

Retrospective Evaluation of Intestinal Protozoa Parasites in Patients Presenting to Kafkas University Health Research and Application Hospital Between 2019-2022

2019-2022 Yılları Arasında Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi'ne Başvuran Hastalarda Bağırsak Protozoon Parazitlerinin Retrospektif Olarak Değerlendirilmesi

© Hilal Bedir, © Neriman Mor, © Ahmet Deniz, © Mükremin Özkan Arslan
Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye

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ABSTRACT

Objective: This study aimed to retrospectively evaluate the prevalence of protozoan parasites in stool samples collected from patients presenting with various gastrointestinal complaints to the Medical Parasitology Laboratory of Kafkas University Research and Application Hospital between 2019 and 2022.

Methods: Stool samples were initially examined using the native-Lugol method for the detection of protozoan parasites, followed by the formol-ethylacetate sedimentation method, Giemsa, and carbol fuchsin staining methods. Specific immunochromatographic card tests were used for the diagnosis of *Entamoeba histolytica*, *Cryptosporidium* spp., and *Giardia intestinalis*.

Results: Of the 2.267 stool samples examined over the four-year period from January 2019 to December 2022, 7.63% were found to contain one or more protozoan parasites. Among these parasites, *Entamoeba histolytica* was detected at the highest rate of 4.06%. The other parasite species were identified as follows: *Blastocystis* spp. 1.15%, *Entamoeba* spp. and *Entamoeba coli* each 0.52%, *Giardia intestinalis* 0.48%, *Endolimax nana* 0.17%, and *Entamoeba histolytica/dispar* 0.08%.

Conclusion: This study indicates that despite a decrease in the prevalence of intestinal protozoan infections in the Kars region, these infections remain a significant public health issue. Therefore, improvements in hygiene and sanitation conditions, increased public health education, and the widespread implementation of early diagnosis and treatment methods are necessary. Special measures should be taken to protect vulnerable groups, particularly children and the elderly.

Keywords: Protozoan parasites, microscopic examination, Kars

ÖZ

Amaç: Bu çalışmada, 2019-2022 yılları arasında Kafkas Üniversitesi Araştırma ve Uygulama Hastanesi Tıbbi Parazitoloji Laboratuvarı'na çeşitli gastrointestinal şikayetlerle başvuran hastalardan alınan dışkı örneklerinde protozoon parazit prevalansının retrospektif olarak değerlendirilmesi amaçlanmıştır.

Yöntemler: Protozoon parazitlerin tespiti için dışkı örnekleri öncelikle Nativ-Lugol yöntemiyle incelenmiş, ardından formol etil asetat çöktürme yöntemi, Giemsa ve karbol fuksin boyama yöntemleri uygulanmıştır. *Entamoeba histolytica*, *Cryptosporidium* spp. ve *Giardia intestinalis*'in tanısında spesifik immünokromatografik kart testleri kullanılmıştır.

Bulgular: Ocak 2019 ile Aralık 2022 tarihleri arasındaki dört yıllık dönemde incelenen 2267 dışkı örneğinin %7,63'ünde bir veya birden fazla protozoon parazit tespit edilmiştir. Bu parazitler arasında %4,06 ile *Entamoeba histolytica* en yüksek oranda saptandı, diğer parazit türleri ise sırasıyla *Blastocystis* spp., %1,15 *Entamoeba* spp. ve *Entamoeba coli* %0,52 *Giardia intestinalis* %0,48, *Endolimax nana* %0,17 ve *Entamoeba histolytica/dispar* %0,08 oranında tespit edildi.

Sonuç: Bu çalışma, Kars bölgesinde bağırsak protozoon enfeksiyonlarının prevalansının azalmış olmasına rağmen, bu enfeksiyonların hala önemli bir halk sağlığı sorunu olduğunu ortaya koymaktadır. Bu nedenle, hijyen ve sanitasyon koşullarının iyileştirilmesi, halk sağlığı eğitimlerinin artırılması ve erken tanı ve tedavi yöntemlerinin yaygınlaştırılması gerekmektedir. Özellikle çocuklar ve yaşlılar gibi savunmasız gruplara yönelik özel önlemler alınmasına ihtiyaç vardır.

Anahtar Kelimeler: Protozoon parazitler, mikroskopik inceleme, Kars



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Address for Correspondence/Yazar Adresi: Hilal Bedir, Kafkas University Faculty of Medicine, Department of Medical Parasitology, Kars, Türkiye
Phone/Tel: +90 506 420 89 40 E-mail/E-Posta: bedirhilal@gmail.com ORCID ID: orcid.org/0000-0002-1583-3385



INTRODUCTION

Intestinal protozoan infections constitute a significant global public health concern in developing countries, where water and sanitation facilities are often inadequate (1). In developing countries, factors such as low hygiene standards, environmental fecal contamination, and insufficient infrastructure have been reported to increase the prevalence of these infections (2). Although individuals of all ages are at risk, the most vulnerable groups include young children, individuals with low socio-economic status, those with limited education, the elderly, individuals with chronic health conditions, and migrants (3).

Transmission of intestinal protozoan parasites primarily occurs through contaminated water and food (4) and is clinically characterized by diarrhea. Diarrhea can be chronic or severe and is often associated with symptoms such as nausea, vomiting, low-grade fever, abdominal cramps, loss of appetite, fatigue, and weight loss. Prolonged infections can lead to serious anemia or malnutrition and may impair psychomotor development in children (5).

Enteric protozoan parasites are among the leading causes of the 1.7 billion annual cases of diarrhea worldwide, resulting in 842,000 deaths annually, making it the second leading cause of death among children under five years old. The most common etiological agents are *E. histolytica*, *G. intestinalis*, and *Cryptosporidium* spp. (6). These parasites are transmitted primarily through direct contact with infected individuals or animals or indirectly through the consumption of contaminated water or food; transmission typically occurs via the fecal-oral route (7). *E. histolytica* is the pathogenic species responsible for amebiasis worldwide (8). According to estimates from the Global Burden of Disease Study 2010, amebiasis accounts for 22 million disability-adjusted life years and approximately 55,500 deaths annually (1). *G. intestinalis* is a significant cause of chronic infectious diarrhea in both developed and developing countries, causing approximately 200-300 million clinical cases annually worldwide (9). It is also suggested to be responsible for ~15% of childhood diarrhea cases in developing countries (10). Data from the Global Enteric Multicenter Study indicate that *Cryptosporidium* spp. is a leading cause of moderate-to-severe diarrhea among children under two years old in developing countries (11).

Although there are many studies on the prevalence of intestinal protozoa in the literature, data from the Kars region of Türkiye is limited. The last significant study conducted in the region was in 2008, which reported a high prevalence of protozoan infections (12). This study aims to retrospectively evaluate the prevalence of protozoan parasites in stool samples collected from patients presenting with various gastrointestinal complaints at the Medical Parasitology Laboratory of Kafkas University Research and Application Hospital between January 2019 and December 2022.

METHODS

A retrospective analysis was conducted on stool samples from 2,267 patients presenting with various gastrointestinal complaints at the Kafkas University Medical Faculty Health Research and Application Hospital Parasitology Laboratory between January 2019 and December 2022. The inclusion criteria encompassed all patients who provided stool samples within

the study period, while exclusion criteria included incomplete patient records or insufficient sample quality. Since our study is retrospective, patient consent was not required.

Stool samples were initially examined macroscopically, followed by microscopic evaluation using native-Lugol and formalin-ethyl acetate concentration techniques. Samples suspected of containing protozoa were stained and evaluated using Giemsa and Carbol Fuchsin staining methods. Prepared native-Lugol smears were examined under a light microscope at 40x magnification, concentration method smears at 10x magnification, and permanently stained smears at 100x magnification. For the qualitative detection of *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* spp., rapid chromatographic immunological tests were used: *Giardia lamblia* Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BGL 602), *Entamoeba histolytica* Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BEH 602), and *Cryptosporidium* Antigen Rapid Test Cassette (feces) (Acro Biotech Inc., USA, BC 602). All procedures were conducted in accordance with the manufacturer's instructions and standard laboratory protocols.

This study was approved in Research Committee of Kafkas University approval no: 10, date: 28/12/2022.

Statistical Analysis

All cases in which intestinal protozoan parasites were detected were statistically evaluated in terms of age, gender, and presence of parasites over the years. The statistical analysis of the data obtained from the research was performed using the IBM SPSS Statistics for Windows Version 23.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) software program, and Pearson's chi-square test was utilized. In all evaluations, a p-value of <0.05 was considered significant.

RESULTS

During the four-year period from January 2019 to December 2022, our study analyzed a cohort of 2,267 patients, identifying protozoan parasites in 173 individuals (7.63%). Of these cases, 91.2% were infected with a single parasite species, while 8.1% harbored multiple species. The most frequently detected protozoan was *E. histolytica*, present in 4.05% of the cases, followed by *Blastocystis* spp. at 1.14%, *E. coli* and *Entamoeba* spp., each at 0.52%, *G. intestinalis* at 0.48%, *En. nana* at 0.17%, and *E. histolytica/dispar* at 0.08%. The species of parasites identified and their prevalence rates are presented in Table 1.

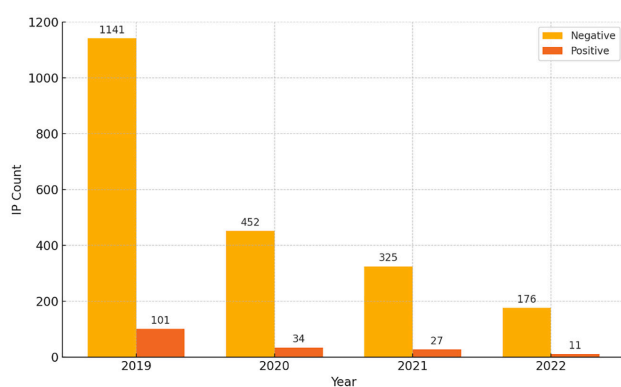
An analysis of the annual distribution of parasite detection rates revealed that parasites were identified in 8.13% of 1,242 cases in 2019, 6.99% of 486 cases in 2020, 7.67% of 352 cases in 2021, and 5.9% of 187 cases in 2022 (Table 1). There was a notable decline in parasite prevalence from 2019 onward, though no statistically significant differences were observed between the years ($p > 0.675$). However, a significant seasonal variation was noted, with higher rates of parasite detection during the autumn-winter months compared to the spring-summer months ($p = 0.02$) (Figure 1).

The overall study population had a mean age of 23.23 ± 21.95 years, ranging from 0 to 98 years, with a male prevalence of 56.6% and female prevalence of 43.4%. Among the parasite-positive patients, 54.9% were male and 45.1% were female, with a mean age of 11.24 ± 14.33 years (ranging from 0 to 79 years). There was

Table 1. Number of patients by year, rates of positive cases, and distribution of intestinal protozoan species

IPP species	2019 (n=1242)		2020 (n=486)		2021 (n=352)		2022 (n=187)		Total (n=2267)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Single infection										
<i>Entamoeba histolytica</i>	67	(5.39)	19	(3.9)	5	(1.42)	1	(0.53)	92	(4.05)
<i>Blastocystis</i> spp.	5	(0.4)	2	(0.41)	10	(2.84)	9	(4.81)	26	(1.14)
<i>Entamoeba</i> spp.	8	(0.48)	3	(0.61)	3	(0.85)	-	-	14	(0.61)
<i>Entamoeba coli</i>	10	(0.8)	2	(0.41)	-	-	-	-	12	(0.52)
<i>Giardia intestinalis</i>	5	(0.4)	4	(0.82)	2	(0.56)	-	-	11	(0.48)
<i>Endolimax nana</i>	3	(0.24)	1	(0.2)	-	-	-	-	4	(0.17)
Double infection										
<i>Entamoeba histolytica</i> + <i>Giardia intestinalis</i>	-	-	-	-	3	(0.85)	-	-	3	(0.13)
<i>Giardia intestinalis</i> + <i>Blastocystis</i> spp.	-	-	1	(0.2)	1	(0.28)	-	-	2	(0.08)
<i>Entamoeba</i> spp.+ <i>Blastocystis</i> spp.	-	-	-	-	1	(0.28)	-	-	1	(0.04)
<i>Giardia intestinalis</i> + <i>Entamoeba</i> spp.	-	-	-	-	1	(0.28)	-	-	1	(0.04)
<i>Entamoeba histolytica</i> + <i>Blastocystis</i> spp.	1	(0.08)	-	-	-	-	-	-	1	(0.04)
<i>Entamoeba histolytica</i> + <i>Endolimax nana</i>	1	(0.08)	-	-	-	-	-	-	1	(0.04)
<i>Entamoeba coli</i> + <i>Blastocystis</i> spp.	-	-	-	-	-	-	1	(0.53)	1	(0.04)
<i>Entamoeba coli</i> + <i>Endolimax nana</i>	1	(0.08)	-	-	-	-	-	-	1	(0.04)
<i>Giardia intestinalis</i> + <i>Iodamoeba butschlii</i>	-	-	1	(0.2)	-	-	-	-	1	(0.04)
Triple infection										
<i>Entamoeba</i> spp.+ <i>Blastocystis</i> spp. + <i>Chilomastix mesnili</i>	-	-	1	(0.2)	-	-	-	-	1	(0.04)
<i>Entamoeba histolytica</i> + <i>Giardia intestinalis</i> + <i>Cryptosporidium</i> spp.	-	-	-	-	1	-	-	-	1	(0.04)
Total	101	(8.13)	34	(6.99)	27	(7.67)	11	(5.9)	173	(7.63)

IPP: Intestinal protozoa parasite

**Figure 1.** The total number of cases and the distribution of positive samples by year

no statistically significant difference in the prevalence of parasites between genders ($p=0.345$) (Table 2).

Further age stratification showed that parasites were most prevalent in individuals aged 0-15 years (72.3%), followed by those aged 16-30 years (9.9%), 31-45 years (7.6%), 46-55 years (2.9%), and over 56 years (7.6%). The highest frequency of parasitic infections was observed in the 0-15 year age group, with significantly higher infection rates in the 46-55 and over 56 age groups compared to other age groups ($p=0.0001$) (Table 3).

Table 2. Distribution of patients with and without detected parasites by gender

		n	%	Age average	Age range	p*
Positive	Male	95	54.9	11.24±14.33	0-79	0.345
	Female	78	45.1			
Negative	Male	1189	56.8	23.23±21.95		0.345
	Female	905	43.2		0-98	
Total		2267	100	11.24±23.23	0-98	

p*: $p<0.05$

In reviewing the annual parasite distribution, no statistically significant differences were found in the numbers of detected parasites from the onset of the study in 2019 through 2022 ($p>0.675$). Nevertheless, a significant increase in parasite numbers was observed during the autumn-winter months compared to the spring-summer months ($p=0.02$) (Figure 2).

DISCUSSION

Recent assessments of disease burden and epidemiological studies have highlighted the role of intestinal protozoa as significant etiological agents in low- and middle-income countries. Insights

Table 3. Prevalence of positive samples and negative samples by age

Age group	Negative number	Negative (%)	Positive number	Positive (%)	Total
0-15	1044	49.85%	125	72.25%	1169
16-30	362	17.28%	17	9.82%	379
31-45	315	15.04%	13	7.51%	328
46-55	129	6.16%	5	2.89%	134
>56	244	11.65%	13	7.51%	257
Total	2094	100.0%	173	100.0%	2267

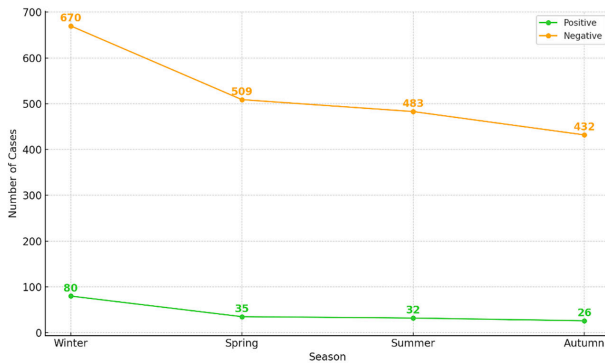


Figure 2. Distribution of total cases and positive samples by seasons

from the second decade of the twenty-first century have brought to light both the unexpected and severe global health impacts of these organisms (1).

In our study, in addition to the traditional method of microscopic examination for the diagnosis of intestinal protozoan parasites, immunochromatographic tests (ICTs) were used as an alternative, particularly for the detection of significant pathogenic intestinal parasites such as *E. histolytica*, *G. intestinalis*, and *Cryptosporidium* spp., which are among the most common etiological agents of diarrhea in humans. While microscopic examination allows for the direct morphological identification of parasites, ICTs operate by detecting specific antigens or antibodies. Microscopy can identify a wide range of parasites, whereas ICTs focus only on specific parasite species. The advantages of microscopy include its broad application and low cost, while ICTs are distinguished by providing rapid results and showing higher sensitivity in detecting low-intensity infections (12).

In our study, the overall prevalence rate of intestinal protozoan parasites was 7.63%, a significant reduction from the 34.1% reported in 2008 (13). This finding indicates that intestinal protozoan infections continue to be a significant public health issue in the region. The observed decrease in prevalence compared to previous years suggests that improvements in infrastructure and sanitation, as well as public health interventions, have been effective across the province. This study aligns with global efforts to mitigate the impact of protozoan parasites and emphasizes the need for ongoing interventions to sustain and improve public health outcomes.

The prevalence of protozoan parasites in our country shows significant geographical variation. Recent studies demonstrate that the incidence of these parasites fluctuates by year and

region. The overall prevalence of protozoan infections in our study is consistent with the rates reported by Manisa Celal Bayar University Hafsa Sultan Hospital at 9.72% (14), Sivas Cumhuriyet University Faculty of Medicine at 8.65% (15), and Dicle University Faculty of Medicine at 5.64% (16). These rates are lower than those reported by Ege University Faculty of Medicine at 10.23% (17) and Gaziantep University Faculty of Medicine at 14.61% (18), yet higher than those found at Cerrahpaşa Faculty of Medicine at 2.49% (19), Yüzüncü Yıl University Faculty of Medicine at 2.62% (20), Fırat University Faculty of Medicine at 1.21% (21), and Hacettepe University Faculty of Medicine at 4.30% (22). These findings underscore the ongoing significance of protozoan parasite infections, which are influenced by geographical and socio-economic factors. Variations in prevalence rates may also stem from differences in stool examination techniques employed. Since 2020, our research has documented a consistent decline in both the number and prevalence of parasitic infections. Lifestyle changes during the Coronavirus disease-2019 pandemic, such as improved hand hygiene, social distancing, and quarantine measures, have contributed to a reduction in intestinal parasite infections (23). It is likely that heightened public awareness during the pandemic has led to a reduction in fecal-oral transmission of pathogens, which correlates with the observed decrease in the quantity of samples analyzed and the number of parasites detected. This trend underscores the impact of behavioral health interventions on controlling infectious diseases.

The global molecular prevalence of *Entamoeba* species in humans is estimated at 3.5%, with *E. histolytica* and *E. dispar* comprising 81.7% of this prevalence, with *E. dispar* being significantly more prevalent than *E. histolytica* worldwide (24). In Kars, the prevalence of *Entamoeba* species has been reported with *E. histolytica/dispar* at 10.1% and *E. coli* at 8% (12). Our study confirms the presence of both pathogenic and non-pathogenic *Entamoeba* species, specifically *E. histolytica* at 4.06%, *E. coli* at 0.52%, other *Entamoeba* spp. at 0.52%, and *E. histolytica/dispar* at 0.08%. Accurate diagnosis of *E. histolytica* is essential to avoid unnecessary treatment of individuals infected with non-pathogenic *Entamoeba* species (25). The risk of *E. histolytica* infection is higher among individuals living in households with a middle socio-economic status (26). Our findings indicate the hygiene and sanitation conditions in the province, suggesting that individuals with lower socio-economic status are at a higher risk, underscoring how socio-economic disadvantages can contribute to the spread of *E. histolytica*.

G. intestinalis, a significant pathogenic intestinal protozoan, is one of the most common causes of waterborne disease outbreaks related to drinking water (27). In a previous study, the prevalence of *G. intestinalis* was reported to be as high as 10.9% (12). In contrast, our study found the prevalence rate of *G. intestinalis* to be significantly lower at 0.48%. The low prevalence of *G. intestinalis* observed in our study may reflect improvements in public health measures, such as enhanced management of water quality and increased awareness of personal hygiene.

In our study, the second most commonly encountered parasite was identified as *Blastocystis* spp., with a prevalence rate of 1.15%. *Blastocystis* spp. is known as the most frequently detected unicellular parasite in human fecal samples worldwide. The highest reported prevalence of this parasite was 100% in a child population in Senegal (28). Although the majority of individuals infected with *Blastocystis* spp. do not show intestinal symptoms,

infections are associated with non-specific gastrointestinal symptoms such as diarrhea, abdominal pain and urticaria (29). Studies conducted in Türkiye have reported significant positive rates for *Blastocystis* spp., varying between 6.63% and 71.6% (14,15,17,18,22). Higher prevalence rates of *Blastocystis* infections have previously been reported among individuals in close contact with animals, especially animal caretakers (30). The Kars Plateau, an area with extensive agriculture and animal husbandry, constitutes a high-risk environment for *Blastocystis* spp. infections, particularly among farmers. This suggests a high likelihood of transmission between humans and animals in rural settings. The low prevalence observed in our study may not be generalizable to the broader population, but could reflect improvements in public health measures. Research into the sources and transmission routes of *Blastocystis* spp. is essential to prevent the spread of infections between humans and animals.

Regarding co-infections with pathogenic and non-pathogenic parasites, our results are comparable with other studies (13,14). Co-infections with protozoan species demonstrate their opportunistic and recurrent nature, which may proliferate under favorable conditions, leading to intestinal disorders.

Regarding the risk factors associated with protozoan infections, our data analysis has shown that protozoan parasites can infect all age groups. Age is a potential risk factor for intestinal infections (31). In our study, the high prevalence rate observed among children aged 0-15 years (72.03 %) is likely due to this age group's immature immune systems, consumption of unwashed vegetables and fruits, reluctance to wash hands before meals and after using the toilet, nail-biting, and poor personal hygiene habits. Previous studies have identified *G. intestinalis* and *E. histolytica/dispar* as common species in child patients (13). These species are among those detected in this age group in our study. Family size, source of drinking water, handwashing habits before meals, wearing shoes, and nail cleanliness are strongly associated with the presence of intestinal parasitic infection (32). The possible explanation for this relationship is that as family size increases, overcrowding, malnutrition, and problems with personal and household hygiene may arise; these conditions create ideal environments for parasite transmission and increase the susceptibility of family members to parasitic infections. Additionally, the prevalence of parasites was found to be higher in the 46-55 age groups and those over 56 years. This can be associated with the increasing prevalence of chronic diseases and the weakening of the immune system with age (33).

When all cases were evaluated by gender, 54.9% of the patients were male and 45.1% were female. This situation can be associated with the increased exposure to environmental factors and infection risk due to men spending more time outdoors. Men involved in outdoor activities such as agriculture, construction, and animal husbandry are more exposed to environments where parasites are present. Although some studies have reported gender differences, in general, parasitic infections are not directly related to gender, and hygiene and sanitation conditions are more determining factors (14,20,34). Consequently, our study did not observe a significant difference between genders in the frequency of parasitic infections, which is consistent with other literature findings. The management of protozoan infections should focus on improving hygiene and sanitation conditions rather than targeting specific genders. Additionally, raising public awareness

among those involved in outdoor and animal husbandry activities is important to reduce the prevalence of these infections.

In our study, a significant increase in the number and prevalence of parasites was observed during the autumn-winter seasons compared to the spring-summer seasons. This increase can be attributed to factors such as spending more time indoors, changes in hygiene practices, and differences in seasonal food consumption. Contrary to our findings, other studies conducted in Türkiye have reported higher rates of parasitic infections during the spring and summer seasons (16,19). These differences highlight the potential impact of regional and environmental factors on the seasonality of parasitic infections.

CONCLUSION

The findings of this study provide current data on the prevalence and distribution of intestinal protozoan infections in the Kars region. In conclusion, the significant presence of protozoan parasites such as *E. histolytica* and *Blastocystis* spp. in Kars province highlights the ongoing public health issue posed by intestinal protozoan infections. This underscores the importance of continuous efforts to improve sanitation, water quality, and public awareness. The lower prevalence of *G. intestinalis* compared to previous studies may reflect improved public health measures and hygiene practices. Addressing these factors comprehensively is crucial for reducing the prevalence of *E. histolytica* infections, particularly among children, and for improving overall community health.

*Ethics

Ethics Committee Approval: This study was approved in Research Committee of Kafkas University approval no: 10, date: 28/12/2022.

Informed Consent: Retrospective study.

*Authorship Contributions

Concept: H.B., N.M., A.D., M.Ö.A., Design: H.B., N.M., A.D., M.Ö.A., Data Collection or Processing: H.B., N.M., A.D., M.Ö.A., Analysis or Interpretation: H.B., N.M., A.D., M.Ö.A., Literature Search: H.B., N.M., A.D., M.Ö.A., Writing: H.B., N.M., A.D., M.Ö.A.

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