoeba coli** (36,7%).

A cultura de larvas de ancilostomídeos nas fezes — método de Harada — revelou que **Necator americanus** (84,4%) é a espécie mais comum nesta região seguida pelo **Strongyloides stercoralis** (5,8%).

Foram ainda realizados testes sorológicos — imunodifusão em gel (GDP) e enzima imunoensaio (ELISA) — em 334 soros, para o diagnóstico de amebíase, todavia apenas 24 (7,2%) dos soros apresentaram positividade no teste de ELISA e nenhuma positividade foi encontrada nos mesmos soros testados pelo GDP.

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