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DETECTION OF INTESTINAL PARASITES IN ADULTS FROM TERNOPIL, UKRAINE: INSIGHTS FROM MICROSCOPY AND REAL-TIME PCR

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Background. *Intestinal parasitic infections remain an important public health concern in Ukraine, yet data on their prevalence and the performance of diagnostic methods in adults are limited. This study aimed to compare microscopy and real-time PCR for detecting intestinal parasites in stool samples collected from adults in the Ternopil region, and to investigate the presence of protozoa and helminths in the study population.*

Materials and methods. *Stool samples from 48 adults were analyzed by microscopy and real-time PCR. Fresh samples were examined on-site in Ukraine by direct smear, while preserved samples (i.e. samples preserved in 96 % ethanol or on Whatman FTA Classic Cards) were analyzed in a laboratory in Poland using microscopy and real-time PCR.*

Results. *Microscopy detected Blastocystis spp. in 8 fresh (16.7 %) and 4 ethanol-preserved (8.3 %) stool samples. Real-time PCR confirmed all microscopy-positive cases and detected additional infections, identifying Blastocystis spp. in 12 (25 %) and Dientamoeba fragilis in 13 (27.1 %) samples, with higher detection rates observed in FTA Card-preserved material. No helminths or other intestinal protozoa were detected in any of the collected samples.*

Conclusions. *Blastocystis spp. and D. fragilis were the only intestinal protozoa detected among adults from Ternopil, Ukraine. The absence of helminths may reflect low infection intensity or the characteristics of the studied cohort. Molecular techniques proved useful for detecting fragile protozoa that may be missed by conventional microscopy.*

Key words: *Blastocystis spp., Dientamoeba fragilis, FTA Cards, microscopy, real-time PCR, Ukraine.*

Intestinal parasitic infections remain an important public health concern in Ukraine. Recent epidemiological data indicate a rising trend in infectious and parasitic diseases, with overall incidence increasing from 12 669 per 100 000 population in 2022 to 13 149 per 100 000 in 2023. Helminth infections, particularly ascariasis and enterobiasis, are among the most commonly reported parasitic diseases. In the Ternopil region, the incidence rates of ascariasis were 148.25 and 131.87 per 100 000 in 2022 and 2023, respectively, while enterobiasis increased from 59.18 to 69.12 per 100 000 population [1]. Protozoal infections are less frequent but still reported, with 2 431 cases of giardiasis and 20 cases of cryptosporidiosis nationwide in 2025 (January–September) [2]. This local epidemiological context highlights the need for effective diagnostic approaches to detect intestinal parasites in the region.

Accurate diagnosis of intestinal parasites is critical for understanding their true prevalence. Conventional microscopy is limited by sample freshness, preparation, and observer expertise [3, 4], whereas molecular methods such as real-time PCR can improve sensitivity [5], particularly when samples are preserved appropriately [6]. However, data comparing these two methods in Ukrainian populations are scarce.

The aim of this study was to compare microscopy and molecular methods for detecting intestinal parasites in stool samples collected from adults in the Ternopil region, with particular focus on the effect of sample preservation, and to assess the presence of intestinal parasites, including helminths, in this Ukrainian population.

Materials and Methods

The study was initiated on 29 September 2025 at the Department of Infectious Diseases with Epidemiology, Dermatology and Venerology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. A total of 48 adult volunteers from Ternopil provided fresh stool samples collected over the preceding weekend. Each participant gave informed consent for inclusion in the study.

On the day of collection, a portion of each stool sample was examined on-site by an experienced clinical laboratory specialist using direct smear in Lugol's solution under light microscopy at 10× and 40× magnification. The remaining material was processed for preservation: a portion was applied onto Whatman FTA Classic Cards and left to dry, and the rest was covered with 96 % ethanol until completely immersed. The FTA cards were stored in sealed bags with desiccant. All preserved samples were then transported to Poland for further laboratory analysis.

Preserved stool samples (on FTA Classic Cards and in 96 % ethanol) were transported to the Department of Epidemiology and Tropical Medicine, Military Institute of Medicine – National Research Institute, Poland. Laboratory analyses included microscopy and molecular methods. Microscopy was performed using three techniques: direct smear in Lugol's solution, decantation in distilled water, and flotation according to the Fülleborn method.

For molecular analyses, DNA was extracted from both FTA Classic Cards and ethanol-preserved stool samples using the Bosphore Nucleic Acid Extraction Versatile Spin Kit (Anatolia Geneworks, Turkey). For FTA Card samples, the area containing stool material was cut out with metal scissors, which were disinfected between samples using sodium hypochlorite solution and distilled water. The pieces which had been cut out from FTA Cards were placed in 2 ml microcentrifuge tubes, suspended in 1 ml distilled water, and eluted with gentle shaking at room temperature for 24 hours. After elution, 400 µl of the supernatant was transferred to a new tube, mixed with 400 µl LTX buffer (Anatolia Geneworks, Turkey) and 20 µl proteinase K, and incubated at 56 °C for 1 hour. Subsequently, 400 µl LB1 buffer and 20 µl proteinase K were added, and the procedure continued according to the manufacturer's protocol.

Ethanol-preserved stool samples were transferred to 2 ml microcentrifuge tubes, and centrifuged at 14,500 RPM for 5 minutes to remove the supernatant containing ethanol. After decanting the supernatant, 1 ml of distilled water was added to the remaining pellet, mixed thoroughly, and centrifuged again under the same conditions. This washing step was repeated three times to ensure complete removal of ethanol residues. Following the final wash, 400 µl of LTX buffer and 20 µl of proteinase K were added to the pellet and incubated at 56 °C for 1 hour. After incubation, the tubes were centrifuged again, and the supernatant was transferred to a new microcentrifuge tube, where 400 µl of LB1 buffer and 20 µl of proteinase K were added. The extraction was then continued according to the manufacturer's instructions, as described for samples collected on FTA Cards.

Real-time PCR assays were then performed on all extracted DNA to detect both protozoan and helminth parasites.

Real-time PCR analyses were performed using two commercial multiplex kits. The first, Bosphore Parasitic GI Panel Kit v1 (Anatolia Geneworks, Turkey), was designed for the detection of *Ascaris* spp. (ITS-1 gene), *Taenia* spp. (ITS1 & 5.8S rRNA genes), *Enterobius vermicularis* (COX1 gene), *Entamoeba histolytica* (5.8S rRNA–ITS2 region), *Cryptosporidium* spp. (18S rRNA gene), and *Giardia duodenalis* (18S rRNA gene).

The second kit, AmpliTest Digestive System Parasites II (Real-Time PCR) (Amplicon, Wrocław, Poland), targeted *Dientamoeba fragilis* and *Blastocystis hominis*; the manufacturer does not disclose the target gene sequences.

For the Bosphore Parasitic GI Panel, the thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 97 °C for 15 s and annealing/extension at 60 °C for 1 min, with a final hold at 32 °C for 2 min.

For the AmpliTest Digestive System Parasites II assay, the thermal profile consisted of an initial denaturation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 10 s and 58 °C for 25 s. All reactions were performed on the Aria MX Real-Time PCR System (Agilent Technologies, USA).

Descriptive statistics were used to summarize the data. Frequencies and percentages were calculated for the detection of intestinal parasites by different methods and preservation techniques. Given the small sample size and the methodological aim of the study, no inferential tests were performed. Data management and visualization were performed using Microsoft Excel (Microsoft Corporation, USA).

Research Results and their Discussion

A total of 48 adult residents of Ternopil participated in the study.

Microscopic examination of fresh, unpreserved stool samples using a direct smear in Lugol's iodine detected *Blastocystis* spp. (*B. hominis*) in 8 participants (16.7 %). When stool samples preserved in 96 % ethanol were examined microscopically using three methods – direct smear in Lugol's solution, flotation (Fülleborn method), and decantation in distilled water – *Blastocystis* spp. were observed in 4 samples (8.3 %). Of these, three coincided with positive findings from the fresh stool smears, and one was an additional positive result. No other intestinal protozoa or helminths were detected in any of the examined samples by light microscopy.

Real-time PCR analysis of ethanol-preserved stool samples detected *Blastocystis* spp. in 4 participants (8.3 %), all of whom were also positive by microscopy of fresh stool samples.

PCR performed on FTA card–preserved samples confirmed these four cases and additionally detected *Blastocystis* spp. in eight more participants, giving a total of 12 (25.0 %) positive samples. All samples that were microscopy-positive were also confirmed by PCR,

regardless of preservation method. In addition, real-time PCR identified *D. fragilis* infections. PCR analysis of ethanol-preserved stool detected *D. fragilis* in 9 participants (18.8 %), while PCR on FTA card–preserved stool confirmed all these cases and detected four additional infections, resulting in a total of 13 (27.1 %) positives.

None of the tested samples were positive for *Ascaris* spp., *Taenia* spp., *E. vermicularis*, *G. intestinalis* (*G. duodenalis*), *Cryptosporidium* spp. or *E. histolytica*.

Table 1 summarizes the results obtained using all diagnostic methods and sample preservation techniques, while Figure 1 presents pie charts illustrating the proportion of detected intestinal parasites versus negative results.

Table 1

The results obtained using all diagnostic methods and sample preservation techniques, Ternopil 2025 (n=48)

Agent	Preservation method	Microscopy		Real-time PCR	
		n	%	n	%
Blastocystis spp. (Blastocystis hominis)	native smear	8	16.7	0	0.0
	96 % ethanol	4	8.3	4	8.3
	FTA card	0	0.0	12	25.0
Dientamoeba fragilis	native smear	0	0.0	0	0.0
	96 % ethanol	0	0.0	9	18.8
	FTA card	0	0.0	13	27.1

Note. *Giardia intestinalis (Giardia duodenalis), Entamoeba histolytica, Cryptosporidium* spp., *Ascaris lumbricoides, Taenia* spp., *Enterobius vermicularis* and other intestinal protozoa and helminths were not detected.

Although national surveillance data indicate relatively high incidence rates of ascariasis and enterobiasis in Ukraine, particularly in western regions such as Ternopil [1], no helminth infections were detected in the present study. This discrepancy may reflect differences between population-level surveillance data, which often include children and symptomatic individuals, and the current study group consisting of asymptomatic adults. Additionally, the absence of helminths could be related to the small sample size. No cases of giardiasis or cryptosporidiosis were detected either, contrasting with several thousand cases reported nationwide in 2025 [1, 2]. In contrast, intestinal parasites such as *Blastocystis* spp. stramenopile and*D. fragilis* protozoa were detected, which is consistent with the findings from previous studies by Kyrychenko et al. [7] and Korzeniewski et al. [8], which reported a predominance of these protozoa among Ukrainian adults.

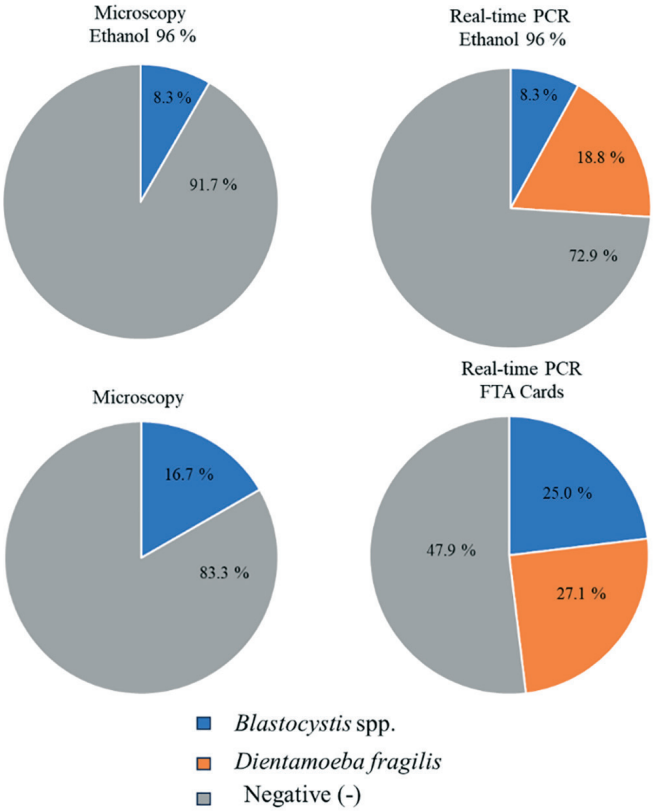

Fig. 1. Pie charts illustrating the proportion of detected intestinal parasites versus negative results, Ternopil 2025 (n=48).

The clinical relevance of both *Blastocystis* spp. and *D. fragilis* remains debated [9]. Infections may range from asymptomatic carriage to nonspecific gastrointestinal symptoms, including abdominal pain, diarrhea, bloating, nausea, and vomiting [10, 11]. Observational studies indicate that the parasite burden, particularly of *D. fragilis*, correlates with symptom severity: individuals with higher numbers of trophozoites tend to exhibit gastrointestinal symptoms more frequently than those with low parasite load [12]. This highlights that quantitative assessment of parasites can provide useful context when evaluating potential clinical impacts, although many carriers remain asymptomatic.

Detection of fragile protozoa such as *D. fragilis* poses a challenge for conventional light microscopy, particularly in unstained preparations or samples preserved in ethanol, as trophozoites may degrade rapidly during collection, transport, and storage [13]. Recent studies on *D. fragilis* in humans often employ qPCR techniques, as they are considered the most sensitive methods [14–16].

In our study, PCR confirmed all microscopy-positive *Blastocystis* cases and additionally identified *D. fragilis* in

samples which tested negative by microscopy. Real-time PCR allows detection of parasite DNA even when trophozoites are no longer morphologically intact.

However, its interpretation requires caution: high cycle threshold (Ct) values can reflect non-specific amplification, and PCR results alone do not necessarily correlate with parasite load or clinical manifestations [17]. Accurate interpretation depends on proper laboratory practice, operator training, and consideration of the clinical context. High-resolution microscopy of permanently stained smears (e.g., modified Wheatley trichrome or Heidenhain's iron hematoxylin) can improve detection rates of both *Blastocystis* spp. and *D. fragilis* [7], but requires expertise.

Overall, our findings indicate that potentially pathogenic parasites were detectable in the adult population sampled in Ternopil. Both *Blastocystis* spp. and *D. fragilis* were present, with *D. fragilis* detected only by molecular methods. The fragile nature of *D. fragilis* and the unknown conditions and duration of sample storage prior to processing may have contributed to its non-detection by conventional microscopy, while molecular methods allowed identification of its genetic material. The absence of helminths and other protozoa, such as *G. intestinalis* and *Cryptosporidium* spp. may be explained by the limited sample size, the adult cohort and the specific epidemiological context in Ternopil.

Conclusions

In the adult population sampled in Ternopil, only potentially pathogenic intestinal protozoa were detected. Conventional light microscopy may underestimate the presence of fragile protozoa, emphasizing the importance of proper sample preservation, staining, and skilled interpretation. Real-time PCR can enhance detection sensitivity for *D. fragilis* and confirm microscopy-positive *Blastocystis* spp., but results should be interpreted with caution due to the possibility of non-specific amplification and the lack of direct correlation with clinical manifestations. Future studies with larger sample sizes, systematic quantitative assessment, and optimized sample handling are warranted to better understand the prevalence, distribution, and potential clinical relevance of intestinal protozoa in adult populations in Ukraine, particularly in the Ternopil region, which is considered at higher risk for ascariasis and enterobiasis.

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ВИЯВЛЕННЯ КИШКОВИХ ПАРАЗИТІВ У ДОРОСЛИХ МЕШКАНЦІВ ТЕРНОПОЛЯ, УКРАЇНА: ПОРІВНЯЛЬНИЙ АНАЛІЗ ДАНИХ МІКРОСКОПІЇ ТА ПЛР У РЕАЛЬНОМУ ЧАСІ

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РЕЗЮМЕ. Кишкові паразитарні інфекції залишаються важливою проблемою для охорони здоров'я в Україні, проте дані щодо їхньої поширеності та ефективності методів діагностики серед дорослого населення обмежені. Метою дослідження було порівняти мікроскопію та полімеразну ланцюгову реакцію (ПЛР) у реальному часі для виявлення кишкових паразитів у зразках калу, зібраних у дорослих мешканців Тернополя, а також встановити наявність найпростіших і гельмінтів у дослідній популяції.

Зразки калу 48 дорослих осіб були проаналізовані за допомогою мікроскопії та ПЛР у реальному часі. Свіжі зразки були досліджені на місці в Україні методом прямого мазка, тоді як законсервовані зразки (збережені в 96 % етанолі або на картках Whatman FTA Classic Cards) були вивчені в лабораторії в Польщі із застосуванням мікроскопії та ПЛР у реальному часі.

При мікроскопії виявлено Blastocystis spp. у 8 (16,7 %) свіжих і 4 (8,3 %) консервованих етанолом зразках калу. ПЛР у реальному часі підтверджено всі випадки, позитивні за мікроскопією, і виявлено додаткові інфекції, ідентифікувавши Blastocystis spp. у 12 (25,0 %) і Dientamoeba fragilis у 13 (27,1 %) зразках, причому вищі показники виявлення відзначали в матеріалі, консервованому на FTA картках. Гельмінтів або інших кишкових найпростіших не виявлено в жодному із зібраних зразків.

Blastocystis spp. і D. fragilis були єдиними кишковими найпростішими, виявленими в дорослих мешканців Тернополя, Україна. Відсутність гельмінтів може відображати низьку інтенсивність інфекції або особливості досліджуваної когорти. Молекулярні методи виявилися корисними для виявлення нестійких найпростіших, яких можна не знайти за допомогою традиційної мікроскопії.

Ключові слова: *Blastocystis spp., Dientamoeba fragilis, FTA картки, мікроскопія, ПЛР у реальному часі, Україна.*

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Отримано