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PREVALENCE AND PATHOGENICITY OF *ENTAMOEBA HISTOLYTICA* IN THREE DIFFERENT REGIONS OF PERNAMBUCO, NORTHEAST BRAZIL (1)

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SUMMARY

Parasitological examinations were carried out on 663 individuals of three different cities of Pernambuco State, Northeastern Brazil: Recife, Palmares and Bodocó. The population from a drought area of Pernambuco State, Bodocó, was investigated for amoebiasis and compared with Recife, metropolitan city (about 1.3 million of inhabitants) and another inland community, Palmares, located inside of the sugar-cane plantation region of the State. No evidence of invasive strains of *E. histolytica* were found in these inhabitants, provided that the isolated zymodemes I, III, IV, VIII, IX, X, XVII and XVIII are recognized as nonpathogenic strains of *E. histolytica*. Furthermore, the prevalence of intestinal helminths and other protozoan infections showed that these individuals are infected by other agents responsible for diarrhoeal diseases.

KEYWORDS: *Entamoeba histolytica*; Protozoan pathogenicity; Amebiasis; Northeast Brazil.

INTRODUCTION

Amoebiasis is an important public health problem in developing countries and it is the third cause of death among parasitic diseases. The percentage of the world population infected by *Entamoeba histolytica* is calculated to be 10% and 90% of these individuals are not symptomatic (WALSH, 1986).

Recent epidemiological studies on the occurrence of pathogenic and non pathogenic *E. histolytica* have been carried out in low income population (OKAZAKI et al., 1988; GONÇALVES et al., 1990; MAGALHÃES et al., 1990); mentally handicapped patients (NAGAKURA et al., 1990; SARGEAUNT et al., 1982); homosexuals (SARGEAUNT et al., 1983; TAKEUCHI et al., 1990); foreign students from tropical and Southern Africa and from Southern Asia (GIBODA et al., 1990); individuals returning from trips to tropical countries (WEINKE et al., 1990).

In Brazil, studies on pathogenicity of *E. histolytica* among low income population have shown a difference between the North and Northeast regions (CUNHA et al., 1977; NOZAKI et al., 1990). In the last eight years, 1987-1993, epidemiological studies of serology and zymodeme profiles on amoebiasis of Recife and its environs, in Pernambuco, have suggested that the majority of individuals infected with *E. histolytica* were asymptomatic carriers caused by nonpathogenic strains

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of *E. histolytica* (OKAZAKI et al., 1988; NOZAKI et al., 1990).

In this work, the prevalence and pathogenicity of *E. histolytica* strains was studied in low income populations of three cities of Pernambuco State (Northeastern Brazil): Recife, Palmares and Bodocó. These cities are located in the three different climatic regions of this part of the country.

MATERIAL AND METHODS

Fecal samples were collected from 663 individuals living in three different environmental areas of Pernambuco State, Northeast Brazil (Fig. 1): cities of Recife, Palmares and Bodocó. These individuals were selected among poor families living in either urban (Recife) or urban and rural areas (Palmares and Bodocó) under identical water supplies, sanitation, hygiene and crowding conditions. Recife is the capital. It is localized on the coast and has 1,296,195 inhabitants. Palmares is 125 km away from Recife, population 58,807, surrounded by sugar cane plantation, with a temperature of 24°C and high humidity. Bodocó is 649 km away from Recife and it is inside of the drought region of Northeastern Brazil, population of 28,523. The annual precipitation of Recife, Palmares and Bodocó is 2,243; 1,465 and 648 mm, respectively.

The fecal samples were examined by the direct smear (LENNETTE et al., 1974), by the formalin-ether centrifugation technique (MGL method, RITCHIE, 1948), and by the test tube cultivation method (HARADA et al., 1951) to distinguish filariform larvae of hookworms and *Strongyloides*. Fecal samples were also introduced into Robinson medium and incubated at 37°C and analyzed electrophoretically according to SARGEAUNT et al. (1984) for *E. histolytica* zymodeme characterization. Serological evaluation for the presence of antibodies against *E. histolytica* was performed in all individuals presenting *E. histolytica* in the stool. This evaluation was proceeded according to Gel Diffusion Precipitation Test (MADDISON, 1965) and Enzyme Linked Immunosorbent Assay (ELISA) (MATSUDA et al., 1984). The antigens used in this work were prepared as preconized by TAKEUCHI & KOBAYASHI (1980) and obtained from *E. histolytica* axenic strains (1:M-1-IMSS) grown in BI-S-33 axenic medium.

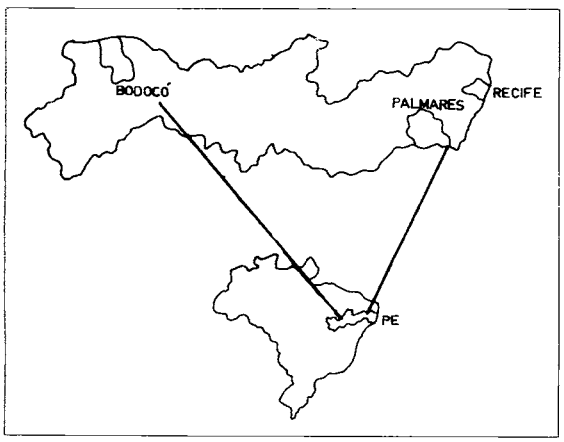

Fig. 1 - Map of Brazil showing the localization of Recife, Palmares and Bodocó inside of the Pernambuco State.

RESULTS

Table 1 shows the *E. histolytica* positivity among fecal samples of the individuals from Recife, Palmares and Bodocó, according to sex and age distribution of the inhabitants in these cities. It is worthwhile to notice that children younger than 10 years old represented half (51.34%) of the investigated population and only 10.6% of the individuals were older than 40 years. The different prevalence of *E. histolytica* in Recife, Palmares and Bodocó (18.0%, 31.5% and 36.3%, respectively) showed to be statistically significant (chi-square = 21.514; p=0.00002). However, there was no statistical significance in the sex and age distribution.

Table 2 presents the number of isolated isoenzyme zymodemes. The most prevalent zymodemes were found to be I and XVII. This represents 78.7% of the isolated zymodemes. Furthermore, the analysis of these two zymodemes prevalence showed values of 63.3%, 91.4% and 79.0% for Recife, Palmares and Bodocó, respectively.

The prevalence of intestinal helminths in the three cities is shown in Fig. 2. Firstly, one can notice that the prevalence profile in Recife and Palmares are similar. However, Bodocó showed lower intestinal helminths infection than Recife and Palmares. Furthermore, it is evident that *Necator americanus* and *Hymenolepis nana* are the most common infection in Bodocó whereas *Ascaris lumbricoides*, *Trichuris thichiura* and *Necator americanus* are more prevalent in Recife and Palmares. The statistical analysis of these different patterns showed to be significant, except for the *Strongyloides stercoralis*, *Necator americanus* (Recife

TABLE 1

Sex and age distribution of amoebiasis prevalence in the inhabitants of Recife, Palmares and Bodocó*

	Recife	%	Palmares	%	Bodocó	%	Total	%
Sex								
Male	24 (94)	25.5	21 (60)	35.0	43 (109)	39.4	88 (263)	33.5
Female	20 (150)	13.3	20 (70)	28.6	51 (150)	34.0	91 (370)	24.6
Total	44 (244)	18.0	41 (130)	31.5	94 (259)	36.3	179 (633)	28.3
Age (years)								
0-4	18 (116)	15.5	4 (30)	13.3	14 (55)	25.4	36 (201)	17.9
5-9	11 (44)	25.0	10 (29)	34.4	23 (51)	45.1	44 (124)	35.4
10-19	5 (30)	16.6	8 (23)	34.7	19 (61)	31.1	32 (114)	28.0
20-29	6 (20)	30.0	11 (26)	42.3	15 (40)	37.5	32 (86)	37.2
30-39	1 (14)	7.1	3 (10)	30.0	9 (17)	52.9	13 (41)	31.7
≥ 40	3 (20)	15.0	5 (12)	41.6	14 (35)	40.0	22 (67)	32.8
Total	44 (244)	18.0	41 (130)	31.5	94 (259)	36.3	179 (633)	28.3

The income of the families was not higher than US$ 1,000 per year

*- The number of examined individuals is in brackets.

versus Bodocó) and *Hymenolepis nana* (Palmares versus Bodocó) prevalences ($p \geq 0.05$).

Fig. 3 shows the prevalence of protozoan infection in the present study. Again, Bodocó showed a different pattern of prevalence compared with Recife and Palmares. Protozoan infections are more frequent among Bodocó inhabitants. *Entamoeba coli* and *histolytica* are the most prevalent protozoan infections even higher than in Recife and Palmares. It is also worthwhile to observe that some of protozoa were only found in Bodocó inhabitants (*Dientamoeba fragilis, Balantidium coli* and *Isospora belli*). The chi-square analysis of these different patterns also showed to be significant ($p \leq 0.05$).

DISCUSSION

Previous studies have shown that the zymodemes analysis of *E. histolytica* isolates in Pernambuco State are related to nonpathogenic strains (NOZAKI et al., 1990). Serologic studies also showed that antibodies against *E. histolytica* were not detected in any of the serum samples of the investigated individuals (OKAZAKI et al., 1988; GONÇALVES et al., 1990). According to these data, the invasive amoebiasis seems to be rare in the studied regions. This can be attributed to the absence of pathogenic strains or to the different infectivity between the pathogenic and nonpathogenic strains. In fact, the pathogenicity of 47 strains of *E. histolytica* isolated in Pernambuco, Brazil, was examined using the polymerase chain reaction (PCR) followed by restriction-endonuclease digestion (TACHIBANA et al., 1992). This study also demonstrated that nonpathogenic strains of *E. histolytica* predominate in northeastern Brazil. Although *E. histolytica* is highly prevalent in northeastern Brazil it seems that diarrhoeal diseases can be caused by other agents. In this region, the most frequently identified pathogen in

TABLE 2

Numbers of isolated isoenzyme zymodemes of *E. histolytica* in the inhabitants of Recife, Palmares and Bodocó

	Zymodemes								
City	I	III	IV	VIII	IX	X	XVII	XVIII	Total
Recife (18%)	11	3	0	3	0	0	8	5	30
Palmares (31.5%)	16	2	0	0	1	0	16	0	35
Bodocó (36.3%)	22	1	1	3	0	1	12	3	43
Total	49	6	1	6	1	1	36	8	108

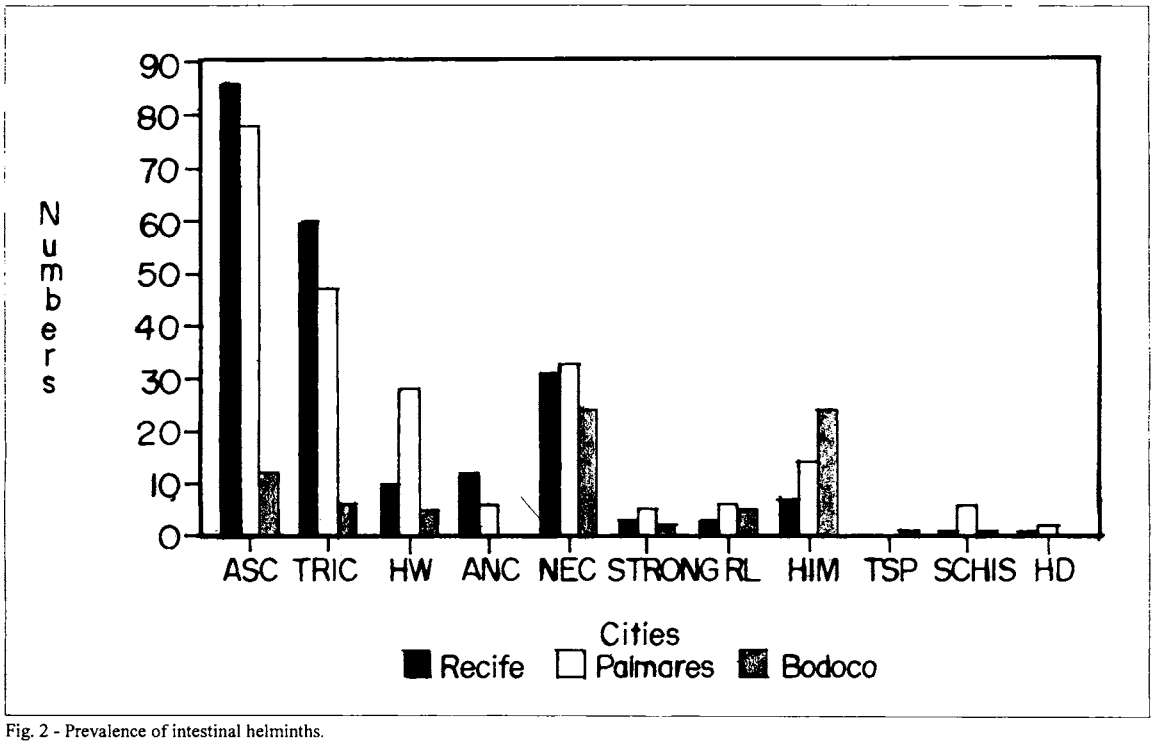

Fig. 2 - Prevalence of intestinal helminths.
ASC - *Ascaris lumbricoides*; TRIC - *Trichuris trichiura*; HW - *Hookworm*; ANC - *Ancilostoma duodenalis*; NEC - *Necator americanus*; STRONG - *Strongyloides stercoralis*; RL - *Rabditoides larva*; HIM - *Hymenolepis nana*; TSP - *Taenia sp*; SCHIS - *Schistosoma mansoni*; HD - *Hymenolepis diminuta*.

children was Enteropathogenic *Escherichia coli* and Rotavirus where *E. histolytica* did not show significant case control difference (MAGALHÃES et al., 1990). Furthemore, in this work, the prevalence of intestinal helminths and other protozoan infections in these same populations showed that these individuals are usually hosts of several other agents responsible for diarrhoeal diseases.

In the present study, a population from a drought area of Pernambuco State, Bodocó, was investigated and compared with Recife, metropolitan city about 1.3 milion of inhabitants) and another inland community, Palmares, located inside of the sugar-cane plantation region of the state, where poverty is a dramatic social problem. Again, no evidence of invasive strains of *E. histolytica* were found in these inhabitants, provided that the isolated zymodemes I, III, IV, VIII, IX, X, XVII and XVIII are recognized as nonpathogenic strains of *E. histolytica* (SARGEAUNT et al., 1984).

These results represent one more evidence for the high prevalence of nonpathogenic strains of *E. histolytica* in Northeastern Brazil. The relevance of such observation can play an important role on new clinical approach towards the treatment of diarrhea in this region of Brazil.

RESUMO

Prevalência e patogenicidade da *Entamoeba histolytica* em três regiões diferentes do estado de Pernambuco, Nordeste Brasileiro

Exames parasitológicos foram realizados em amostras fecais de 663 indivíduos residindo em três cidades do estado de Pernambuco, Nordeste brasileiro: Recife, Palmares e Bodocó. A população de Bodocó, situada na região do semi-árido de Pernambuco, foi investigada para amebíase e comparada com a do Recife, cidade metropolitana com cerca de 1,3 milhões de habitantes, e com outra da zona de plantação de cana-de-açucar do estado, Palmares. Não foi observada evidência da presença de cêpas invasivas de *E. histolytica* nesses habitantes desde que aquelas isoladas das amostras fecais, com zimodomas dos tipos I, III, IV, VIII,

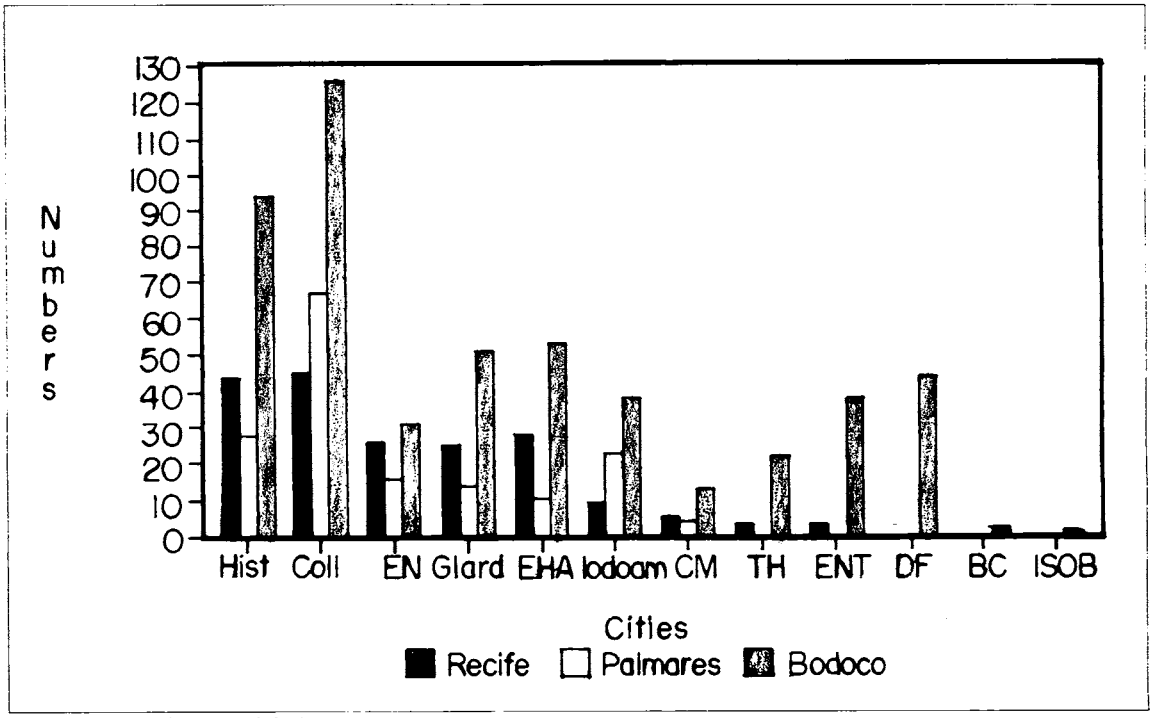

Fig. 3 - Prevalence of protozoan infection.
Hist - *Entamoeba histolytica*; Coli - *Entamoeba coli*; EN - *Endolimax nana*; Giard - *Giardia lamblia*; EHA - *Entamoeba hartmanni*; Iodoam - *Iodamoeba butschlii*; CM - *Chilomastix mesnili*; TH - *Trichomonas hominis*; ENT - *Enteromonas*; DF - *Dientamoeba fragilis*; BC - *Balantidium coli*; ISOB - *Isospora belli*.

IX, X, XVII e XVIII, são reconhecidas como não patogênicas. Ademais, a prevalência de helmintos intestinais e de infecções por protozoários mostrou que esses indivíduos são infectados por outros agentes responsáveis por quadros diarréicos.

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