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BRIEF COMMUNICATION

PREVALENCE OF *Entamoeba histolytica/Entamoeba dispar* IN THE CITY OF CAMPINA GRANDE, IN NORTHEASTERN BRAZIL

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SUMMARY

There is a clear need to perform epidemiological studies to find the true prevalence of *Entamoeba histolytica* around the world. The evaluation of this prevalence has been hindered by the existence of two different species which are morphologically identical, but genetically different, namely *E. histolytica,* which causes amebiasis, and *E. dispar,* which is non-pathogenic. In Brazil, the *E. dispar* has been detected in communities in the Southeastern (SE) and Northeastern (NE) regions with poor sanitation. However, individuals infected with *E. histolytica* have been identified in other regions. There is an absence of reports on the prevalence of these parasites in the state of Paraíba, which also has areas with poor sanitary conditions where a high prevalence of the *E. histolytica/E. dispar* complex has been detected in children from urban slums. The present study evaluated the prevalence of *E. histolytica* and *E. dispar* in 1,195 asymptomatic children between two and 10 years of age, living in a sprawling urban slum in Campina Grande, in the state of Paraíba, in Northeastern Brazil. These children were examined and their feces samples were analyzed microscopically. A total of 553 children tested positive for the *E. histolytica/E. dispar* complex, and 456 of the positive samples were tested with the *E. histolytica* II® ELISA kit. All 456 samples were negative for the presence of the adhesin *E. histolytica* specific antigen. The evidence suggests that in this community *E. histolytica* is absent and *E. dispar* is the dominant species.

KEYWORDS: *Entamoeba histolytica; Entamoeba dispar*; Prevalence; Adhesin detection immunoassay.

INTRODUCTION

Until 1993, the studies on the prevalence of amoebiasis showed that approximately 10% of the world's population was infected with *Entamoeba histolytica*. However, only 1% of these infections developed into an invasive form of the disease, with a mortality factor of about 100,000 cases per year[26,27]. *Entamoeba histolytica* was redescribed and separated into two species: *Entamoeba dispar,* non-pathogenic to humans; and *Entamoeba histolytica*, pathogenic and the cause of invasive intestinal and extra intestinal disease[8,16]. These species are morphologically identical, but new methods for parasite detection based on enzymes, lectin antigens, or DNA sequencing have been extremely helpful in determining the most current data[1,11,18,21,27]. These studies show that *E. dispar* infection is more frequent than *E. histolytica* infection in most of the endemic regions[2,12,13,14,15,20]. An accurate worldwide distribution of these species has not yet been determined. It is important to obtain complete epidemiological profiles, particularly in case of pediatric patients, to avoid unnecessary antiamoebic pharmacotherapy. The Pan American Health Organization does not recommend treatment to asymptomatic individuals presenting cysts of *E. histolytica/E. dispar* in stools without the specific identification of *E. histolytica*[26].

The prevalence of *E. histolytica* infection is variable among different geographic regions in Brazil due to differences in sanitation and socioeconomic conditions. For example, the city of Belém, in the state of Pará, in Northern Brazil, presents a high frequency of hepatic amoebiasis cases (29.35%)[19,22]. In neighboring regions, such as in the city of Manaus, Amazonas State, the *E. histolytica* prevalence is 6.8%, whereas in the Northeast city of Fortaleza, Ceará, the prevalence is 14.9%[3,5]. On the other hand, in the city of Maceió, Alagoas, and in several cities, in Pernambuco (in Northeastern Brazil), several parasites are endemic due to poor sanitation. However, no autochthonous cases of *E. histolytica* are present in spite of the great prevalence of non-pathogenic amoebas[9,10,17]. Campina Grande in the state of Paraíba is located in the same Northeastern region of Brazil and displays the same sanitary and socioeconomic conditions; however, the distribution of *Entamoeba* species is unknown, particularly in children. The current study was designed to determine the prevalence of *E. histolytica* in an urban slum of this city.

METHODS

The population chosen for this study was the community of Pedregal, in the city of Campina Grande. The *E. histolytica/E. dispar* complex was

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detected through microscopic examinations in a previous survey[23] of this community (in 89.9% of the children). Campina Grande is a city located at 7°13'50''S and 35°52'52''W, at 552 meters above sea level, with temperatures varying between 15 °C and 30 °C, and a relative humidity of 82%. The city's average pluvial index is approximately 810 mm/year[24]. It has a population of around 400,000 inhabitants. Pedregal has 9,267 inhabitants, of which 16.62% are under the age of 10. This community lacks proper waste disposal, sewage, and drainage systems. Most of the sewage is discharged in a central channel that overflows during the rainy season, flooding major access routes to school and homes used by the whole community. The study was conducted from January to November 2007, when researchers and a Community Health Worker visited children between two and 10 years of age in their homes. The children were clinically examined for amoebiasis symptoms: dysentery, diarrhea, cramping abdominal pain, rectal tenesmus, vomiting and hepatomegaly[18].

The samples of feces were recovered in containers supplied during the visit. Every child that brought their stools became part of the study. The study was approved by the Ethics Committee in Human Research of the Federal University of Campina Grande on May 22nd, 2006, and authorized by the Secretary of Municipal Health in Campina Grande (protocol # 20060314-001, 05/30/2006). Detailed explanations of the protocols to be used in the study were provided to the parents or guardians who voluntarily signed the form of consent. The 1,195 fecal samples collected from children between two and 10 years of age were separated into two aliquots: one aliquot was submitted to the Ritchie Formalin-Ether concentration method, and the pellets examined for the presence of erythrocytes, *E. histolytica/E. dispar* complex cysts and trophozoites, with 0.9% saline and Lugol's iodine solutions. The second aliquot was stored without preservatives at -20 °C for the ELISA *Entamoeba* differentiation test.

The *E. histolytica* II® (TechLab, USA) ELISA kit was used for the detection of *E. histolytica* specific adhesin antigen in 456 stool samples. This test was performed on thawed samples randomly selected from the population that had been identified as positive for the *E. histolytica/E. dispar* complex by microscopic examination; the test was performed strictly according to the manufacturer's instructions.

RESULTS

Light microscope examination: Analysis of 1,195 stool samples demonstrated that 314 (26.3%) were free of any kind of intestinal parasite and 553 (46.3%) were positive for the presence of four-nuclei amoeba. *Giardia lamblia (*229 samples, 19.2%) and *Entamoeba coli* (369, 30.9%) were the next most prevalent protozoa observed in the stool samples. *Ascaris lumbricoides* (314, 26.3%) and *Trichuris trichiura* (198, 16.6%) were the most prevalent helminths; *Ancylostoma sp* (6, 0.5%), *Strongyloides stercoralis* (5, 0.4%), *Hymenolepis s*p (12, 1%), and *Taenia* sp (4, 0.3%) were also observed in the samples. Associations of parasites were observed among 492 (88.9%) children infected with the *E. histolytica/E. dispar* complex. Forty-five children showed unformed feces. Six of them showed the presence of numerous bacteria, moderate leucocytes and mucus in the feces. No erythrocytes were observed. The remainder 39 unformed feces were concomitantly parasitized by the *E. histolytica/E. dispar* complex and by *G. lamblia* and/or helminths, mostly *Ascaris lumbricoides.* Infected children were pharmacologically treated according to the parasite infection detected. The symptoms evaluated - diarrhea, abdominal pain, vomiting and anorexia - were common to all children, but nothing was identified as gastrointestinal amoebic infection. No rare symptoms, such as mucous sanguineous evacuation, rectal tenesmus, cramping abdominal pain or hepatomegaly were observed.

***Entamoeba* differentiation test**: 456 (82.5%) positive stool samples (showing the presence of *E. histolytica/E. dispar* complex cysts through microscopy) were submitted to the *E. histolytica* differentiation test. None of these samples were reactive to the antibody against the specific *E. histolytica* adhesin antigen.

DISCUSSION

Considering the sanitary conditions of endemic regions, the Brazilian Ministry of Health recommends the treatment of every child resulting positive for amoebas to avoid spreading of this parasite[6]. The accurate identification of the amoeba species found in stools and the true determination of *E. histolytica* prevalence in endemic regions become necessary, once studies have shown the neurotoxicity of metronidazole - the first line antibiotic drug to treat amoeba infections[4].

In the previous study conducted in 2005[23], a prevalence of 89.9% for the *E. histolytica/E. dispar* complex was determined in children living in this community, through microscopic examination of stool samples. These children were treated with metronidazole[23]. In this study, analyses of the feces of 1,195 children under the age of 10 were performed, revealing that 46.3% of the population remains infected. All of them were asymptomatic for amoebiasis as observed through clinical examination. The presence of parasites in 73.7% of the population under study, demonstrates that the poor hygienic conditions, important risk factor of fecal-oral transmission, were not improved in the community. The socio-economic and environmental conditions were the same. In this context, the observed decrease in *E. histolytica/E. dispar* complex prevalence is probably due to treatments conducted previously with metronidazole.

Only one stool sample was collected once the community declined to provide more samples, even after extensive explanations on the benefits of the examination of multiple samples for confirmatory diagnosis. Ritchie's concentration method has showed limitations, associated with the preservation of trophozoites. However, this technique allowed us to observe four nuclei-amoeba cysts. The microscopic examination was performed by two experienced parasitologists to minimize the reported low sensitivity of the methodology used[7].

No specific *E. histolytica* adhesin antigens were detected in the 456 cyst positive stool samples for the E. *histolytica/E. dispar* complex. Based on the high specificity and sensitivity of the ELISA test used[11], it could be assumed that in this population, the high prevalence of *Entamoeba* cysts (46.3% of the samples studied) does not represent cases of *E. histolytica* infection. Although precise identification of the *E. dispar* species requires the use of molecular methods, this evidence suggests that in this community *E. dispar* is the dominant species. The absence of *E. histolytica* in the universe under study is in accordance with reports from Pernambuco[9], Maceió in Alagoas[10], and São Leopoldo in Rio Grande do Sul[25], using the same ELISA method for *E. histolytica* adhesin; and with results from Salvador in Bahia[21], and the state of Pernambuco[17]using PCR. The analyzed sample (1,195 children) represents 77.57% of the

child population in this urban slum (1,540 children); therefore, the results obtained are representative for this community. Most of the reports for the prevalence of *E. histolytica* were obtained from cities situated in coastal regions. The city of Campina Grande is in the "agreste" region, inland and in between humid Atlantic Forest and the semiarid region of the Northeast. Nevertheless, the sanitary conditions and risk factors[23,24] in this city are similar to other regions such as Fortaleza[5], Manaus[3], and Belém[22], displaying a high prevalence of *E. histolytica*. There are no records of the prevalence of *E. histolytica* in the states of Paraíba, Rio Grande do Norte and Maranhão. Hence, it is highly recommended that a national epidemiological survey be performed to determine with accuracy the prevalence of *E. histolytica* in the various regions of the country, and assist in reviewing the adopted strategies for medicating asymptomatic cyst carriers.

RESUMO

Prevalência de *Entamoeba histolytica/Entamoeba dispar* na cidade de Campina Grande, Nordeste do Brasil

A prevalência mundial de *Entamoeba histolytica* não está bem estabelecida. Este fato deve-se à complicação derivada da existência de duas espécies morfologicamente idênticas, mas geneticamente diferentes: a *E. histolytica* que causa amebíases e a *E. dispar* descrita como não patogênica. No Brasil, em comunidades com precárias condições sanitárias e endêmicas para várias parasitoses, localizadas nas regiões Sudeste (SE) e Nordeste (NE), somente *E. dispar* tem sido encontrada, porém outras regiões, apresentam indivíduos infectados por *E. histolytica*. Na região agreste do Estado da Paraíba (NE) que apresenta as mesmas precárias condições sanitárias, não tem sido reportada prevalência específica destes parasitos, embora fosse encontrada alta prevalência do complexo *E. dispar/E. histolytica* em crianças em favela urbana. O presente estudo foi realizado em favela da cidade de Campina Grande, Estado da Paraíba, onde 1.195 crianças de dois a 10 anos sem sintomatologia foram examinadas. Amostras de fezes destas crianças foram analisadas microscopicamente, encontrando-se 553 positivas para o complexo *E. dispar/E. histolytica*. Do total de amostras positivas, 456 foram submetidas à pesquisa do antígeno especifico para *E. histolytica* pelo teste ELISA *E. histolytica* II®,obtendo-se resultado negativo para a presença do antígeno adesina específico de *E. histolytica,* em todas as amostras testadas. Os resultados sugerem que nesta comunidade não há infecção por *E. histolytica*, e que *E. dispar* é a espécie dominante na região.

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