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PARASITOLOGICAL AND SEROLOGICAL STUDIES ON AMOEBIASIS AND OTHER INTESTINAL PARASITIC INFECTIONS IN RECIFE AND ITS SUBURBAN AREA, NORTHEAST BRAZIL

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SUMMARY

Parasitological examinations were carried out during April to August, 1987, with 187 out-patients of the IMIP hospital, located in the center of Recife City, and 464 inhabitants of several villages around Cabo City, 50 Km southeast of Recife, Pernambuco, Brazil. Approximately 71% of the IMIP patients and 92% of the Cabo inhabitants were infected with at least one species of intestinal parasite. There was minimum difference in the prevalence rate of **Trichuris trichiura** between two areas, whereas the prevalence rates of **Ascaris lumbricoides**, hookworms, **Strongyloides stercoralis, Schistosoma mansoni** and **Entamoeba histolytica** were higher in the inhabitants of the Cabo City area. Only **Giardia lamblia** was more prevalent in the out-patients of IMIP hospital. Test tube cultivation revealed that the prevalence rate of **Necator americanus** in both areas was much higher than that of **Ancylostoma duodenale** , and also that the prevalence rate of **S. stercoralis** of the IMIP patients and Cabo inhabitants were 4.5% and 9.6%, respectively.

Six hundred and fifteen sera were serologically examined for amoebiasis by the gel diffusion precipitation test (GDP) and enzyme linked immunosorbent assay (ELISA) using the antigen prepared from axenically cultured trophozoite of **E. histolytica** (strain HM-1:IMSS). No positive reaction was observed in all of the sera as examined by GDP, while 32 out of 615 sera were positive on ELISA.

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

INTRODUCTION

An analysis of the annual statistics on the population in northeast Brazil demonstrated that there has been a high level of infantile mortality. Main causes of this high mortality were

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assumed to be malnutrition and infectious diseases by bacteria, virus and parasite [30]. Parasitic infections do not necessarily cause serious disease in the human host, but they interfere with the nutritional status and ultimately lead them more susceptible to other diseases. Prevalence rate of parasitic infections can, therefore, be one of the major parameters of local public health of the inhabitants anywhere.

The prevalence of **Entamoeba histolytica** in developing countries is often assumed to be high, frequently without supporting data. Major reasons for this may be that proper identification of **E. histolytica** cyst in stool is difficult without professional experience, and that reliable diagnosis of amoebiasis is often not attainable by stool examination alone. Now, serologic procedures have been commonly utilized for the diagnosis of amoebiasis in many countries because these tests are highly specific and have a sufficient sensitivity[9, 10, 13, 16, 19, 25]. In northeast Brazil, the prevalence of **E. histolytica** has been estimated to be high, but the incidence of amoebic liver abscess at autopsy was reported to be very low[20]. Such discrepancies are apparently due to lack in reliable data on the epidemiology and estimation on annual morbidity by this parasite, since serological examination for amoebiasis is still not common in this area.

Parasitological examination of intestinal helminths and protozoa, and serological examination for amoebiasis were carried out during April to August, 1987, in Recife and its suburban area. This report presents the results of these studies as an attempt to define a reliable prevalence rate in intestinal helminths and protozoa, in particular that of amoebiasis in this area.

MATERIALS AND METHODS

From April to August, 1987, the out-patients of the hospital of Instituto Materno Infantil de Pernambuco (IMIP), located in the center of Recife, were selected at random (10 to 30 patients per day) and their general health conditions were preliminarily checked. On this occasion, age, sex, the place of residence as well as socio-economic state of the family of the patient were recorded, and the peripheral blood was collected. Sera were separated by centrifugation at the Laboratório de Imunopatologia Prof. Keizo Asami (LIKA), Federal University of Pernambuco. Fresh stools were collected within a couple of days after the first visit. Inhabitants in several villages around Cabo City, located 50 Km southeast of Recife, also primarily received general medical examination, and their age, sex, medical history as well as socio-economic state were recorded. Blood and stools were collected at this time, and sera were separated in LIKA within 4 hours after bleeding.

A single stool specimen collected as above was examined by the direct smear[11], by the formalin-ether centrifugation technique (MGL method)[21], and by test tube cultivation method[8] to distinguish filariform larve of hookworms and **Strongyloides.** Ninety-eight stools, including 16 positive stools for **E. histolytica** cysts, were inoculated into the Balamuth medium[3] and the trophozoite growth were examined after cultivation for 72 hours at 27°C. All parasitological examination of stools were done by a single medical parasitologist, who had sufficient experience on parasitological diagnosis.

The antigen used for serologic test of amoebiasis was prepared by the method of TAKEUCHI & KOBAYASHI[25] from axenic strain of **E. histolytica** (HM 1:IMSS), cultured in BI-S-33 medium[6]. The lyophilized antigen was dissolved in 20 mM sodium phosphate buffer, pH 7.2. After stirring for 30 min under ice cold condition, the solution was centrifuged at 15,000 g for 30 min at 4 °C. The supernatant fluid was filtered by Millex-GS filter (Millipore Co. Bedford, MA, USA) at 0.2μm porosity and kept at — 70°C until use.

All sera were tested for antibodies to **E. histolytica** by the gel diffusion precipitation test (GDP)[13] and enzyme linked immunosorbent assay (ELISA) according to the modified method of MATSUDA et al.[15]. ELISA was performed in 96-well microtiter flat-bottomed plate (NUNC Co. Denmark). The optimum antigen concentration was determined by checker board titration between serial dilution of antigen against positive and negative reference sera. Half number of wells in a plate were sensitized with 100μl of the antigen prepared as above at concentration of 10μg protein per ml in 50 mM carbonate buffer, pH 8.15, in a moist chamber at 37°C for 2 hours. Non-sensitized wells were filled with 100μl of car-

bonate buffer alone. The plate was washed three times with 150 mM sodium phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 (PBS/T). After washing the wells, 200 μl of PBS/T containing 2% bovine serum albumin (BSA, Sigma Chem., St Lois, MO, USA) was added to all wells to block non-specific immunoglobulin binding, and the plate was incubated at 37 °C for 60 min. After washing the wells with PBS/T, test sera diluted 200 times with PBS/T containing 1% BSA were placed in a sensitized and non sensitized wells and the plate further incubated at 37°C for 60 min. After washing with PBS/T, 100 μl of horseradish peroxidase (HRP) labelled anti-human IgG rabbit immunoglobulin (Miles Scientific, Naperville, IL, USA), 10^4-fold diluted, was added and incubation was carried out at 37°C for 60 min. After washing the wells 3 times with PBS/T, 200 μl of the substrate solution, i.e., 30 mg diammonium salt of 2,2'-azino-bis (3-ethylbenzthiazoline-sulfonic acid) (Sigma Chem.) dissolved in a mixture of 100 mM citric acid and 200 mM sodium phosphate dibasic, pH 4.6, with 10 μl of H_2O_2 (30%), was added to each well and then the plate was kept at room temperature for 60 min. Subsequently, the absorbance of reaction mixture was measured by EIA Reader (Model 2550, Bio Rad Lab., Tokyo) at 405 nm. To correct daily variation of absorbance, triplet of positive reference serum (isolated from a Japanese patient with confirmed amoebic liver abscess) were included on each plate, and the corrected absorbance of sample was calculated according to the modified method of VOLLER et al[29]. as follows. Coefficient value was first calculated by the equation (1), and then the corrected absorbance of sample serum was calculated by multiplying the absorbance of sample serum by the coefficient value according to equation (2).

$$a = 1.5 / (Pa - Pb) \quad (1)$$

$$Sc = (Sa - Sb) \times a \quad (2)$$

in which

a: the coefficient value
Pa: mean absorbance of positive controls at antigen positive well
Pb: mean absorbance of positive controls at antigen-free well
Sa: absorbance of sample serum at antigen positive well
Sb: absorbance of sample serum at antigen-free well
Sc: the corrected absorbance of sample serum

Protein concentration was determined by the method of LOWRY et al.[12], with bovine serum albumin (fraction V) as a standard.

RESULTS

Sex, age distribution and socio-economic condition of the out-patients of IMIP hospital and the inhabitants in Cabo City area were summarized in Table 1. Virtually all of the IMIP patients were under 9 years old, and only 4% of them were from 10 to 12 years old. Most of the IMIP patients inhabited in the downtown or urban area of Recife. Approximately one-third of the Cabo inhabitants who took our health examination were under 9 years old, another one-

TABLE 1
Sex, age distribution and socio-economic feature of the out patients of IMIP hospital and the inhabitants of Cabo City area

		IMIP patients	Cabo inhabitants
Sex	Male	100 (53.5%)	178 (38.4%)
	Female	87 (46.5%)	286 (61.6%)
Age	0-9	179 (96.0%)	156 (33.6%)
	10-19	8 (4.0%)	153 (33.0%)
	20-39	0	81 (17.5%)
	40 ≤	0	74 (15.9%)
Occupation			Farm hand (100%)
Income (US $/year)	0-400	127 (67.9%)	464 (100%)
	401-1000	53 (28.3%)	0
	1,001 ≤	7 (3.7%)	0
House	Wooden	54 (28.8%)	128 (27.6%)
	Brick	131 (70.0%)	336 (72.4%)
	Apartment	2 (1.2%)	0
Number of room	1	31 (16.6%)	47 (10.2%)
	2-3	151 (80.7%)	406 (87.5%)
	4 ≤	5 (2.7%)	11 (2.3%)
Toilet	present	142 (76.0%)	293 (63.2%)
	absent	45 (24.0%)	171 (36.8%)
Number of family member	1-3	20 (10.8%)	
	4-7	125 (66.9%)	
	8 ≤	42 (22.3%)	
Total		187	464

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

third were 10 to 19 years old, and the remainder were over 20 years old. All of them inhabited in the several villages located in the rural area around Cabo City and their family members were employed by the farm of sugar-cane as a farm hand. Also demonstrated in Table 1 was that the social condition of the families of IMIP patients and Cabo inhabitants seemed to be low judging from their socio-economic characteristics. There was, however, a little difference in the total amounts of income per year between the families of IMIP patients and Cabo inhabitants.

Prevalence of intestinal parasites of the IMIP patients and the Cabo inhabitants were summarized in Table 2. Approximately 71.2% of the IMIP patients and 91.9% of the Cabo inhabitants were infected with at least one species of intestinal parasite. The lower prevalence of intestinal parasites in the IMIP patients was also found as compared with that of the Cabo inhabitants utilizing comparable age groups, under 12 years old. There was no significant difference in the prevalence rate between males and females in both groups.

Prevalence of intestinal helminths was summarized in Table 3. **Trichuris trichiura** infections, with a rate of 48.1%, was the most prevalent parasite in the IMIP patients, followed by **Ascaris lumbricoides** (26.9%), hookworm (6.4%), **Strongyloides stercoralis** (4.5%) and **Schistosoma mansoni** (1.3%). In contrast, the Cabo inhabitants, regardless of age, showed much higher prevalence rate of intestinal helminths. Hookworm infections, with a rate of 66.7%, was the most prevalent one, followed by **A. lumbricoides** (62.1%), **T.trichiura** (60.1%), **S. stercoralis** (9.6%) and **S. mansoni** (9.6%). Two cases of **Enterobius vermicularis** and one case of **Hymenolepis diminuta** infections were also found in the Cabo inhabitants.

Test tube cultivation revealed that **Necator americanus** infection was much more common than of **Ancylostoma duodenale** in both areas (87.5% vs. 12.5% in the IMIP patients and 93.0% vs. 7.0% in the Cabo inhabitants). This procedure also demonstrated a high prevalence rate of **S. stercoralis** infection in both IMIP patients (4.5%) and Cabo inhabitants (9.6%).

Prevalence of intestinal protozoa was summarized in Table 4. **Giardia lamblia** infection (26.3%) was the most prevalent one in the IMIP patients, followed by **Entamoeba coli** (17.3%), **Endolimax nana** (13.5%), **Entamoeba histolyti-**

TABLE 2
Prevalence of intestinal protozoa and helminths in the out-patients of IMIP hospital and the inhabitants of Cabo City area

	IMIP patients	Cabo inhabitants		
	0-12 yrs	0-12 yrs	13 yrs ≦	Total
Total number of stools examined	156	170	289	459
Number of positive cases for parasitic infections	111 (71.2%)	159 (93.5%)	263 (91.0%)	422 (91.9%)
Male	58 (68.2%)	59 (89.4%)	104 (92.9%)	163 (91.6%)
Female	53 (74.6%)	100 (96.2%)	159 (89.8%)	259 (92.2%)
Number of positive cases for helminthous infections	92 (58.9%)	149 (87.6%)	242 (83.7%)	391 (85.2%)
Number of positive cases for protozoan infections	66 (42.3%)	89 (52.4%)	151 (52.2%)	240 (52.3%)

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

TABLE 3
Prevalence of helminths in the out patients of IMIP hospital and the inhabitants of Cabo City area

Parasites	IMIP patients	Cabo inhabitants		Total
	0-12 yrs	0-12 yrs	13 yrs ≤	
Ascaris lumbricoides	42 (26.9%)	121 (71.1%)	164 (56.7%)	285 (62.1%)
Trichuris trichiura	75 (48.1%)	107 (62.9%)	169 (58.5%)	276 (60.1%)
Hookworm	10 (6.4%)	110 (64.7%)	196 (67.8%)	306 (66.7%)
Necator americanus	7 (4.5%)	95 (55.9%)	184 (63.7%)	279 (60.8%)
Ancylostoma duodenale	1 (0.6%)	2 (1.2%)	4 (1.4%)	6 (1.3%)
unknown	2 (1.3%)	14 (8.2%)	7 (2.4%)	21 (4.6%)
Strongyloides stercoralis	7 (4.5%)	18 (10.6%)	26 (9.0%)	44 (9.6%)
Enterobius vermicularis	0	2 (1.2%)	0	2 (0.4%)
Schistosoma mansoni	2 (1.3%)	19 (11.2%)	25 (8.7%)	44 (11.2%)
Hymenolepis diminuta	0	1 (0.6%)	0	1 (0.2%)

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

TABLE 4
Prevalence of intestinal protozoas in the out patients of IMIP hospital and the inhabitants of Cabo City area

Parasites	IMIP patients	Cabo inhabitants		Total
	0-12 yrs	0-12 yrs	13 yrs ≤	
Giardia lamblia	41 (26.3%)	24 (14.1%)	23 (8.0%)	47 (10.2%)
Entamoeba coli	27 (17.3%)	74 (43.5%)	91 (31.5%)	165 (35.9%)
Entamoeba histolytica	12 (7.7%)	18 (10.6%)	36 (12.5%)	54 (11.8%)
Entamoeba hartmanni	0	7 (4.1%)	8 (2.8%)	15 (3.3%)
Endolimax nana	21 (13.5%)	35 (20.6%)	57 (19.7%)	92 (20.0%)
Chilomastix mesnili	1 (0.6%)	2 (1.2%)	10 (3.5%)	12 (2.6%)
Iodamoeba buetschlii	2 (1.3%)	17 (10.0%)	26 (9.0%)	43 (9.4%)
Trichomonas hominis	4 (2.6%)	7 (4.1%)	14 (4.8%)	21 (4.6%)
Isospora belli	2 (1.3%)	0	0	0
Balantidium coli	0	0	2 (0.7%)	2 (0.4%)

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

ca (7.7%), and **Trichomonas hominis** (2.6%). **Chilomastix mesnili** (one case), **Iodamoeba buetschlii** (2 cases) and **Isospora belli** (2 cases) were also found. In the Cabo inhabitants, **E. coli** infection (35.9%) was the most prevalent, followed by **E. nana** (20.0%), **E. histolytica** (11.8%), **G. lamblia** (10.2%), **I. buetschlii** (9.4%), **T. hominis** (4.6%) and **Entamoeba hartmanni** (3.3%). Trophozoite of **Balantidium coli** was also found in two primary school pupils in Cabo.

Multiple infection was observed at 75.8% of the IMIP patients with parasitic infection, while a higher rate of multiple infection (84.6%) was demonstrated in the Cabo inhabitants (Fig. 1).

the upper limit of negative sera. Fig. 2 also showed that serum samples isolated from the IMIP patients infected with various parasites did not show a positive reaction except for one case with trichuriasis. From this observation, it is likely that there are scarcely cross reactions between **E. histolytica** antigen and sera isolated from various parasitic infections, and that our ELISA seems to be specific and have a sufficient sensitivity on epidemiological survey of amoebiasis.

As shown in Table 5, the positive reaction of ELISA was observed in 32 out of 615 sera examined. Nine (13.6%) out of 66 sera isolated from the IMIP patients and the Cabo inhabitants, who discharged **E. histolytica** cystis in their stools, exhibited positive reaction on ELISA, while twenty-three sera (4.1%) out of 549 cyst-negative subjects were also judged positive on ELISA.

Ninety-eight stool specimens were inoculated into Balamuth medium and then trophozoite growth was examined. No trophozoite growth was observed in all stools examined although 16 out of 98 stools were positive for **E. histolytica** cysts.

DISCUSSION

Our present investigation indicates that the out-patients of IMIP hospital and the inhabitants of Cabo City area were heavily infected with intestinal parasites. The overall prevalence rate of the IMIP patients and Cabo inhabitants were 71.2 and 91.9%, respectively, which are much higher than those of central and southern Brazil[7, 18, 27, 28]. There was significant difference in the prevalence rate of hookworms, **A. lumbricoides** and **S. mansoni**, but not of **T. trichiura**, between the IMIP patients (urban area) and the Cabo inhabitants (rural area). The higher prevalence of these parasites in such rural sectors suggests that these infections are a particular problem in agricultural areas with abundant water resource, which play an important role in maintaining these parasitic infections.

Test tube cultivation method revealed that approximately 87 to 93% of the IMIP patients and Cabo inhabitants, who discharged hookworm eggs, were infected with **N. americanus**. Using the same technique, ASAMI et al.[1] already demonstrated that prevalence of **N. americanus** in the patients of UFPE hospital was much higher than that of **A. duodenale**. Since MARZOCHI & CHIEFFI[14] also demonstrated higher prevalence rate of **N. americanus** in the inhabitants of Paraná State, southern Brazil, it seems likely that **N. americanus** is the main hookworm species in Brazil. This technique also demonstrated the high prevalence rate of **S. stercoralis** in both areas. In Cabo area, approximately 10% of the inhabitants were infected with this parasite. Since test tube cultivation method already showed 5.1% of patients in the UFPE hospital were infected with **S. stercoralis**[1], **S. stercoralis** infection appears still common in Recife area.

S. mansoni egg was detected in only 2 out-patients of IMIP hospital, whereas approximately 10% of the inhabitants in Cabo City area were found positive for the eggs by stool examination. An epidemiological study on schistosomiasis in São Lourenço village in Pernambuco, in 1975 and 1979, ASAMI et al.[2] detected schistosome eggs from over 40% of primary school pupils. BARBOSA[4] also demonstrated high prevalence rate of **S. mansoni** infection in the primary school pupils in Pernambuco State. Schistosomiasis is, therefore, still considered to be one of the most important parasitic diseases in the rural sector of Pernambuco State.

Our present examination also demonstrated a high prevalence rate of intestinal protozoa. In the rural area of Pernambuco, the prevalence rate of all intestinal protozoa except for **E. histolytica** reported here were much higher than those published before[2, 7, 18]. The high prevalence rate of intestinal protozoa, including pathogenic and non-pathogenic, is considered to be dependent on the contamination of drinking water and food with protozoan cyst in accordance with incomplete stool disposal by poor sanitary facilities. Among these intestinal protozoa, **G. lamblia** and **E. histolytica** were considered important in this area in respect to their pathogenicity. The difference in prevalence rate of **G. lamblia** between the IMIP patients and the Cabo inhabitants is interesting. Although this may be at least partially dependent on the difference in age distribution between these two groups, there was still a difference in the prevalence rate of

G. lamblia in comparing the same age groups from two areas. Therefore, the direct transmission (person to person)[5], in large communities such as school and/or playground, may be worth consideration for prevalence of this parasite.

Sixty-six out of 615 persons were demontrated to be positive for **E. histolytica** cyst by stool examination. It is, therefore, assumed that **E. histolytica** infection still distributed widely in Recife area. To estimate the reliable morbidity of invasive amoebiasis, serological tests were employed. As shown in Table 5, none of the sera was positive on GDP although these specimens included sera from the subjects with **E. histolytica** cyst in their stools and/or with various intestinal symptoms. In the previous studies[13, 19, 26] GDP was considered to be one of the most reliable serological tests for diagnosis of amoebiasis, since it is well correlated with the clinical course of symptomatic amoebiasis. Our observation, therefore, may be explained as follows: there was no case with invasive amoebiasis among the subjects examined in this study; rather, all of them with cyst in stool may be asymptomatic cyst carrier according to the concept established by SARGEAUNT and his colleagues[22, 23, 24]. In other words, avirulent strains of **E. histolytica** primarily distribute around Recife and its suburban area. This speculation is supported by previous observation that the incidence of amoebic liver abscess at autopsy was extremely low[20]. And by our preliminary finding that neither GDP nor ELISA detected anti-amoebic antibody in serum samples isolated from 20 or more hospitalized patients who were clinically diagnosed to have amoebic liver abscess or colitis.

As shown in Fig. 2, evaluation of ELISA method as a tool for sero-diagnostic method indicates that this method appears specific to **E. histolytica** antigen. However, ELISA could detect anti-amoebic antibody in 9 out of 66 persons with **E. histolytica** cyst in their stools and in 23 of 549 persons without cyst. Recently OKUSAWA et al.[17] reported that ELISA could demonstrate an IgG class anti-amoebic antibody over 80% of sera isolated from the asymptomatic cyst carrier. The difference between these two observations can be explainable by the difference in employed ELISA method. To characterize the epidemiological feature of amoebiasis in northeast Brazil, an extensive epidemiological survey utilizing serological and parasitological techniques as well as an establishment of axenic or monoxenic strain of **E. histolytica** isolated from this region seems to be absolutely necessary.

RESUMO

Estudos sorológicos e parasitológicos na Amebíase e em outras infecções parasitárias intestinais em Recife e áreas circunvizinhas, nordeste do Brasil

Exames parasitológicos foram realizados em 187 pacientes do Hospital do IMIP e 464 habitantes de vários vilarejos no município do Cabo, 50 Km à sudeste de Recife, durante os meses de abril a agosto. Aproximadamente 71% dos pacientes examinados do IMIP e 92% dos examinados do Cabo apresentavam-se infectados com, no mínimo, uma espécie de parasita intestinal.

Houve uma diferença mínima na taxa de prevalência de **Trichuris trichiura** entre as duas áreas, entretanto a prevalência de **Ascaris lumbricoides**, família **Ancylostomidae, Strongyloides stercoralis, Schistosoma mansoni** e **Entamoeba histolytica** foi maior entre os habitantes do Cabo. Somente a **Giardia lamblia** apresentou uma taxa de prevalência maior nos pacientes do IMIP.

O cultivo em tubo de ensaio revelou que a prevalência do **Necator americanus** em relação a do **Ancylostoma duodenale** era muito maior em ambas as áreas e que a do **S. stercoralis** entre os pacientes do IMIP e dos habitantes do Cabo era, respectivamente, 4.5% e 9.6%.

A amebíase foi verificada através de exames sorológicos, imunodifusão em gel (GDP) e enzima imunoensaio (ELISA), usando como antígeno extrato bruto preparado a partir dos trofozoitos de **E. histolytica** (cepa HM-1: IMSS), realizados em 615 soros, onde nenhuma reação positiva aparente foi observada através da imunodifusão, contudo foram observados resultados positivos em 32 dos 615 casos através da enzima imunoensaio.

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