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Full Length Research Paper

Prevalence of ıntestinal parasites in Bursa Province of Turkey

Oktay Alver[1]*, Yasemin Heper[2], Iker Ercan[3], Halis Akalın[2] and Okan Töre[1]

[1]Department of Medical Microbiology, Uludag University, Faculty of Medicine, Gorukle, Bursa 16059, Turkey.
[2]Department of Microbiology and Infectious Diseases, Uludag University, Faculty of Medicine, Gorukle, Bursa 16059, Turkey.
[3]Department of Biostatistics, School of Medicine, Uludag University, Faculty of Medicine, Gorukle, Bursa 16059, Turkey.

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This study was designed to determine the prevalence of intestinal parasites in relation to personal and environmental risk factors in Bursa province of Turkey and to compare wet mount + Lugol's iodine, formalin ethyl acetate concentration, trichrome staining and enzyme-linked immunosorbent assays (ELISA) methods used in *Entamoeba histolytica/ Entamoeba dispar* diagnosis. For this purpose a total of 176 faecal samples from people were randomly collected from regions of the Bursa City Centre, where crowded families of low socio- economic levels reside. All faecal samples were examined by wet mount + Lugol's iodine formalin ethyl acetate concentration, trichrome staining methods and ELISA. 66 faecal samples were infected with one or more parasite species and the overall prevalence rate was 37.5%. Nine species of intestinal parasites, including six protozoon and three helminth species were found. The most prevalent species was *Blastocystis hominis* (17.04%) and others were found with the following frequencies: 9.09% *Enterobius vermicularis*, 7.38% *Giardia intestinalis,* 5.68% *Entamoeba coli,* 2.27% *E. histolytica/E.dispar*, 1.13 % *Entamoeba hartmani,* 1.13% *Taenia spp.,* 0.56% *Hymenolepis nana.* 6.2% of examined samples had mixed infections. Overall prevalence of the detected parasites did not differ significantly in different demographic groups. Except for *B. hominis* and *G. intestinalis*, there was no evidence for sex, age and other demographic- related differences in the prevalence of these parasites. Statistically, *B. hominis* and *G. intestinalis* were higher in 20 to 49 and in 1 to 19 year age groups, respectively than in any other age group. Microscopical examination and ELISA revealed that one sample (0.5%) was found to be positive using all 4 methods, and 162 samples (92%) were found to be negative with all 4 methods applied. *E. histolytica/E. dispar* complex was detected in 0.56% (1); in 0.56% (1), in 0.56% (1) and in 2.27% (4) of the fecal samples examined by wet mount + Lugol's iodine, formalin ethyl acetate concentration, with trichrome staining, and ELISA respectively. The wet mount + Lugol's iodine, formalin ethyl acetate and trichrome staining methods had a sensitivity of 25% each, a specificity of 94.1, 99.4 and 98.2%, compared to the results of the *E. histolytica/E. dispar* ELISA, respectively.

Key words: *Entamoeba histolytica/ Entamoeba dispar,* enzyme-linked immunosorbent assays (ELISA), intestinal parasites, microscopy, prevalence.

INTRODUCTION

Amebiasis is a parasitic infection leading to various clinical manifestations, from asymptomatic colonization in humans by the protozoon *Entamoeba histolytica* to severe fulminant colitis and non-intestinal amebiasis. The disease is more common in tropical and subtropical regions and the number of cases is reported to be higher

in developing countries (WHO/PAHO/UNESCO, 1997). Amebiasis can be transmitted through fresh food and drinks containing *E. histolytica* cysts with four nuclei (Pickering et al., 1986). In developed countries such as the United States of America and Japan, amebiasis is frequently seen among homosexuals and institutionalized patients (Phillips et al., 1981). It is estimated that more than 10% of the world's population is colonized with *E. histolytica* and *Entamoeba dispar*, each year 50 million people develop invasive diseases, and that 40,000 to 100,000 people die from it each year (Ravdin, 2000). According to the World Health Organization (WHO), amebiasis is the third most lethal disease after malaria and schistosomiasis (World Health Organization; Amoebiasis, 1997). Intestinal amebiasis diagnosis is confirmed with the presence of *E. histolytica/E. dispar* cysts or trophozoites in feces, and tests examining ameba antigens in feces are reported to be useful (Haque, 2006). Microscopy remains the main method for the diagnosis of amebiasis and is used in most developing countries.

However, it cannot differentiate between *E. dispar* and *E. histolytica*, and the accuracy of this method in detecting *E. histolytica* depends heavily on the skills of the technician and has been shown to be less sensitive and less specific compared with other methods such as immunoflorescence (IFA), antigen detection, and polymerase chain reaction (PCR) (Haque at al., 2003). Intestinal parasites are a significant health problem today in poorly developed or developing countries. In studies held in different regional hospitals in Turkey, the prevalence of intestinal parasites ranged between 4.1 to 96% depending on the age group, the laboratory method used in diagnosis, regional differences, socioeconomic level and whether pathogenic species are included in the study or not (Yılmaz et al., 2002). This study aimed to: (i) determine the frequency of *E. histolytica/E. dispar* carriers in regions with a low socioeconomic level and inadequate sanitation conditions in three central districts of Bursa Province, located on the North-eastern part of Turkey; (ii) compare wet mount + Lugol's iodine, formalin ethyl acetate concentration, trichrome staining, and enzyme- linked immunosorbent assays (ELISA) methods used in *E. histolytica/E. dispar* diagnosis; and (iii) establish the relationship between the presence of the intestinal parasites and socio-demographic factors.

MATERIALS AND METHODS

The laboratory examinations for the study were performed in various Health Centers in the Bursa City center and at the Parasitology and ELISA Laboratories of the Uludag University, Faculty of Medicine's Department of Microbiology.

*Corresponding author. E-mail: oktayalver@uludag.edu.tr. Tel: +90 224 295 03 22. Fax: +90 224 442 91 81.

Study area and population

The study was conducted during the months of October 2004 and January and May 2005, in the communities within Bursa City, Turkey. Throughout the year in this area, the weather is cool but is coolest during the months of December to February with an average temperature of 6.2°C. The area is densely populated with a population of 1,229,454 people and a density of 898 people/km^2 (http://www.tuik.gov.tr/VeriBilgi. do?tb_id=11- 2010). Our hospital offers tertiary level health services. The individuals living in the regions included in the study refer mostly to primary and secondary level health institutions due to socioeconomic reasons. For this reason, face-to-face interviews with individuals included in the study were performed in the health centers where they receive health services in order to inform them about the study, receive informed consent, and collect samples. Furthermore, demographic features such as age, sex, and factors related to the living environment of these individuals (e.g., quality of drinking water and toilet location) were also recorded.

Fecal sampling and storage and laboratory procedures

A prospective study was conducted on 176 stool samples collected from cases who had no complaints for intestinal parasite. All fecal samples were collected in lidded plastic containers and were examined within 30 min with 40X objectives by wet mount + Lugol's iodine in the health centers. The samples were then fixed in polyvinyl alcohol solution and transferred to the Parasitology Laboratory of the Department of Microbiology at the Uludag University, Faculty of Medicine. Each fixed fecal sample was microscopically evaluated using formalin ethyl acetate concentration and trichrome staining (Garcia, 2001) . The unfixed portions of the fecal samples were kept for 30 days at -20°C until studied with ELISA for antigen detection. Spheric structures of 12 to 15 µm in diameter with centrally located karyosomes and peripheral uniform chromatin bodies containing one or two immature and four mature nuclei were evaluated as *E. histolytica/E. dispar* cysts and were diagnosed differentially from other commensal parasites such as *Entamoeba coli* (Tanyuksel 2003) . The measurements were conducted oculometrically (CHWK, Olympus, Japan).

ELISA

E. histolytica/E. dispar antigens in the feces were detected with Ridascreen® (Entamoeba R-Biopharm AG, Darmstadt, Germany), a commercial ELISA kit. ELISA tests were performed according to the instructions of the manufacturer, and all samples were studied once.

Statistical analysis

The number of samples to be included in the study was calculated out of a population of 1,229,454 people with p = 0.07, and an error rate of d = 0.05. Prevalence levels were estimated according to each of the variables studied. In the statistical evaluation of the differences between groups, chi-square (^{2}) and Fisher's exact tests were used and differences at a level of $p<0.05$ were considered significant.

RESULTS

A total of 176 faecal samples were examined by three microscopic methods and ELISA of which 66 (37.5%)

Table 1. Prevalence of intestinal parasites in 176 faecal samples in a urban population in Bursa Province.

Parasite species	Prevalence N (%)	*p* value
Protozoa		
Blastocystis hominis	30 (17.04)	0.555
Giardia intestinalis	13 (7.38)	0.967
Entamoeba coli	10 (5.68)	0.171
Entamoeba histolytica/E. dispar [a]	4 (2.27)	0.348
Entamoeba hartmani	2 (1.13)	0.476
Chilomastix mesnili	2 (1.13)	1.000
Helminthes		
Enterobius vermicularis	16 (9.09)	0.899
Hymenolepis nana	2 (1.13)	0.476
Taenia spp.	1 (0.56)	1.000

[a]Positive by ELISA for Entamoeba histolytica/E. dispar.

Table 2. The prevalence of intestinal parasites according to demografic factors in infected individuals (n=66).

Characteristic	No. examined	No. of positive	Prevalence rate	*p* value
Age group (years)				
1-19	50	24	48.00	0.181
20-49	93	35	21.21	0.983
50	33	7	37.63	0.072
Gender				
Male	102	36	35.29	
Female	74	30	40.54	0.478
People living in the house (n)				
<4	81	35	43.20	
5	95	32	33.68	0.149
Immigration (residential area)				
Native	76	29	38.15[a]	0.921
Western	5	1	20.00[b]	0.653
Eastern	54	25	46.29[b]	0.297
Abroad	37	10	27.02[b]	0.268
Northern	4	1	25.00	1.000

([a]) native inhabitants.. ([b]) inhabitants of immigrants from Turkey. No statistically significant differences were detected between the prevalence of intestinal parasites and personal factors such as age, sex, household size, locality, water supply.

were infected with one or more intestinal parasites, pathogenic or non pathogenic. The prevalence of parasite species found in the sample of individuals is presented in Table 1. Nine parasite species were iden-tified, including six protozoon and three helminth. The most common intestinal parasites detected were *Blastocystis hominis* (17.04%), followed by *Enterobius vermicularis* (7.38%) and *Giardia intestinalis* (5.68%). Generally, mix infections were detected in 11 (6.2%) faecal samples. Overall prevalence of intestinal parasite in relation to different sociodemografic factors such as age, sex, household size, immigration (residential area) and water supply are summarised in Table 2. Although, the prevalence rates were found to be higher in the 1 to 19 age group (48%), female (40.5%), people who migrated from east of Turkey (45.4%) and people drinking well-water (66%), no statistically significant differences were detected between the overall

Table 3. The prevalence of parasitic species according to gender, age, household size, immigration (residential area) and water supply in infected individuals (n= 66).

Characteristics	No. of examined	Identified parasites, No. of positive (%)								
		Bh	Gi	Ev	Ec	Ehi/Ed	Eha	Cm	Tsp	Hn
Age group (years)										
1-19	50	6(12)	10(20) [a]	8(16)	3(6)	2(4)	0	0	0	1(2)
20-49	93	22(23.6) [b]	2(2.1)	7(7.5)	5(5.4)	1(1.07)	2(2.14)	1(1.0)	2(2.14)	0
50	33	2(6.06)	1(3.0)	1(3.0)	2(6.0)	1(3.03)	0	1(3.0)	0	0
Gender										
Male	102	16(15.7)	6(5.88)	9(8.82)	2(1.9)	3(2.94)	2(1.96)	1(0.98)	0	1(0.98)
Female	74	14(18.91)	7(9.45)	7(9.45)	8(10.8)	1(1.35)	0	1(1.3)	2(2.7)	0
People living in the house (*n*)										
<4	81	17(20.1)	3(3.7)	8(9.87)	5(6.2)	1(1.2)	1(1.2)	1(1.2)	1(1.2)	0
5	95	13 (13.7)	10(10.5)	5(5.3)	5(5.3)	3(3.1)	1(1.05)	1(1.05)	1(1.05)	1(1.05)
Immigration (residential area)										
Native	76	13(17.1)	7(9.2)	8(10.52)	5(6.57)	1(1.3)	1(1.3)	1(1.3)	1(1.3)	0
Western	5	1(20)	0	1(20)	0	0	0	0	0	0
Eastern	54	10(18.5)	4(7.4)	6(11.1)	3(5.5)	2(3.7)	1(1.85)	1(1.85)	1(1.85)	1(1.85)
Northern	37	5(13.5)	1(2.7)	1(2.7)	2(5.4)	1(2.7)	0	0	0	0
Abroad	4	1(25)	0	0	0	0	0	0	0	0
Water supply										
Municipal	161	29 (18.0)	12(7.4)	16(9.9)	8(4.9)	3(1.8)	2(1.2)	2(1.2)	2(1.2)	1(0.62)
Commercial/Municipal	12	1(8.3)	1(8.3)	1(8.3)	1(8.3)	0	0	0	0	0
Well	3	0	0	0	0	0	0	0	0	0

Bh= *Blastocystis hominis*; Gi= *Giardia intestinalis*, Ev= *Enterobius vermicularis*; Ehi/ Ed= *Entamoeba histolytica/E. dispar*; Eha= *Entamoeba hartmani*; Cm= *Chilomastix mesnili*; Tsp= *Taenia* spp; and, Hn= *Hymenolepis nana*

([a]) Significantly higher than the other age groups (p= 0.001). ([b]) Significantly higher than the other age groups (p= 0.005)

prevalence of intestinal parasites and these factors. Also, it was noticed that infection decreased when family size increased and no significant difference was found (p=0.149). Prevalence of intestinal parasite species in different demographic groups are presented in Table 3. Statistically, *B. hominis* and *G. intestinalis* were higher in the 20 to 49 and 1 to19 year age groups, respectively. Only *E. coli* and *Taenia spp*. was more frequent in females than males, while the prevalence of other parasites was higher in males than females (with the exception of *C. mesnili*, which had a similar frequency in both sexes). However these differences were not statistically significant.

As seen in Table 4, positive results for the *E. histolytica/E. dispar* complex were observed in 0.56% (1) of the fecal samples examined with wet mount + Lugol's iodine, in 0.56% (1) of those examined with formalin ethyl acetate concentration, in 0.56% (1) of those examined with trichrome staining, and in 2.27% (4) of those examined with ELISA. Of the samples, 92.04% (162) yielded negative results for all 4 tests. Only one sample (0.56%) showed positive results with all four methods. The wet mount + Lugol's iodine, formalin ethyl acetate and trichrome staining methods had a sensitivity of 25% each, a specificity of 94.1, 99.4 and 98.2%, a positive predictive value of 9.09, 50 and 25% and a negative predictive value of 98.1, 98.2 and 98.2% compared to the results of the *E. histolytica/E. dispar* ELISA, respectively.

DISCUSSION

Intestinal parasitosis, a major public health problem, particularly in developing countries, affects 3.5 billion people globally (WHO's World Health Report, 2000). Recent studies reported that the prevalence rates of the

Table 4. Comparison of four different diagnostic method in detection of *E. histolytica/E*. *dispar*.

Diagnostic test	Result	
	Positive (%)	Negative (%)
Wet mount + Lugol's iodine	1(0.56)	165(99.43)
Formalin ethyl acetate concentration	1(0.56)	174(99.43)
Trichrome staining metod	1(0.56)	172(99.43)
ELISA[a]	4(2.27)	172(97.72)

[a] Specific for *E. histolytica*/*E. Dispar*.

intestinal parasite infections were 51.7% in China (Ning and Nian, 2003), 44.3% in Bangladesh (Sultana-Azam and Rahman-Bhuiyan, 2007), and 83% in Ethiopia (Mengistu et al., 2007). A national epidemiological survey (1999 to 2000) showed that intestinal parasitic infections affect more than 19% of the Iranian population (Sayyari et al., 2005). In Turkey, previous studies revealed high prevalence rates of infection with intestinal parasites. Two community-based studies have reported the preva-lence rates as 41.4 and 37.2% (Ak et al., 2007; Celiksoz et al., 2005). Although hospital based studies were carried out in the Bursa Province (Alver et al., 2005; Alver and Töre, 2006), there are no available data from the urban settings of this region. In this study, we examined 176 faecal samples and the prevalence of intestinal parasites found was 37.5%. This prevalence value indicate how widespread parasitic diseases are in the region. The prevalence in our study was also higher com-pared to other community-based studies conducted in zmir (Dagci et al., 2008) showing an overall prevalence of 25.6% but lower than in Sivas (Celiksoz et al., 2005). Migrations not only cause large shifts in global population but also create new public health problems. It has been reported that the prevalence of parasitic infections was found to be elevated among immigrants from Eastern to Western Anatolia (Demirel et al., 2002) . In a study conducted in school children in Manisa, it was revealed that the prevalence of intestinal parasites was higher among immigrants from Eastern Anatolia (Balcioglu et al., 2007). The rapid extension of industriali-zation of this region causes thousands of migrant families to migrate to urban areas of Bursa. Although living standards in Bursa are higher than in other Eastern provinces, the prevalence of the intestinal parasites was found to be high there.

In our study, *B. hominis* was the predominant parasite followed by *E. vermicularis* and *G. intestinalis*. Similar studies conducted in other counties in Turkey revealed that the predominant parasites were *B. hominis, G. intestinalis,* and *E. coli* among the general population (Celiksoz et al., 2005; Okyay et al., 2004*).* In this study, the observed *H. nana*, *G. intestinalis*, and *B. hominis* coinfections with *E. histolytica*/*E. dispar* could be explained by the facts that these parasites have the same mode of transmission and that hygiene is poor in these areas. *B. hominis* which is transmitted by faecal-oral route was commonly found together with other intestinal pathogenic protozoa. Therefore, infection with *B. hominis* is an indicator of poor personal hygine and warning sign of intestinal parasitic infection (Saksirisampant et al., 2003; Nascimento and Moitinho, 2005). The communities with high prevalence of *B. hominis* infection have to improve their sanitation to prevent not only *B. hominis* but other pathogenic intestinal protozoa. Although a few reports found that *B. hominis* infection was common in the over 30 year- old group (Kain et al., 1987; Doyle et al., 1990), our study showed the highest prevalence to be in the 20 to 49 year-old age group ($p<0.005$) . In our study population, the 1- 19 year-old group showed a significantly high risk for *Giardia* infection ($p<0.001$). No significant differences were noted among the age groups with regard to other parasitic infections. However, statistical significances might have been obtained if a larger number of infected subjects with each species had been observed. Overall, our study shows that females were more likely to be infected (40.5%) than males (35.2%) without statistically significant differences; this is in agreement with the findings of population studies in Western Turkey (zmir) (Balcioglu et al., 2007) and China (Ning and Nian, 2003). These differences in sex groups may be attributed to different occupational activities of males and females. Intestinal parasites are known to be closely related to hygiene behaviour and as such, members of a family can easily infect each other. A large family size or living in relatively crowded conditions are also a risk factor for intestinal parasites (Holland et al., 1988). However, in this study, it was noticed that intestinal parasite prevalance decreased with increased family size. It could not be explained on the basis of the information available from the questionnaire. The reason for this is not entirely clear but may include differences in host factors.

Infection with *E. histolytica* is a severe health problem in many tropical and subtropical areas of the world, especially in developing countries. While *E. histolytica*, one of two *Entamoeba* species that infect humans and cannot be differentiated morphologically causes amebic colitis and liver abscess, *E. dispar* is noninvasive. Laboratory diagnosis of intestinal amebiasis is performed by native-lugol staining and examination of the stained slides for cysts and/or trophozoites under a light microscope (Haque et al., 1995). *E. histolytica* cannot be distinguished under the microscope from the morphologically

similar apathogenic *E. dispar* (WHO/PAHO/UNESCO, 1997). The World Health Organization stated that distinguishing *E. histolytica* from *E. dispar* is important both in deciding the type of treat-ment and in epidemiological terms and recommended that only cases with *E. histolytica* be treated (World Health Organization, 1997). In recent years, differen-tiation of pathogenic species has been achieved by molecular methods [Polymerase Chain Reaction (PCR), DNA probes, ribotyping], detection of parasitic antigens in feces with enzyme immunoassay methods, and by isoenzyme analysis after fecal culturing (Tanyuksel and Petri, 2003; Tachibana et al., 2000).

Different studies have been conducted in various countries to compare microscopical methods, serologic methods and PCR. The results of a study reported by Evangelopoulos et al. (2006) indicated that the prevalence of *E. histolytica* and *E. dispar* was very low in Greece and that PCR and ELISA were by far better detection methods than microscopy. Visser et al. (2005) postulated that in carriers of *E. histolytica/E. dispar* from non-endemic countries, the high serology specificity can be used to establish the diagnosis of *E. histolytica* infection if antibodies are present. Haque et al. (1997) reported that the frequency of asymptomatic colonization with *E. histolytica/E. dispar* was found to be 3.5% with microscopy and 4.2% with culture in 987 children between the ages of 1 to 14 in Bangladesh. When the same samples were screened with ELISA, 8% antigen positivity for *E. histolytica/E. dispar* and 1% antigen positivity for *E. histolytica* were detected. In a study by Nesbitt et al. (2004), *E. histolytica* was detected in 8.7% of the 842 samples using microscopic examination, whereas the prevalences of *E. histolytica* and *E. dispar* were reported to be 0.8 and 7.4% respectively, based on ELISA. Comparing antigen tests and microscopy for the detection of *E. histolytica/E. dispar* complex to the gold standard of microbial culture, Nesbitt et al. (2004) also found that the antigen detection test was more sensitive (80% vs. 60%) and more specific (99% vs. 79%) than microscopy. When Haque et al. (1995) analyzed the *E. histolytica*-specific antigen detection test for the discrimination of *E. histolytica* from *E. dispar*, they found 95% sensitivity and 93% specificity. While the probability of detecting protozoan agents with a single microscopic examination in symptomatic cases is 13%, this rate was reported to increase to 19% with 2 fecal examinations at different times and to 65% with 3 examinations (Hiatt et al., 1995). However, this rate is higher (up to 85 to 95%) for other intestinal parasites (Li and Stanley, 1996) . In Turkey, Delialioglu et al. (2004) detected 20.4% *E. histolytica/E. dispar* antigen positivity with trichrome staining and 29.5% positivity with ELISA. The researchers found that trichrome staining had a sensitivity of 53.8% and a specificity of 94% with a positive predictive value of 78% and negative predictive value of 17% compared to ELISA. In a study held in anlıurfa, 21% *E. histolytica/E. dispar* antigen positivity was detected with ELISA, compared to 26.4% with microscopy (Zeyrek et al., 2006). Tanyuksel et al. (2005) detected *E. histolytica/E. dispar* with microbiological methods in 91 (24%) of the 380 fecal samples studied; they also detected positivity for *E. histolytica* antigens in 14 of the samples detected to be positive with ELISA and microscopic methods and in 37 of the samples detected to be negative with microscopic methods. The sensitivity of microscopic examination varies from 10 to 60% even in the best conditions, and detection of leukocytes, macrophages and other apathogenic *Entamoeba* species in feces could lead to false positive results (Haque et al., 1997; Delialioglu et al., 2004). Discrimination of the pathogenic *E. histolytica* and apathogenic *E. dispar* cannot be achieved by microscopy and can lead to false positive and false negative results. In this study, positivity for *E. histolytica/E. dispar* antigen was detected by ELISA in only 4 of the 14 samples found to be positive with at least 1 of the 3 other methods. This difference can be attributed to a low number of cysts, non-homogenous distribution of the cysts and single examination of the samples. On the other hand, in the present study, the cases that were microscopy-positive but ELISA-negative could be accounted for by misdiagnosis of microscopy.

From the data obtained in this study, it can be concluded that we detected a high rate of intestinal parasites without discriminating between pathogenic and apathogenic species and may suggest high fecal-oral transmission and be indicative of the poor socio-economic conditions of these individuals. In addition, the study suggests that *E. histolytica/E. dispar* complex antigen detection with ELISA can be used for screening purposes, as it is more cost-effective compared to the microscopic examination methods previously mentioned and does not require experienced microscopists, since it provides objective results. In the present survey of 166 individuals, using an ELISA based antigen detection kit on stools, approximately 2.2% were *E. histolytica/E. dispar* complex positive in the Bursa city centre. We are aware of the fact that the data obtained in our study cannot in general reflect the overall prevalence in the Northwest Region of Turkey as the samples analysed are random but belong to individuals not suspected of harboring intestinal parasitosis.

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