

# A parasite that should not be neglected in geriatric individuals

## *Entamoeba histolytica*

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### ABSTRACT

**الأهداف:** تسليط الضوء على أهمية العدوى الممهلة لحالة النسيج (*E. histolytica*) في الفئة العمرية للمسنين، والتي تمثل نسبة متزايدة من سكان العالم.

**المنهجية:** أجريت هذه الدراسة خلال الفترة من يناير 2022 وديسمبر 2023م في جامعة فان يوزونكو بيل، كلية الطب، مختبر الطفيليات، فان، تركيا. شملت الدراسة 96 مريضاً من كبار السن يعانون من الإسهال (مجموعة كبار السن). واشتملت المجموعتين الضابقتين المختلفتين (CGs) في الدراسة، اشتملت مجموعة CG1 على 92 مريضاً تتراوح أعمارهم بين 18-64 عاماً يعانون من الإسهال ومجموعة CG2 على 50 فرداً من كبار السن دون إسهال وغيره من شكاوى الجهاز الهضمي. أجرينا تحليل العينات مجهرياً ومن ثم تقييمها عن طريق المقايسة المناعية المجهريّة المرتبطة بالإنزيم وتفاعل البوليميراز المتسلسل.

**النتائج:** كشفت هذه الدراسة عن بكتيريا *E. histolytica* في 31.3% من مجموعة كبار السن و 14.1% من المرضى في مجموعة CG1  $p=0.004$ . لم يتم اكتشاف *Entamoeba histolytica* إيجابية في مجموعة CG2. وفقاً لتحليل المراسلات المتعدد، كان هناك ارتباط وثيق بين إيجابية الحالة للأنسجة والإسهال الدموي والإسهال المخاطي في مرضى الشيخوخة. وقد تقرر أيضاً أن *E. histolytica* يمكن أن تسبب آلاماً في البطن، وانتفاخ البطن، وآلماً شرسوفي عند مرضى الشيخوخة.

**الخلاصة:** وجدنا أن كلا من خطر عدوى *E. histolytica* وزيادة العدوى لدى الأفراد المسنين. وبذلك، نستنتج أن داء الأميبات يمثل مشكلة صحية خطيرة لدى كبار السن ولا ينبغي إهماله.

**Objectives:** To highlight the importance of neglected *Entamoeba histolytica* (*E. histolytica*) infections in the geriatric age group, which is an increasing proportion of the world's population.

**Methods:** This study was carried out between January 2022 and December 2023 at Van Yüzüncü Yil University, Faculty of Medicine, Parasitology Laboratory, Van, Turkey. The study included 96 geriatric patients with diarrhea (geriatric group). Two different control groups (CGs) were also included in the study, comprising 92 patients aged 18-64 years with diarrhea as CG1 and 50 geriatric individuals without diarrhea and other gastrointestinal complaints as CG2. Samples were analysed macroscopically

and then evaluated by microscopic, enzyme-linked immunoassay, and polymerase chain reaction.

**Results:** This study detected *E. histolytica* in 31.3% of the geriatric group and 14.1% of the patients in CG1 ( $p=0.004$ ). *Entamoeba histolytica* positivity was not detected in CG2. According to the multiple correspondence analysis, there was a close association between *E. histolytica* positivity and bloody diarrhea and mucous diarrhea in the geriatric patients. It was also determined that *E. histolytica* can cause abdominal pain, abdominal distension, and epigastric tenderness in geriatric patients.

**Conclusion:** Both the risk of *E. histolytica* infection and the pathogenicity of the infection increase in geriatric individuals. Therefore, it was concluded that amoebiasis is a serious health problem in the geriatric population and should not be neglected.

**Keywords:** amoebiasis, diarrhea, elderly, neglected, prevalence

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The geriatric population, defined by the World Health Organisation (WHO) as those aged 65 and over, is growing every day around the world.<sup>1</sup> According to the Organisation for Economic Co-operation and Development, people aged 65 and over made up 9.82% of the world's population in 2022.<sup>2</sup> According to the WHO, the growth of the elderly population will continue and by 2050, the majority of the elderly population will live in low- and middle-income countries.<sup>3</sup> This WHO prediction raises the question: how will intestinal parasites (IP), which are a major health problem in low- and middle-income countries, affect geriatric individuals?

Worldwide, more than 3.5 billion people are infected with IP each year, with clinical symptoms occurring in 4.5 million cases.<sup>4</sup> Amoebiasis caused by *Entamoeba histolytica* (*E. histolytica*) is one of the leading IP infections around the world.<sup>5</sup> Amoebiasis of the intestine can also spread to other organs, such as the liver, lungs, or skin.<sup>6</sup> Amoebiasis, a neglected emerging disease, is the leading cause of death from parasitic diseases worldwide, after malaria and schistosomiasis.<sup>6,7</sup>

The prevalence of *E. histolytica* is increasing in both the paediatric and the geriatric age groups.<sup>8</sup> Weakening of the immune system is one of the most common adverse effects in geriatric individuals. Because of the immune deficits that occur, diseases can lead to poor clinical outcomes in a much shorter period of time. However, most of the studies on amoebiasis have focused on the paediatric age group. Therefore, there is little information on *E. histolytica* in geriatric patients, who are more likely to have a poor prognosis. The aim of this study was to highlight the importance of neglected *E. histolytica* infections in the geriatric age group and discuss the impact that amoebiasis has on these individuals.

**Methods.** This cross-sectional study was carried out between January 2022 and December 2023 at the Parasitology Laboratory, Faculty of Medicine, at Van Yüzüncü Yıl University, Van, Turkey. The study was designed with one patient group and 2 control groups (CGs). Geriatric individuals with gastrointestinal complaints were included in the patient group. In CG1, patients aged 18-64 years with gastrointestinal complaints were included to compare *E. histolytica* positivity in geriatric individuals and middle-aged individuals. A second CG was formed to determine the clinical course of *E. histolytica* positivity in geriatric patients. The CG2 included geriatric patients without gastrointestinal complaints.

The sample size of this prospective study was calculated using G\*Power (ver.3.1.9.7) statistical

software within the scope of Chi-square ( $\chi^2$ ) goodness-of-fit tests.<sup>9</sup> In the calculations, "a minimum of 50 samples in each group" was determined when the Power was 0.80, the effect size was 0.4 ( $\chi^2$  test effect size interval value) and the type 1 error ( $\alpha$ ) was 0.05.

As it was difficult to reach geriatric people who volunteered for the study without gastrointestinal complaints, the number of people in the CG2 group was kept lower than in the other groups.

The study included 96 geriatric patients with diarrhea (geriatric group) referred to the Parasitology Laboratory at Van Yüzüncü Yıl University Dursun Odabaş Medical Centre, Van, Turkey. The CG1, which comprised 92 patients with diarrhea aged 18-64 years referred to the same laboratory. Information on age, gender, clinical complaints, and chronic diseases of the patients was obtained from the hospital's automated system.

The CG2, which included 50 geriatric patients without diarrhea and other gastrointestinal complaints, was included. Geriatric individuals without any gastrointestinal complaints such as abdominal pain, diarrhea, constipation, nausea, vomiting, anorexia, nausea, and vomiting were included in CG2 by direct interview. Individuals with any gastrointestinal complaints and who had used antibiotics, antiprotozoa, antidiarrhoeal compounds, barium, bismuth, or mineral oil in the last 3 months before the study were not included in the CG2. In addition, individuals whose stool samples were not shaped were not included in the study.

This study was approved by Van Yüzüncü Yıl University non-interventional clinical research ethics committee, Van, Turkey (01/10/2019-E.13580). All individuals included in the study signed an informed consent form.

The stool samples were macroscopically examined and evaluated for *Entamoeba* species. The stool samples were analysed by both nativ-lugol and trichrome staining methods to detect different numbers of nucleated cysts and trophozoites of *Entamoeba* species. Trichrome staining was carried out using Wheatley's trichrome staining kit (Gul Biology Laboratory, Istanbul, Turkey), following the manufacturer's instructions.

Adhesin antigen was detected for *E. histolytica* seropositivity in stool samples using an enzyme-linked immunoassay (ELISA) kit (TechLab Systems Inc., Blacksburg, VA, USA), following the manufacturer's instructions.

The DNA isolation from stool samples was carried out using DNA extraction kit (EURx GeneMATRIX Stool DNA Purification Kit, Poland)

according to Aydemir et al.<sup>10</sup> The SYBR Green reverse transcription polymerase chain reaction (RT-PCR) was carried out with the obtained DNA using EhF3 5'-CAGTAATAGTTTTCTTTGGTTTAGTAAAA-3' and EhR3 5'-CTTAGAATGTCATTTCAATTTCTCAATTCA-3' primers to differentiate *E. histolytica* from *E. dispar*, *E. moshkovski*, and *E. bangladeshi*.<sup>11</sup> The RT-PCR was carried out using a 20- $\mu$ L mixture containing 10  $\mu$ L of 2X SYBR Green Master Mix (Thermo Fisher, UK), 1  $\mu$ L each of 20  $\mu$ M right and left primers, and 5  $\mu$ L of genomic DNA. All of the RT-PCR experiments were carried out on a Rotor Gene Q (Qiagen, Germany) thermal cycler. Thermal cycles of RT-PCR were carried out according to Ali et al.<sup>11</sup>

**Statistical analysis.** In the statistical the analyses,  $\chi^2$  test, 2-ratio Z test of the ratios, and Fisher's exact test were used. In the calculations, statistical significance was considered to be <5%. Multiple correspondence analysis (Permutation tests) was used to determine the fit between the variables. The Statistical Package for the Social Sciences, version 26.0 (IBM Corp., Armonk, NY, USA) and MINITAB, version 14.0 were used for the calculations.

**Results.** Of the 96 geriatric patients with diarrhea, 45 (46.9%) were male and 51 (53.1%) were female. Of the 92 patients with diarrhea aged 18-64 years included as CG1, 44 (47.8%) were male and 48 (52.2%) were female. Of the geriatric patients without diarrhea and gastrointestinal complaints included as CG2, 27 (54%) were male and 23 (46%) were female. A total of 10

(10.4%) of the geriatric patients, 17 (18.5%) of the CG1 patients and 3 (6%) of the CG2 individuals had a chronic disease that could affect the immune system. The macroscopic and microscopic examination of the stool samples showed blood in 78 (81.3%) and mucus in 75 (78.1%) of the geriatric patients and blood in 67 (72.8%) and mucus in 62 (67.4%) of the CG1 patients. No blood or mucus was found in any of the individuals in CG2. No significant difference was found between the frequency of blood and mucus, chronic diseases, and gender in geriatric group and CG1 (Table 1).

Microscopically, *Entamoeba* spp. was detected in 79 (33.2%) of a total of 238 patients, including 43 (44.8%) of the geriatric patients, 32 (34.8%) of the patients in CG1, and 4 (8%) of the individuals in CG2. The difference between the geriatric group and CG1 was not statistically significant ( $p=0.159$ ), whereas the difference between CG2 and the other groups was statistically significant ( $p=0.001$ ). Microscopic examination also revealed *Blastocystis* in 40 (16.8%), *Giardia duodenalis* in 10 (4.2%), *Iodamoeba butschlii* in 5 (2.1%), and *Chilomastix mesnili* in 2 (0.8%) of the 238 patients.

*Entamoeba histolytica* adhesin antigen was detected by ELISA in a total of 43 (18.1%) patients; 30 (31.3%) in the geriatric group and 13 (14.1%) in CG1. All 43 of the patients who were positive for *E. histolytica* by ELISA were also positive by RT-PCR. *Entamoeba histolytica* was not detected in CG2. A statistically significant difference was found between the positivity rates for *E. histolytica* detected in the geriatric group and those detected in CG1 (Table 2).

**Table 1** - Evaluation of the gender, the presence of blood and mucus in the stool samples, and the presence of chronic diseases in the geriatric between the groups.

Variables	Geriatric group	CG1	CG2	P-values
<i>Gender</i>				
Female	51 (53.1)	48 (52.2)	23 (46.0)	0.896
Male	45 (46.9)	44 (47.8)	27 (54.0)	
<i>Blood in the stool</i>				
Yes	78 (81.3)	67 (72.8)	0 (0.0)	0.169*
No	18 (18.7)	25 (27.2)	50 (100)	
<i>Mucus in the stool</i>				
Yes	75 (78.1)	62 (67.4)	0 (0.0)	0.098*
No	21 (21.9)	30 (32.6)	50 (100)	
<i>Chronic disease</i>				
Yes	10(10.4)	17 (18.5)	3 (6.0)	0.062**
Cancer	4 (4.2)	9 (9.9)	0 (0.0)	
Chronic kidney disease	1 (1.0)	4 (4.3)	0 (0.0)	
Diabetes	5 (5.2)	4 (4.3)	3 (6.0)	
No	86 (89.6)	75 (81.5)	48 (94.0)	

Values are presented as numbers and percentages (%). \*The geriatric group was compared with the CG1. \*\*This value was calculated by taking into account the total number of individuals with chronic diseases between the groups. CG: control group

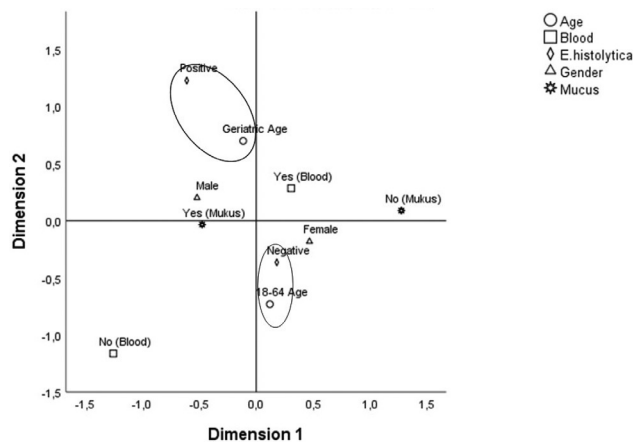
Multiple correspondence analysis was carried out for all of the patients with diarrhea to determine the association between *E. histolytica* positivity and the age group, gender, presence of mucus in the stool, and presence of blood in the stool. According to the multiple correspondence analysis diagram (Figure 1), there was an association between *E. histolytica* positivity in the geriatric group patients with diarrhea and *E. histolytica* negativity in the CG1 patients.

While bloody diarrhea was detected in 27 (90%) of the geriatric patients who were positive for *E. histolytica*, bloody diarrhea was detected in 9 (69.2%) of the patients who were positive in CG1. In addition, mucus was detected in 27 (90%) of the geriatric patients who were positive for *E. histolytica*, whereas mucus was detected in 7 (53.9%) of the patients who were positive in CG1. According to the multiple correspondence

**Table 2 -** Distribution of the *Entamoeba histolytica*-positive patients according to the study group and gender.

Group	<i>E. histolytica</i>		Total	P-values
	Positive	Negative		
<b>Research group</b>				
Geriatric group	30 (31.3)	66 (68.7)	96 (40.3)	0.004
CG1	13 (14.1)	79 (85.9)	92 (38.7)	
CG2	0 (0.0)	50 (100)	50 (21.0)	
<b>Gender</b>				
Female	17 (13.9)	105 (86.1)	122 (51.3)	0.089
Male	26 (22.4)	90 (77.6)	116 (48.7)	
Total	43 (18.1)	195 (81.9)	238 (100)	

Values are presented as numbers and percentages (%).  
*E. histolytica*: *Entamoeba histolytica*

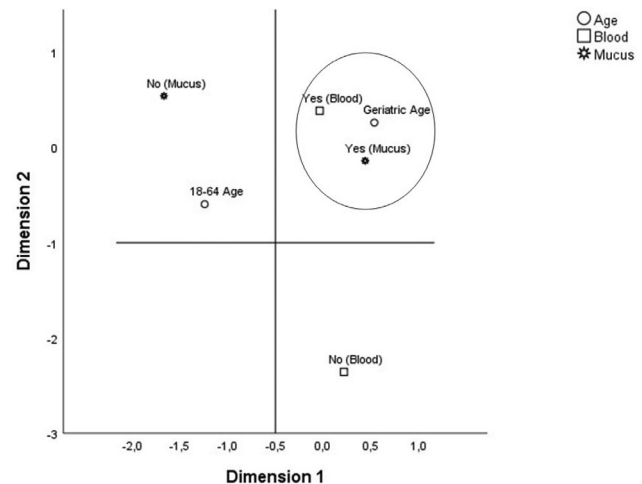


**Figure 1 -** Diagram of the multiple correspondence analysis between *Entamoeba histolytica* positivity and the variables in all of the diarrhea patients. Dimension 1 explains 27.1% of the total change, while Dimension 2 explains 26.5% of the total change. *E. histolytica*: *Entamoeba histolytica*

analysis diagram between the variables (Figure 2), a significant association was found between the incidence of *E. histolytica* and bloody diarrhea and diarrhea with mucus in the geriatric patients.

Statistical analysis showed significant correlations between the positivity of *E. histolytica* and abdominal pain, abdominal distension, and epigastric tenderness (each separately) in the geriatric group. However, no statistically significant association was found between positivity of *E. histolytica* and weight loss and itching (Table 3). In addition, no statistical difference was found in the comparison of *E. histolytica* positivity in the geriatric group with CG1 according to chronic diseases (Table 4).

**Discussion.** This study detected *E. histolytica* in 31.3% of geriatric patients with diarrhea and 14.1% of patients aged 18-64 with diarrhea. *Entamoeba histolytica* positivity was not detected in geriatric patients without gastrointestinal symptoms. Studies have reported a higher prevalence of *E. histolytica* in the elderly.<sup>12,13</sup> The current study also shows that the risk of transmission of *E. histolytica* is higher in the geriatric age group than in those aged 18-64 years old. Thus, this study showed that amoebiasis, which is usually emphasised as being more important in the paediatric age group, is also an important health problem in the geriatric age group and should be kept in mind in these individuals.<sup>14</sup> In one study, it was stated that the prevalence of *E. histolytica* in humans has decreased from 1990-2019, but *E. histolytica* infections in adults and the elderly in societies with a high sociodemographic index tend to increase.<sup>15</sup> On the



**Figure 2 -** Diagram of the multiple correspondence analysis between the variables in the *Entamoeba histolytica*-positive patients. Dimension 1 explains 33.7% of the total change, while Dimension 2 explains 27% of the total change.



**Table 3 -** The relationship between the clinical findings in the geriatric group and *Entamoeba histolytica*.

Clinical symptoms	<i>E. histolytica</i> in the geriatric group		P-values
	Positive	Negative	
<i>Abdominal pain</i>			
Yes	13 (50.0)	13 (50.0)	0.016*
No	17 (24.3)	53 (75.7)	
<i>Abdominal distension</i>			
Yes	9 (69.2)	4 (30.8)	0.001**
No	21 (25.3)	62 (74.7)	
<i>Epigastric tenderness</i>			
Yes	9 (100)	-	0.001**
No	21 (24.1)	66 (75.9)	
<i>Itching</i>			
Yes	3 (27.3)	8 (72.7)	0.999**
No	27 (31.8)	58 (68.2)	
<i>Weight loss</i>			
Yes	10 (28.6)	25 (71.4)	0.668*
No	20 (32.8)	41 (67.2)	

Values are presented as numbers and percentages (%). \*Chi-square test. \*\*Fisher exact test.  
*E. histolytica*: *Entamoeba histolytica*

**Table 4 -** Comparison of *Entamoeba histolytica* positivity in the geriatric group with the CG1 according to chronic diseases.

Chronic diseases	Geriatric group		CG1		P-values*
	<i>E. histolytica</i>		Positive	Negative	
	Positive	Negative	Positive	Negative	
Cancer	0 (0.0)	4 (100)	1 (11.1)	8 (88.9)	0.999
Chronic kidney disease	0 (0.0)	1 (100)	1 (25.0)	3 (75.0)	0.999
Diabetes	2 (40.0)	3 (60.0)	1 (25.0)	3 (75.0)	0.999
Total	2 (20.0)	8 (80.0)	3 (17.6)	14 (82.4)	0.999

Values are presented as numbers and percentages (%). \*Fisher exact test. *E. histolytica*: *Entamoeba histolytica*, CG: control group

other hand, there are studies reporting the importance of amoebiasis and that it should not be neglected due to new reports of the emergence of hypervirulent strains, the lack of other effective options in case of resistance to currently used drugs, and the lack of a viable vaccine candidate ready.<sup>16</sup> Proof also that amoebiasis should not be neglected in geriatric individuals is the detection of necrotising amoebic colitis in the autopsy of a geriatric patient with unknown cause of death.<sup>17</sup> That study raised the question of whether amoebiasis plays a role in causing unexplained deaths in geriatric individuals. Consequently, as not considering amoebiasis in the geriatric population is detrimental to both patient health and the national economy, we believe it should be considered in this patient population.

In the present study, a close relationship was found between *E. histolytica* positivity and bloody diarrhea and diarrhea with mucus in geriatric individuals. It was also determined that *E. histolytica* may cause abdominal pain, abdominal distension, and tenderness in the

epigastric region in geriatric patients. Trophozoites of *E. histolytica*, a pathogenic parasite, begin to destroy the muco-epithelial barrier, thus causing excessive mucus production, killing host cells, and causing inflammation and dysentery. Human immunity and gut microbiota play a role in preventing these effects of the parasite and can influence its pathogenicity.<sup>18</sup> The gut microbiota protects the host by inhibiting the growth of harmful pathogens in the gut through the production of short-chain fatty acids across the mucosa, promoting the regeneration of intestinal epithelial cells, and thus maintaining the integrity of the intestinal barrier.<sup>19</sup> Small molecules originating from bacteria, such as oxaloacetate and queuine, manipulate the parasite by controlling different aspects of its physiology.<sup>20</sup> Age is an important factor modifying the gut microbiota. Several studies have shown that the gut microbiota of the geriatric population is different than that of the young healthy population.<sup>19</sup> Amoebiasis is likely to be more severe in geriatric individuals with altered microbiota.

Furthermore, since protection against amoebiasis relies mainly on cell-mediated responses, immune senescence may contribute to the transition from chronic latent intestinal infection to the invasive form of amoebiasis. Impairment of the host's immune defence by the use of certain drugs, such as corticosteroids in the elderly, or the combination of certain drugs with tissue damage caused by the parasite can increase the pathogenicity of amoebiasis.<sup>17</sup>

Older people differ from middle-aged people in terms of polypharmacy, pharmacokinetics, comorbidity, and greater susceptibility to adverse drug reactions. The use of more than one drug can lead to delirium, which significantly increases mortality rates among elderly people. In addition, *Clostridium difficile* infections, often associated with the unnecessary use of broad-spectrum antibiotics, affect elderly people, leading to prolonged hospital stays and serious morbidity and mortality.<sup>21</sup> For these reasons, the treatment of geriatric patients with gastrointestinal complaints should be started after the definitive diagnosis of the disease. Considering the results of this study, we believe that clinicians should definitely consider amoebiasis before using broad-spectrum antibiotics in geriatric patients with diarrhea, abdominal pain, abdominal distension, and epigastric tenderness. It should be kept in mind that microscopic examination for the diagnosis of amoebiasis is of low sensitivity and false negative results may be obtained with poor quality ELISA tests.<sup>11</sup>

**Study limitations.** The first limitation of the study is that the patients included in the study were from a single hospital. The second limitation of the study is the lack of information regarding the socioeconomic status of the individuals included in the study.

In conclusion, both the risk of *E. histolytica* infection and the pathogenicity of the infection increase with the weakening of the immune system and the disruption of the microbiota in geriatric individuals. The fact that the incidence of amoebiasis in geriatric individuals with gastrointestinal complaints was as high as 31.3% revealed both that amoebiasis is a serious health problem in geriatric individuals and that studies on other pathogenic agents should be carried out in geriatric individuals whose health is under threat due to the weakening of the immune system with advancing age. In addition, further studies are needed using a larger study population in different regions in order to confirm this hypothesis.

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