A Field Study of the Distribution of *Entamoeba histolytica / dispar* Cyst Passers in Northern, Central, and Southern Iran

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Abstract

Amoebiasis is one of the most important human diseases in many countries especially in tropical and sub-tropical regions. This study was carried out from August 1999 to February 2002 in order to determine the ratio of *Entamoeba histolytica / Entamoeba dispar* in some regions of Iran. A total of 16,592 stool samples were randomly collected from different age-groups in central, northern and southern Iran both from urban and rural areas. The samples were examined by direct and formalin-ether concentration methods. Two hundred and twenty six samples (1.36%) were positive for *E. histolytica/E. dispar* cyst (C.I = 1.18-1.54%). The prevalence of infection with *E. histolytica/E. dispar* was 0.78%, 3.9% and 4.6% for central, northern and southern part of Iran, respectively. The minimum rate of prevalence was 0.6% in Tehran, Yazd and Ardekan (central Iran), while the highest rate (8.3%) was seen in rural areas of Ahwaz (southern Iran).The study showed that ratio of *E. histolytica /E. dispar* was higher in southern regions (tropical and subtropical) than other regions. It seems that more sanitary facilities and health trainings are needed in different parts of the country, especially in southern Iran, where the rate of infection is high.

Keywords: Entamoeba histolytica, Entamoeba dispar*, Epidemiology, Iran*

Introduction

Intestinal and extra-intestinal amoebiasis still remains a significant health problem around the world, especially in developing countries. Asymptomatic cyst passing is the most common manifestation of the intestinal *Entamoeba* infection.

An estimated 10% of world's population is infected with *E. histolytica / E. dispar*, and between 40,000 to 110,000 individuals die of invasive amoebiasis annually (1-3). It has been known that many people who are apparently infected with *E. histolytica* never develop symptoms and spontaneously clear their infection. It was recently shown that the parasite previously named as *E. histolytica* actually consists of two morphologically identical but genetically distinct species, one *E. histolytica*, which is the causative organism of invasive intestinal and extra-intestinal amoebiasis. The other is *E. dispar*, a non-pathogenic intestinal commensally protozoan organism (4).

Differential diagnosis of *E. histolytica* and *E. dispar* in stool samples is not easy on the basis of microscopy alone, except for the case of hematophagous trophozoites in acute dysentery. Currently some expensive methods such as amoebic antigen and DNA detection, isoenzyme electerophoretic pattern, PCR-basis methods, are available to differentiate both non-pathogenic *E. dispar* from pathogenic *E. histolytica* (5, 6)

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Differentiation of these two organisms is of great importance in clinical and epidemiological studies. An expert consultation on amoebiasis in Mexico City stressed needs for improved methods for the specific diagnosis of *E. histolytica* infections based on technologies appropriate for use in developing world (7). Also, this meeting recommended that when diagnosis is made by light microscopy, the cysts of two species are indistinguishable and should be reported as *E. histolytica / E. dispar*.

In Iran, there have been several studies on the prevalence of *E.histolytica/E.dispar* complex, before the concept of two species was confirmed, in which the prevalence of infection varied between 2.2% to 30% (8-10).

To date, there is no data on the estimated prevalence of *E.histolytica/E.dispar* in different parts of Iran.

The aim of this study, however, was to address the prevalence of *E.histolytica/E.dispar* in each climatic region, and its relation with some epidemiological factors such as age, sex, and climatic conditions.

Materials and Methods

Cross-sectional and descriptive study was carried out in three different regions of Iran, including central (semi-temperate zone), northern (temperate zone) and southern region (tropical and sub-tropical region) from August 1999 to February 2002. A total of 16,952 stool specimens randomly were collected from rural and urban areas in three different climatic regions of the country. The samples were taken from persons who had given their informed consent prior to the collection. Each stool sample then was examined microscopically for the presence of red blood cells and *E.histoltica /E.dispar* complex cysts and trophozoites using direct and formalin-ether concentration methods (11). Microscopy diagnosis of cysts was performed after staining with 5% Lugol's iodine

solution. The criteria for identification of *E. histolytica/E. dispar* were the cysts measurement ranging from 10 to 20 µm with 1 to 4 nuclei with central karyosome and presence of choromatoidal bars in cytoplasm (12).

For statistical analysis, we used X^2 and a 95% confidence interval (C.I). All *P* values less than 0.05 were considered to indicate statistically significant.

Results

Of 16,952 samples collected, 13,945, 2,184, and 436 were from central, southern, and northern Iran, respectively.

A total of 226 individuals (1.36%) were infected by *E. histolytica/E. dispar* complex cysts .The prevalence of the infection with *E. histolytica/E. dispar* complex cysts was 0.78% (C.I: 0.63%- 0.93%), 3.9% (C.I: 2.14%-5.7%), and 4.6 % (C.I: 3.7%-4.5%) for central, northern, and southern parts of Iran, respectively. The rate of infection in northern and southern Iran was significantly greater than that of central Iran (*P*<0.001). The data of 16,952 individuals studied and prevalence of infection according to geographical location is presented in Table 1.

Based on data in this table, the highest rate of infection was 8.3% in rural area of Ahwaz and Khorram-Shaher (southern Iran), 4.85% in rural area of Tonekabon (northern Iran), 2.74% in Eslam-Shaher (central Iran), whilst the lowest rate of infection was 0.6% in Yazd and Ardekan, two desert cities of Iran (southern Iran), 0.7% in Tehran capital city of Iran (central Iran) and 2% in Langerood (northern Iran). The highest rate of infection was seen at 20-29 year-old age-group (2.2%), but the lowest rate was observed at >60 (Table 2). Infection in females (1.6%) was more prevalent than males (1.16%) (*P*<0.02), and the female/male ratio of persons infected was 1:1.3 (Table 3).

Table 1: Prevalence of *Entamoeba histolytica/E. dispar* in three different regions of Iran

Region		No. Examined	E.histolytica /E.dispar No. (%)	C.I
Central	Tehran	11864	82(0.7%)	0.55-0.85 %
	Karaj &Fardis	284	5(1.76%)	0.23-0.3.3 %
	Kashan	710	8(1.13%)	0.35-1.9%
	Eslamshahr & Varamin	73	2(2.74)	0-6.5%
	Isfahan & Najaf abad	555	5(0.9%)	0.12-1.68%
	Ghazvin	138	2(1.45%)	0-3.4%
	Ghom	125	2(1.6%)	0-3.8%
	Golpaygan	71	1(1.4%)	0-4.1%
	Zanjan	125	1(0.8%)	0-2.36%
	Total	13954	108 (0.78%)	0.63-0.93%
Southern	Ahwaz & Khoramshahr	782	65(8.3%)	6.4-10.23%
	Bandar abass	220	7(3.8%)	1.27-6.3%
	Yazd & Ardekan	332	2(0.6%)	0-1.4%
	Khoram abad &Borujerd	300	14(4.7%)	2.3-7.1%
	Kazeroun	67	2(3%)	0-7.1%
	Shiraz	62	1(1.6%)	0-4.7%
	Kerman	57	2(3.5%)	0-8.3%
	Bushehr &Buerahmad	35	2(5.7%)	0-13.4%
	Sarpol-zehab	44	1(2.3%)	0-6.7%
	Ilam	167	2(1.2%)	0-2.8%
	Dourood	90	1(1.1%)	0-3.25%
	Andimeshk	28	1(3.6%)	0-10.5%
	Total	2184	100(4.6%)	3.7-5.5%
Northern	Tonekabon	227	11(4.85%)	2.1-7.6%
	Roodsar	56	2(3.6%)	0-8.5%
	Babol	33	1(3%)	0-8.8%
	Torkman Sahra	38	1(2.6%)	0-7.7%
	Bandar Anzeli	59	2(3.4%)	0-8%
	Langrood	50	1(2%)	0-5.9%
	Total	463	18(3.9%)	2.14-5.7%
Total		16592	226(1.36%)	1.18-1.54%

Table2: Prevalence of *E. histolytica/E. dispar* according to age in three regions of Iran

Age groups	No. Examined	E. histolytica/E. dispar No. (%)
0-9	3508	38(1.08%)
10-19	4215	57(1.35%)
20-29	2937	65(2.2%)
30-39	2396	23(0.96%)
40-49	1523	19(1.2%)
50-59	902	14(1.5%)
+60	1111	10(0.9%)
Total	16592	226(1.36%)

Table 3: Prevalence of *E. histolytica*/*E. dispar* according to sex in three regions of Iran

Sex	No. Examined	E. histolytica/E. dispar No (%)
Male	8526	99(1.16%)
Female‘	8066	127(1.6%)
Total	16592	226(1.36%)

Discussion

Amoebiasis is one of the health issues in many developing countries. It is the third most common cause or death due to parasitic infection after malaria and schistosomiasis as estimated by the World Health Organization (3). Approximately 10% of the world population is infected with *E. histolytica/E. dispar* (13), but most infection due to the noninvasive species, *latter*. Epidemiological studies have shown that low socioeconomic status and unsanitary condition are significant risk factors for infection. In addition, people living in developing countries have a higher risk of infection than those in developed ones (14).

Until now, several microscopy-based epidemiological surveys on the prevalence of *E. histolytica/E. dispar* have been performed in different parts of the Iran that reported the prevalence of infection up to 30 %(8-10). The higher rates of infections with *E. histolytica/E. dispar* in southern and northern Iran may be explained by a difference in the climatic conditions in these two regions which accelerate the viability of the cysts. Additionally, poor sanitation and densely-populated regions in southern Iran may be taken into account for higher prevalence of infections in this region. Similar observations were made by Sheiban and Rezaian (8) who stated that the prevalence rates of infections by *E. histolytica/E. dispar* in Minab area of Hormozgan province, southern Iran is higher than other regions.

Although in previous studies carried out in different parts of the country no significant differences were observed in prevalence rates between females and males (8,10), but in the present study the infection rate in women was higher than man and was statistically significant (*P*< 0.02). This may be explained by the greater samples examined in the present study. These results are in accordance with studies undertaken in some parts of the world (15). The highest infection rate was seen at 20-29 age-group, and infection rate declines with age increase. A similar age distribution of infection has been observed in previous studies in Iran (8, 10). In endemic region, the highest infection rates have been seen in earlier age, for example in Mexico, 11% of the tested population aged 5 to 9 years was infected (16). The results of the current study showed that the ratio of *E.histolytica/E.dispar* was higher in southern regions (tropical and sub- tropical) than other regions.

It seems that more sanitary facilities and health trainings are needed in different parts of the country, especially in southern Iran, where the rate of infection is high.

Acknowledgments

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