The Investigation of *Entamoeba histolytica* Prevalence in Some Villages of Sivas by ELISA Method

Sivas'ın Bazı Köylerinde *Entamoeba histolytica* Yaygınlığının Elisa Yöntemiyle Araştırılması

Erdoğan Malatyalı, Semra Özçelik, Ali Çeliksöz

Cumhuriyet Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Sivas, Turkey

ABSTRACT

Objective: Humans may be infected with three morphologically identical *Entamoeba* species; pathogenic *E. histolytica*, commensal *E. moshkowskii* and *E. dispar*. The aim of the present study was to determine the true prevalence of the *E. histolytica* using native lugol, trichrome staining and a monoclonal antigen detection kit (ELISA kit E. histolytica-II; Techlab, Inc., Blacksburg, VA) among primary school children living in the rural areas around Sivas.
Methods: A total of 1449 stool samples were examined by native lugol and Trichrome staining, and 312 (22%) samples were positive for one or more parasite species. Additionally, 22 (1.5%) stool samples were found to be positive for the presence of *E. histolytica/dispar* cysts, and these samples were further examined by *E. histolytica* specific antigen based ELISA.
Results: As a result, ELISA test gave negative reactions for all the samples. Also, there was no cross reaction with other luminal protozoa such as *E. coli*, *G. intestinalis*, *B. hominis* and *I. butschlii*.
Conclusion: The data reveals that *E. histolytica* prevalence may be lower than estimated. *(Turkiye Parazitol Derg 2011; 35: 6-9)*

Key Words: ELISA, *Entamoeba histolytica*, prevalence

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ÖZET

Amaç: İnsanlar morfolojik olarak ayırt edilemeyen patojenik *E. histolytica* ve kommensal *E. moshkowskii* ve *E. dispar* türleriyle enfekte olabilir. Bu çalışmanın amacı, Sivas'ın bazı köylerinde yaşayan öğrencilerde monoklonal antijen kitiyle (*E. histolytica*-II; Techlab, Inc., Blacksburg, VA) *E. histolytica*'nın yaygınlığını saptamaktır.
Yöntemler: Bu amaçla toplanan 1449 dışkı örneğinin direkt mikroskobik incelenmesi (nativ lugol ve Trikrom) sonucu 312 (%22)'sinde bir veya daha fazla bağırsak parazitine rastlanmıştır. Ayrıca *E. histolytica/dispar* kisti saptanan 22 (%1.5) dışkı örneği ELISA ile *E. histolytica* varlığı yönünden araştırılmıştır.
Bulgular: Sonuç olarak *E. histolytica/dispar* kisti saptanan örneklerin tamamının ELISA testi ile negatif sonuç verdiği gözlenmiş, bununla birlikte antijen testinin diğer bağırsak protozoonlarıyla (*E. coli, G. intestinalis, B. hominis* ve *I. butschlii*) çapraz reaksiyon vermediği belirlenmiştir.
Sonuç: Elde edilen bulgular bölgemizde *E. histolytica* yaygınlığının tahmin edilenden daha az olabileceğini ortaya koymaktadır.
(Turkiye Parazitol Derg 2011; 35: 6-9)

Anahtar Sözcükler: ELISA, *Entamoeba histolytica*, prevelans

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INTRODUCTION

Entamoeba histolytica is the causative agent of amebiasis that results in dysentery or amebic abscess (1). This infectious agent is known to be common in developing areas; although cases have been described in developed countries among homosexual men, immigrants, HIV infected patients and travelers visiting endemic areas (2, 3). Following malaria and schistosomiasis, amebiasis is the third leading cause of death among parasitic diseases on a global scale; it affects approximately 50 million people each year, resulting in almost 100.000 deaths (4). However, the true distribution of the disease is not clear in most of the countries. This has been particularly complicated by the existence of different species morphologically identical but genetically differ-

Address for Correspondence/ Yazışma Adresi: Dr. Erdoğan Malatyalı, Cumhuriyet Üniversitesi Tıp Fakültesi, Parazitoloji Anabilim Dalı, Sivas, Turkey Phone: +90 346 219 10 10-1044 E-mail: emalatyali@cumhuriyet.edu.tr
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ent; namely *E. histolytica,* which is pathogenic, *E.moshkowskii* and *E. dispar,* which are non-pathogenic species (5). The differentiation of *E. histolytica* and *E. dispar* is necessary to avoid unnecessary treatment of patients infected with the non-pathogenic *E. dispar* and to estimate the real prevalence of *E. histolytica* (6). Currently, microscopy, immunoflorescence (IFA), polymerase chain reaction (PCR) and serological methods including enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA), and latex agglutination are used for the laboratory diagnosis of amebiasis (7). The diagnosis of intestinal amoebiasis is still mostly based on the microscopical detection of organisms in stool samples (8). The disadvantages of microscopy are that it requires a skilled microscopist and has low sensitivity and specifity compared with other methods, such as IFA, antigen detection, and PCR (9). Unfortunately, PCR based methods are still too complicated and expensive for the public health systems of many communities (10). Very few studies have addressed the true incidence and prevalence of *E. histolytica* and *E. dispar* in rural areas. Recent epidemiological surveys have shown that the prevalence of *E. histolytica/dispar* varies between 0.5% and 7.8% in the Sivas province (11, 12).

The aim of the study was to determine the prevalence of *E. histolytica* in children in some villages of Sivas, by a monoclonal antigen detection kit.

MATERIALS AND METHODS

Stool samples were collected from children in the rural areas in the Sivas province during 2008. The study group includes 1449 children educated in six different schools (Karşıyaka, Ahmet Türkseven, Kurtlapa, Demirçelik, Gürçayır Kenan Evren and Cumhuriyet Primary Schools). The schools were selected by the simple random sampling method. A questionnaire was completed with the details of every child. The observations were performed with the aid of the school teacher and the parents were informed about the application and also the required permissions were acquired from Sivas Governorship.

Stool samples were investigated by native-lugol examination and Gomori's Trichrome staining (13). The presence of *E. histolytica*-specific galactose adhesin was determined with a commercially available kit (ELISA kit *E. histolytica*-II; Techlab, Inc., Blacksburg, VA) among the samples positive for the presence of one-four-nuclei amoeba. Fresh samples were used for the assay. All stool samples were examined by ELISA on the same day without prior preservation. Additionally, stool samples that contain other protozoa cysts were also investigated with the same kit. The positive result was determined according to the manufacturer's instructions, an optical density reading >0.05 after subtraction of the negative control optical density.

RESULTS

In the present study, stool samples were collected from 1499 children to determine the prevalence of *E. histolytica*. *E. histolytica/dispar* cyst form was detected in 22 (1.5%) stool samples by native-lugol examination and Trichrome staining. The overall infection rate of intestinal parasitic infection was 22% among the children. In addition, the most frequent parasite species were *G. intestinalis* (10.4%), *E. coli* (8.8%) and *E. vermicularis* (7.7%), respectively. 782 (54%) of children were female and 667 (46%) were male. The infection rate among females was 1.8% which was not significantly higher than that among males 1.2%. (χ^2=0.84, p>0.05) (Table 1).

The children's ages varied from 6 to 15 years. No significant difference was found between the two age groups (6-9 and 10-15) according to the prevalence of *E. histolytica/dispar* (χ^2=0.44, p>0.05) (Table 2). Moreover, it was found that the school success level was not associated with the parasite infection rate (χ^2:7.59, p>0.05) (Table 3). Regarding the monthly income of the children, the results revealed that the rate of *E. histolytica/dispar* infection among those with low incomes (2.3%) was significantly different from that among those with high income (χ^2:6.91, p<0.05) (Table 4). The clinical features of children are documented in Table 6. Of 22 cyst passengers, 6 (27.3%) had abdominal pain according to the survey. There was no statistically significant difference for abdominal pain between the children who were four nuclei cysts passengers and others (χ^2:6.40, p>0.05).

Table 1. The rate of *E. histolytica/dispar* infection according to gender

	Female		Male		Total	
E. histolytica/dispar	**n**	**%**	**n**	**%**	**n**	**%**
Cysts (+)	8	1.2	14	1.8	22	1.5
Cysts (-)	659	98.8	1.8	98.2	1427	98.5
Total	667	46	782	54	1449	100

Table 2. The rate of *E. histolytica/dispar* infection according to age groups

	Younger		Older		Total	
E. histolytica/dispar	**n**	**%**	**n**	**%**	**n**	**%**
Cysts (+)	7	1.2	15	1.7	22	1.5
Cysts (-)	554	98.8	873	98.3	1427	98.5
Total	561	38.7	888	61.3	1449	100

A total of 86 stool samples that contain protozoa cysts (*E. histolytica/dispar, E. coli, B. hominis, G. intestinalis, I. butschlii*) and 2 negative controls were tested for the pathogenic strain by ELISA (Table 5). All 88 samples were negative in the ELISA for the presence of *E. histolytica*-specific galactose adhesin. However, when we also studied a pathogenic strain of *E. histolytica* was cultured in our laboratuary, and it gave positive reaction in antigen test.

DISCUSSION

Our findings are consistent with those previously reported in neighboring cities. The prevalence of *E. histolytica/dispar* complex is reported to be between 0% and 17.4% in these region by microscopical detection (14, 15). Microscopical detection of the organisms in stool is time and labour intensive and depends on the skill of an experienced microscopist (16, 17). Also, it is impossible to distinguish nonpathogenic *E. dispar* (morphological identical) from *E. histolytica*. The presence of *E. histolytica* in stool specimens can be considered only when erythrocytes are observed within trophozoites (18). The sensitivity and selectivity of direct microscopy is reported as 5%-60% and 10%-50%, respectively (7). The diagnosis of parasites with clinical symptoms is difficult. Because, the majority of infected individuals are asymptomatic, even with *E. histolytica*; only 5-10% develop diarrhea or colitis and a smaller subset develop extra intestinal disease, mainly amebic liver abscess (19). A record is available that indicates that *E. histolytica* is more common than *E. dispar* in Zonguldak. Mengeloglu et al. reported that 59.1% of four nuclei cysts was positive by ELISA (Seramun Diagnostica GmbH, Wolzig, Germany) among people with gastrointestinal complaints (20). In the present study, most of the children in the experimental group (cyst passengers) were not suffering from abdominal pain, which supports the outcome of the study. The reason for the difference between results obtained by the present study, and the Mengeloglu et al. study reveals that people with gastrointestinal symptoms have to be tested for *E. histolytica* surface adhesins for a reliable diagnosis.

Table 3. The rate of *E. histolytica/dispar* infection according to school success

	1		2		3		4		5	
E. histolytica/dispar	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Cysts (+)	3	3.9	4	2.6	7	1.6	6	1.8	22	1.5
Cysts (-)	73	96.1	152	97.4	440	98.4	328	98.2	1427	98.5
Total	76	5.2	156	10.7	447	30.8	334	23.0	1449	100

Table 4. The rate of *E. histolytica/dispar* infection according to socio-economical level

	Low income		Avarage income		High income		Total	
E. histolytica/dispar	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Cysts (+)	16	2.3	6	1.1	0	0	22	1.5
Cysts (-)	670	97.7	555	98.9	202	100	1427	98.5
Total	686	47.3	561	38.7	202	13.9	1449	100

Table 5. Distribution of the clinical features of children

	Abdominal pain		Gnashing		Extensive salivation	
E. histolytica/dispar	**n**	**%**	**n**	**%**	**n**	**%**
Cysts (+)	6	27.3	4	18.2	3	13.6
Cysts (-)	481	33.7	165	11.6	235	16.5
Total	487	33.6	169	11.7	238	16.4

Table 6. Distribution of parasites in samples used for immunoenzymatic assay

	E. histolytica/dispar	***E. coli***	***G. intestinalis***	***B. hominis***	***I. butschlii***	***E. coli + G. Intestinalis***	***E. coli + E. histolytica/dispar***	***E. coli + G. intestinalis + E. histolytica/dispa***	***Negative control***	**Total**
Number	14	38	11	3	6	7	5	2	2	**88**
%	15.9	43.2	12.5	3.4	6.8	7.9	5.7	2.3	2.3	**100**

PCR, izoenzyme assays and serologic tests can be used for the differentiation of *E. histolytica* and *E. dispar* (16). Antigen detection methods were reported as a better diagnostic tool than the antibody detection (21). Furthermore, recently developed antigen detection methods such as Tech-Lab ELISA were shown to be a sensitive and specific method for the rapid differentiation of the two species because it is easy to perform and entails low cost compared to others (8). In this study, the entire four nuclei cysts showed no *E. histolytica* pattern. These are thought to be other identical amoebas like *E. dispar* and *E. moshkowskii*. Some records are available that show the occurrence of these organisms in our country, but no data has been found in this region (4, 22-24, 26). No cross reaction was detected with other luminal protozoa (*E. histolytica/dispar, E. coli, B. hominis, G. intestinalis, I. butschlii*) as previously reported (25). Our study indicates that *E.dispar* may be more common in our region than in other countries (26). Recent data point out that *E. dispar* is perhaps 7-10 times more common than *E. histolytica* worldwide (27).

The frequencies of *E. histolytica* using the Tech Lab antigen detection kit were reported as 15.6% in Egypt among symptomatic group and as 8% in Bangladesh among the asymptomatic group (28, 29). Amoebiasis is common in tropical and developing countries due to poor sanitary conditions (24). In the present study, the number of four nuclei cyst passengers was high in the group with low incomes which emphasises that status and specific *socioeconomic* levels influence the parasite distribution. In conclusion, direct microscopic diagnosis of amebiasis is not an efficient method for the diagnosis of *E. histolytica*, so we recommend that ELISA procedures based on reliable antigens such as surface adhesins can be used in this region as in other parts of the world.

Conflict of Interest

No conflict of interest is declared by the authors.

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