

## ANTIBODIES AGAINST *Entamoeba histolytica* IN INDIVIDUALS WITH INTESTINAL AMOEBIASIS PRESENTING CYSTS AND/OR TROPHOZOITES IN THE FECES.

Maria das Graças CARVALHO (1), Semframis GUIMARÃES (3) & Edward Felix SILVA (2).

### SUMMARY

Serum samples were obtained from 154 individuals infected with *Entamoeba histolytica* (78 symptomatic and 76 asymptomatic). Twelve had trophozoites in the feces whereas 142 had only cysts. The sera were used to test the existence of antibodies anti-*Entamoeba histolytica* employing the Indirect Hemagglutination (IHA), Indirect Immunofluorescence (IFAT), Complement Fixation Reaction (CFR) and Counterimmunoelectrophoresis (CIEP). For those individuals with trophozoites in their feces, 75.0 were positive by IHA and IFAT, 83.0 by CFR and 41.7 by CIEP. In individuals who had only cysts, positive results by the same tests were respectively, 5.6%, 12.0%, 19.0% and 5.6%. The difference in relation to the titers of antibodies detected through IHA, IFAT, CFR and CIEP and in relation to the presence of trophozoites or cysts in the feces was significative for four immunological reactions when  $X^2$ , was employed ( $P < 0.05$ ).

**KEY WORDS:** *Entamoeba histolytica*, IHA, IFAT, CFR, CIEP, cysts, trophozoites.

### INTRODUCTION

Amoebiasis, an infection of humans with the protozoan *Entamoeba histolytica*, has a worldwide distribution and remains one of the most important diseases in the tropics. Its pathogenic effects and clinical expression depend on many host and parasite factors and their dynamic interplay, which makes it difficult to draw a clear-cut distinction between infection and disease.

In the human organism, the trophozoites of *Entamoeba histolytica* may be found in the tissues, causing illness, or in the surface of intestinal mucosa, as an apathogenic commensal. The invasive form of amoebiasis causes 40,000 to 110,000 deaths annually, throughout the world<sup>2</sup>, with 2 - 10% of deaths due to liver abscess and of almost 70% from fulminating colitis.

In Brazil, the population of individuals with *E.*

*histolytica* seems large, but up to this moment, data about the frequency of amoebiasis in our country are based on surveys carried out some years ago<sup>20</sup> and not reflect the present situation<sup>5</sup>.

The clinical forms of amoebiasis occurring in Brazil are less serious than those recorded in Asia and Africa countries and Mexico, but the disease is a serious problem of public health in the Brazilian Amazonian region, where FEITOSA<sup>8</sup>, recorded 14 cases of amoebic liver abscess in a hospital, in Manaus, over 21 months.

Traditionally, the diagnosis of amoebiasis is made by identifying the parasite (trophozoite or cyst forms) in the stool, but this is not easy.

In recent years, immunodiagnostic tests have

(1) Department of Clinical and Toxicological Analyses, Pharmacy Faculty, Federal Minas Gerais University, MG, Brazil.

(2) Department of Parasitology, Institute of Biological Science, Federal Minas Gerais University, MG, Brazil.

(3) Department of Parasitology, Bioscience Institute, Estadual Paulista University, Botucatu, São Paulo, Brazil.

Correspondence to: Dr. Edward Felix Silva, Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, C.P. 2486 - CEP 30161, Belo Horizonte, MG, Brasil.

been found to be of great value both for diagnosing amoebic disease, distinguishing between symptomatic and asymptomatic forms in individual patients and for assessing the extent of amoebiasis in populations under seroepidemiological study.

When tissue invasion occurs, parasite antigens more readily reach the blood stream and elicit antibody production by immune system<sup>19</sup>. Thus, symptomatic patients present higher titers of serum antibodies than the asymptomatic ones. Such antibodies can be detected through a series of immunological reactions, such as indirect hemagglutination<sup>11, 13, 15, 21</sup>; indirect immunofluorescent<sup>2, 12, 14</sup>; complement fixation<sup>4, 9, 15</sup>; counterimmunoelectrophoresis<sup>16, 23</sup>; ELISA<sup>1, 15, 22</sup>; indirect immunofluorescence using monoclonal antibodies<sup>27</sup>.

In this study, sera from individuals infected with *E. histolytica* were used with the aim of correlating the antibodies titre levels determined by IHA, IFAT, CFR and CIEP tests, to the presence or absence of cysts and/or for trophozoites in feces of the infected individuals, and evaluating these four immunological tests for their use in amoebiasis diagnostic.

## MATERIALS AND METHODS

**Patients:** A total of 154 individuals belonging to three distinct geographic regions (Belo Horizonte, Minas Gerais, Galiléia, a province of Minas Gerais; and Macapá, Amapá Federal Territory) in which the infection with *E. histolytica* was proven through feces examination using the MIFC method<sup>3</sup> and stained by ferric hematoxylin<sup>25</sup>, but that presented different clinical forms of the disease, was used in this study. Among 154 patients, 78 were symptomatic (2 with dysenteric colitis and 76 with non dysenteric colitis) and 76 were asymptomatic. Sera from individuals parasited by others amoebas of intestinal tract, such as *Entamoeba coli* and *Endolimax nana*, and sera from individuals that provenly were not parasited by *E. histolytica* or other amoebas were used as control.

**Sera:** The sera were obtained from these patients and stored at -20° C until testing.

**Antigen:** Three axenic cultures of *E. histolytica* strains were used to produce the antigens for the immunological tests: HK-9<sup>10</sup>; NIH-200<sup>10</sup> and ICB-462<sup>26</sup>. The first two strains were

axenized by DIAMOND<sup>7</sup> and the last one by SILVA et al.<sup>26</sup>, isolated from cysts found in an individual with asymptomatic amoebiasis. Antigen for indirect immunofluorescence was prepared as described by GOLDMAN<sup>12</sup> and padronized according to this same author. Antigen for CFR, IHA and CIEP was prepared according to KRUPP<sup>16</sup> with some modifications. The trophozoite masses from each *E. histolytica* axenic strain were sonicated and dialized for 24 hours against veronal buffer pH 8.2 at 4° C and then they were centrifugated at 1000 rpm during 15 minutes at 4° C. The pellet was discarded and the protein of the supernatant was adjusted to 5-10 mg/ml using Veronal buffer, by the method for LOWRY et al.<sup>18</sup>. The antigens of each strain was stored at -70° C until testing.

**Immunological Tests:** Four tests were employed to detect the presence of anti-*E. histolytica* antibodies in the sera: indirect immunofluorescence (IFAT)<sup>12</sup>, indirect hemagglutination (IHA)<sup>17</sup>, complement fixation (CFR)<sup>9</sup> and counterimmunoelectrophoresis (CIEP)<sup>24</sup>. The IFAT and IHA tests were considered as positives when presented titres of 1/160 and 1/64 respectively. For CFR and CIEP only qualitative tests were carried out. The results observed were analysed by X<sup>2</sup> test using Yates correction.

## RESULTS

The antigenic preparations obtained from the *E. histolytica* axenic strains, ICB-462 and NIH-200, employed in the four immunological reactions (IFAT, CFR, IHA and CIEP), did not show significative difference ( $P > 0.05$ ) when they were compared with the antigen from the strain HK9 used as a standard strain.

In the sera obtained from individuals with trophozoites in feces was observed a high frequency of positive reaction, in opposite of the sera from individuals with only cysts.

The percentual of positive reactions in the sera taken from individuals with trophozoites in the feces was 75.0% for IHA and IFAT 83.3% for CFR and 41.7% for CIEP. For the individuals that had only cysts, the results found were 5.6% for IHA, 12.0% for IFAT, 19.0% for CFR and 5.6% for CIEP, although some of the individuals that presented cysts had symptoms. The number of positive reactions in the sera of the individuals with

trophozoites or cysts showed a significant difference ( $p < 0.05$ ) (Table 1).

None of the sera of the individuals infected by others amoebas of the intestinal tract was reactive.

TABLE 1  
Positivity of the reactions IHA, IFAT, CFR and CIEP regarding the presence of trophozoites or cysts of *Entamoeba histolytica* in the feces.

Form	Number of cases	REACTIONS							
		HAI		IFAT		CFR*		CIEP*	
		+ve ( $\geq 1/160$ )	-ve	+ve ( $\geq 1/64$ )	-ve	+ve	-ve	+ve	-ve
Trophozoites	12	09 (75.0%)	03 (25.0%)	09 (75.0%)	03 (25.0%)	10 (83.3%)	02 (16.7%)	05 (41.7%)	07 (58.3%)
Cysts	142	08 (5.6%)	134 (94.4%)	17 (12.0%)	125 (88.0%)	27 (19.0%)	115 (81.0%)	08 (5.6%)	134 (94.4%)
TOTAL	154	17 (11.0%)	137 (89.0%)	26 (16.9%)	128 (83.1%)	37 (24.0%)	117 (76.0%)	13 (8.4%)	141 (91.6%)

\*non-dilute serum:

HAI:  $X^2$   $lgI = 50.84$   $P < 0,05$   
 IFAT:  $X^2$   $lgI = 24.57$   $P < 0,05$   
 CFR:  $X^2$   $lgI = 23.46$   $P < 0,05$   
 CIEP:  $X^2$   $lgI = 15.87$   $P < 0,05$

## DISCUSSION

In *E. histolytica* infection, with or without clinical symptoms, one of the most important expression of immune response, in the man, is the appearance of circulating antibodies that are present in high levels in individuals with invasive amoebiasis.

Our study showed that the individuals with trophozoites in feces with only mucous or with mucous and blood, had the highest titres and most the sera gave positive reactions on all four tests studied, except three cases in IHA and IFAT, two cases in CFR and seven in CIEP (Table 1). The presence of trophozoites in the feces is related to tissue invasion and clinical symptoms<sup>5, 6</sup> and may explain the findings of higher titres in the immunological reactions<sup>18</sup>. All those individuals that had trophozoites in the feces showed clinical symptoms of dysentery or nondysenteric colitis. The individuals with only cysts in the feces presented a discrete symptomatology or, in the majority of the cases, were asymptomatic.

The comparison of the quantitative reactions (IHA and IFAT) showed that IFAT seems the most sensitive of the serologic test once this technic detected the highest number of positive cases, show-

ing a positivity of 75.0% and 12.0% for those individuals with trophozoites and with only cysts in the feces, respectively (Table 1).

When the qualitative reactions (CFR and CIEP) were compared with the quantitative tests, it was observed that the CFR detected higher number of positive cases, showing a positivity of 83.3% in the cases with trophozoites in the feces and 19.0% in the cases with only cysts in the feces (Table 1). Because in this technic the sera were not diluted, the higher sensibility observed seems to be due the presence of inespecific antibodies. According to the results, the CIEP showed the lower positivity (8.4%) when compared with the others tests (Table 1).

None of the sera of the individuals infected by others amoebas of the intestinal tract was reactive, thus the occurrence of positive reactions due to antibodies produced against other amoebas was completely discarded showing, the high specificity of the four immunological reactions.

In general, the presence of antibodies to an organism reflects response to tissue invasion. In the case of amoebiasis, where parasitologic diagnosis may be confusing, a demonstrable immunologic response may help confirm or refute the mi-

croscopic findings of the feces and may be of enormous help in the management of patients.

It is important to emphasize the necessity of more detailed studies on the amoebiasis in areas like amazonic regions. According to FEITOSA<sup>8</sup>, in this regions of the country there is a predominance of the symptomatic forms (65.22%) over the asymptomatic ones (34.78%), occurring that among the symptomatic patients the dysenteric form of amoebiasis had a high frequency (26.70%). Besides the frequent dysenteric forms, the author describes also the occurrence of 14 cases of amoebic liver abscess with positive correlation between the immunodiagnosis and the clinical data.

### RESUMO

#### Anticorpos anti-*Entamoeba histolytica* em indivíduos com amebíase intestinal apresentando cistos e/ou trofozoítas nas fezes.

Amostras de soros foram obtidas de 154 indivíduos comprovadamente parasitados pela *Entamoeba histolytica* (78 sintomáticos e 76 assintomáticos). Doze apresentavam trofozoítas nas fezes, enquanto 142 tinham apenas cistos. Os soros foram utilizados para testar a ocorrência de anticorpos anti-*Entamoeba histolytica*, empregando-se para tal, Reação de Hemaglutinação Indireta (HAI), Reação de Imunofluorescência Indireta (RIFI), Reação de Fixação de Complemento (RFC) e Contraímunoeletroforese (CIEP). Entre os soros dos indivíduos com trofozoítas em suas fezes, 75,0% foram positivos para HAI e RIFI, 83,3% por RFC, e 41,7% por CIEP. Nos indivíduos que tinham apenas cistos, resultados positivos pelos mesmos testes foram respectivamente, 5,6%; 12,0%; 19,0% e 5,6%. A diferença em relação aos títulos de anticorpos detectados através de HAI, RIFI, RFC e CIEP e em relação à presença de trofozoítas ou cistos nas fezes foi significativa para as quatro reações imunológicas, quando  $X^2$  foi empregado ( $P < 0,05$ ).

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