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Original Article

Prevalence and molecular identification of protozoan parasites in cancer patients in Jordan

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Abstract

Introduction: Parasitic infections are prevalent in developing countries, and cancer patients with weakened immune systems are particularly vulnerable. This study examined the prevalence of protozoan parasites among cancer patients.

Methodology: A total of 300 stool and heparinized plasma samples were collected from cancer patients at the King Hussien Cancer Center (KHCC). *Cryptosporidium*, *Giardia duodenalis*, and *Blastocystis* were detected by microscopy. Immunodiagnostic tests included electrochemiluminescence immunoassay (ECLIA) for *Toxoplasma gondii* IgG and IgM antibodies in plasma samples, and the ImmunoCard STAT *Cryptosporidium*/*Giardia* Kit for *G. duodenalis* and *Cryptosporidium* detection in stool. DNA extraction from stool samples was followed by nested polymerase chain reaction (PCR) to confirm the presence of intestinal parasites.

Results: *Toxoplasma gondii*, *Cryptosporidium*, *G. duodenalis*, and *Blastocystis* were detected. *T. gondii* was found in 22% of patients via IgG antibodies and in 2.7% via IgM, with the highest IgG seropositivity in multiple myeloma and uterine cancer patients (50%), and the highest IgM seropositivity in multiple myeloma patients (12.5%). The prevalence of *Cryptosporidium* varied depending on the detection method: 8.3% by microscopy, 11% by immunodiagnostic tests, and 12.3% by PCR. The highest infection rate was among colorectal cancer (CRC) patients. *Giardia duodenalis* was detected at rates of 1.7% by microscopy, 2.0% by immunodiagnostic testing, and 0.7% by PCR. *Blastocystis* was most prevalent in CRC patients, with detection rates of 31.0% by microscopy and 48.3% by PCR.

Conclusions: This study highlights the significance of protozoan parasitic infections among cancer patients, emphasizing the need for screening and management to improve patient outcomes.

Key words: parasitic infections; *T. gondii*; *Cryptosporidium*; *G. duodenalis*; *Blastocystis*; immunodiagnosis.

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Introduction

The prevalence of parasitic infections varies significantly across different regions, with particularly high rates observed in developing countries. Globally, approximately 3.5 billion individuals are affected by intestinal parasitic infections, leading to around 200,000 deaths annually. In developing countries, prevalence rates range from 30% to 60%, particularly in tropical and subtropical areas, where socioeconomic factors and hygiene practices play a critical role in infection rates [1,2].

Cancer patients are at a higher risk of developing opportunistic infections due to their weakened immune systems, which results from both the disease itself and the treatments they receive, such as chemotherapy and radiation therapy. These infections can be caused by bacteria, viruses, fungi, and parasites [3].

Parasitic infections have significant public health implications, offering valuable insights into their epidemiology in specific regions [4]. They are recognized as opportunistic infections in cancer patients, as well as in immunocompromised and transplant patients, often leading to prolonged and sometimes fatal diarrhea [5].

The most common parasites in cancer patients include *Giardia duodenalis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, *Entamoeba histolytica*, *Blastocystis*, *Cyclospora belli*, and *Strongyloides stercoralis* [6–8].

Despite limited research, existing data indicate that parasitic infections are common in both urban and rural areas in Jordan [9]. The burden of parasitic diseases on cancer patients in Jordan remains a significant yet unexplored public health issue, with few studies addressing their prevalence and impact in this vulnerable group.

A previous study on the seroprevalence of *T. gondii* in cancer patients reported an IgG seroprevalence at 39.5% and an IgM seroprevalence at 12.2% [10]. In the case of *Cryptosporidium*, a higher prevalence was

reported in pediatric oncology patients (14.4%) compared to non-oncology pediatric patients (5.1%) [11]. However, no studies investigated the prevalence of other protozoan parasites, such as *Giardia duodenalis* and *Blastocystis* in cancer patients in Jordan.

The presence of parasitic infections can complicate treatment outcomes and further compromise the health of cancer patients. Understanding the prevalence and types of these infections in this population is crucial for improving clinical care and developing effective preventive strategies.

This study aimed to investigate the prevalence of protozoan parasitic infections among patients at King Hussein Cancer Center (KHCC) in Amman, Jordan. Additionally, it sought to identify the most common parasites affecting this population and examine demographic factors, such as age, gender, and cancer type, associated with these infections.

Methodology

Ethics statement and sample collection

This cross-sectional study was conducted between June and December 2023 and was approved by the Institutional Review Board (IRB) of the Hashemite University (Ethics permit No. 25/2/2022/2023) in collaboration with the King Hussein Cancer Center (KHCC) in Amman, Jordan (Ethics permit No. 23 KHCC 28).

A total of 300 diarrheal stool samples and 300 heparinized plasma samples were collected from the same 300 cancer patients at the Medical Laboratory Department of KHCC, using a leftover sampling approach that eliminated the need for written consent [12]. Inclusion criteria comprised cancer patients presenting with diarrhea who attended KHCC and provided both stool and plasma samples during the study period. Patients were excluded if their medical records were incomplete or if their samples were insufficient in quantity or quality for analysis. Table 1 presents the demographic and clinical characteristics of the 300 cancer patients included in the study, including age, gender distribution, and type of cancer.

Table 1. Demographic and clinical characteristics of cancer patients included in the study.

Characteristics	Number of patients
Gender	
Male	162
Female	138
Age group (years)	
< 20	76
21–30	30
31–40	30
41–50	42
> 51	122
Cancer type	
Lymphoma	44
Leukemia	38
Breast cancer	33
Colon cancer	32
Colorectal Cancer CRC	29
Lung cancer	18
Bone marrow disorders	12
Ovarian cancer	10
Bladder cancer	9
Multiple myeloma	8
Endometrial cancer	7
Rectal cancer	6
Stomach and gastric cancer	6
Brain tumor	6
Prostate cancer	5
Pancreatic cancer	5
Uterine cancer	4
Thyroid cancer	3
Kidney cancer	3
Others	22

Direct wet mount, staining, and microscopic examination

Direct wet mount, staining, and microscopic examination were performed on all fecal samples to detect *G. duodenalis* and other motile protozoan parasites. For wet mount preparation, 2 mg of fresh, unpreserved stool sample was placed on a glass slide using a wooden stick and emulsified with a drop of physiological saline (0.85%). The sample was then examined under a microscope, starting with the 10x objective lens and progressing to the 40x and 100x objective lenses for detailed observation [13].

A modified acid-fast staining procedure was used to detect *Cryptosporidium* oocysts. An air-dried smear was prepared with 1 to 2 drops of the collected stool sample, fixed in methanol for 30 seconds, and stained with carbol fuchsin for 3–5 minutes. The smear was gently heated to help the dye penetrate the thick cell wall of acid-fast organisms. The smear was rinsed with tap water, decolorized with acid-alcohol for 15–20 seconds, and then stained with methylene blue. The stained, dried slides were labeled and examined under a light microscope Olympus CH40/RF200 (Olympus, Tokyo, Japan) using a 100x oil immersion lens to detect *Cryptosporidium* oocysts.

The trichrome staining method was used to detect *Blastocystis*, and involved several key steps. First, a thin smear of the stool sample was spread on a clean glass slide and allowed to air dry. The slide was then fixed with a sodium acetate-formalin solution to preserve the morphology of the parasites. After fixation, it was immersed in 70% ethanol for 5 minutes. Next, the slide was stained with trichrome stain for 10 minutes to differentiate the parasitic structures. Finally, the slide was rinsed thoroughly to remove any excess

stain. Further dehydration of the slide was achieved by immersing it in 100% ethanol for 3 consecutive 5-minute steps. The slide was then cleared by submerging it in xylene. After dehydration, the slide was mounted with a coverslip using an appropriate mounting medium. The stained slides were examined under a light microscope (Olympus CH40/RF200 (Olympus, Tokyo, Japan) with a 100x oil immersion lens to detect the presence of *Blastocystis*.

ImmunoCard STAT Cryptosporidium/Giardia rapid assay

The ImmunoCard STAT *Cryptosporidium*/*Giardia* is a qualitative immunochromatographic assay that uses specific monoclonal antibodies to detect *Cryptosporidium* and *G. duodenalis* antigens. In this study, the ImmunoCard STAT *Cryptosporidium/Giardia* Rapid Assay Kit (Meridian Bioscience, Boxtel, The Netherlands) was used following the manufacturer's instructions. Briefly, 2 drops of the sample treatment buffer were added to a dilution tube, followed by 60 µL of the diluted fecal sample. Then, 2 drops each of conjugate A and B were added. The suspension was gently mixed by manual swirling and transferred to the test cartridge. The results were observed after 10 minutes. The result was considered positive if both the organism and control lines showed gray-black bands, regardless of color intensity. It was considered negative if only the control line exhibited a gray-black band. The test was deemed invalid if the control line did not appear.

Electrochemiluminescence immunoassay (ECLIA) for the detection of T. gondii IgG and IgM

T. gondii IgG and IgM were tested in all heparinized plasma samples using the Roche Cobas® 801e Automated Analyzer (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. The total assay duration was 18 minutes, as described below: (1) In the first incubation step, 10 µL of the sample was automatically pre-diluted at a 1:20 ratio using the Elecsys universal diluent. The *T. gondii*-specific recombinant antigen labeled with a ruthenium complex was then added. If the sample contained anti-*T. gondii* IgG and IgM antibodies, they reacted with the ruthenium labelled *T. gondii*-specific recombinant antigen. (2) In the second incubation step, biotinylated monoclonal h-IgM-specific antibodies and streptavidin-coated microparticles were added, allowing the complex to bind to the solid phase through the biotin-streptavidin interaction. (3) The reaction mixture was aspired into a measuring cell, where the microparticles were magnetically captured onto the electrode surface. Unbound substances were removed with ProCell washing buffer (Roche Cobas, Basel, Switzerland). A voltage was then applied to the electrode, stimulating chemiluminescent emission, which was measured by a photomultiplier. The results were analyzed using the Elecsys software, which relied on a calibration curve generated through a 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

DNA extraction and nested PCR

Freshly collected stool samples were preserved in 2.5% potassium dichromate. Before DNA extraction, the samples were washed 3 times with 10% phosphate-buffered saline (PBS) and centrifuged at 2000 x g for 2 to 3 minutes to remove any residual potassium dichromate. The stool specimens were then subjected to 5 cycles of freezing and thawing using liquid nitrogen to facilitate DNA release. Total DNA was extracted

Table 2. Primer design targets for *Cryptosporidium.*

Gene		Name	Primer sequence (5` to 3`)	Annealing (°C)	Amplicon size (bp)
18SrRNA	1st primer set	Cryp_F1 Cryp_R1	TTCTAGAGCTAATACATGCG CCCATTTCCTTCGAAACAGGA	58.7	1,325
	2nd (nested) primer set	Cryp_F2 Cryp_R2	GGAAGGGTTGTATTTATTAG ATAAA AAGGAGTAAGGAACAACCTCC	59.7	819–825

Table 3. Primer design targets for *Giardia duodenalis.*

Gene		Name	Primer sequence (5` to 3`)	Annealing (°C)	Amplicon size (bp)
β-giardin	1st primer set	Giardia_bg_F1 Giardia_bg_R1	AAGCCCGACGACCTCACCCGCAGTGC GAGGCCGCCCTGGATCTTCGAGACGAC	69	753
	2nd (nested) primer set	Giardia_bg_F2 Giardia_bg_R2	GAACGAGATCGAGGTCCG CTCGACGAGCTTCGTGTT	62	511

Table 4. Primer design targets for *Blastocystis*.

Gene		Name	Primer sequence (5` to 3`)	Annealing (°C)	Amplicon size (bp)
18SrRNA	1st primer set	Blast_ssu_F1 Blast_ssu_R1	GTAACGAAGAATTTGGGTTCG CAGCCTTGCGACCATACTC	56.5	760
	2nd (nested) primer set	Blast_ssu_F2 Blast_ssu_R2	GGAGGTAGTGACAATAAATC TGCTTTCGCATWGTTCATC	53.5	480

using the DNeasy® PowerSoil® Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted DNA was stored at - 80 ºC until further analysis.

Samples positive for parasitic infections, including *Cryptosporidium*, *G. duodenalis*, or *Blastocystis* (n = 42), along with 50 parasite-negative control samples, were screened at the 18S ribosomal RNA gene locus for *Cryptosporidium* and *Blastocystis*, and at the β-giardin gene locus for *G. duodenalis*. Parasite identification was performed via nested PCR which was performed using the QiAmplifier 96 thermal cycler (Qiagen, Germany). The PCR amplicons were analyzed by 1% agarose gel electrophoresis. The list of primers used for parasite detection is provided in Tables 2, 3, and 4.

Statistical analysis

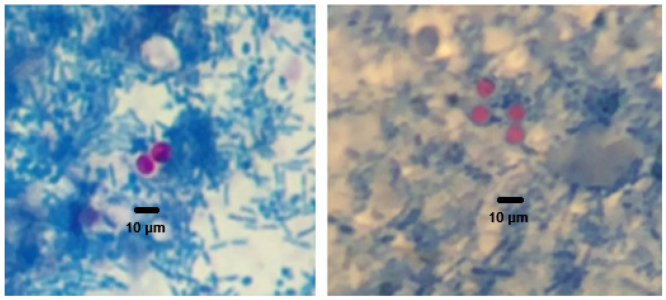
Statistical analyses were conducted using Microsoft Excel 365 for data management and IBM SPSS Statistics software version 29.0 [14] for in-depth statistical evaluation. Prevalence rates were calculated for each variable, and demographic factors were analyzed to assess potential associations with parasitic infections. The Chi-square (χ^2) test was used to determine the significance of differences in prevalence across categorical variables, and p values were calculated to assess statistical significance. A threshold of $p < 0.05$ was considered statistically significant. This approach ensured a rigorous analysis of demographic influences on infection prevalence, allowing for a robust interpretation of the findings.

Results

Cryptosporidium isolates

Cryptosporidium was detected using three different methodologies. Microscopic examination of all collected stool samples after employing the modified acid-fast stain, revealed a prevalence of 8.3% (n = 25/300) (Figure 1). The prevalence was 11% (n = 33/300) using the ImmunoCard STAT! *Cryptosporidium/Giardia* Rapid Assay Kit, while nested PCR detected a higher prevalence of 12.3% (n = 37/300) (Figure 2).

Figure 1. *Cryptosporidium* oocysts stained with modified acid-fast stain, viewed under a light microscope (Olympus CH40/RF200 (Olympus, Tokyo, Japan)) with a ×100 oil immersion lens.



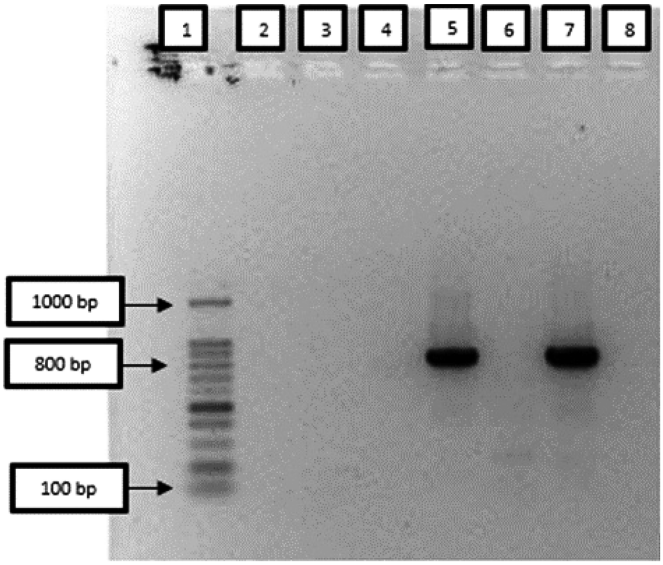
The highest infection rates among all diagnostic methods were observed in patients with colorectal cancer (CRC) and colon cancer. Specifically, CRC patients had infection rates of 27.6% (n = 8/300) by PCR, 24.1% (n = 7/300) by ImmunoCard STAT, and 20.7% (n = 6/300) by the modified acid-fast stain. Notably, no *Cryptosporidium* infections were detected in patients with multiple myeloma, bladder, uterine, thyroid, or kidney cancers.

Prevalence of Cryptosporidium by gender and age

The study results indicated that the prevalence of *Cryptosporidium* was slightly higher among females compared to males; however, the difference was not statistically significant ($p > 0.05$). Additionally, data analysis revealed a significantly higher incidence of cryptosporidiosis in younger patients (< 20 years) ($p < 0.05$).

Among the 300 stool samples analyzed for *Cryptosporidium* infection using PCR, immunodiagnostic testing, and modified acid-fast staining, 23 samples tested positive by all three methods. An additional 9 samples were positive by both PCR and immunodiagnostic testing only. Five samples were positive exclusively by PCR, two samples were positive only by modified acid-fast staining, and one sample tested positive solely by the immunodiagnostic

Figure 2. Agarose gel electrophoresis (AGE) image of the PCR products of *Cryptosporidium* DNA samples. The DNA bands were visualized under UV light after staining with ethidium bromide. Lane 1: DNA ladder. Lanes 5 and 7 showed positive results with fragment sizes 819–825 bp.



method. These findings highlight the partial overlap among the diagnostic techniques and emphasize the importance of combining multiple diagnostic approaches to improve detection accuracy and minimize false-negative results.

Toxoplasma gondii isolates

Toxoplasma gondii antibody assay, performed using ECLIA method, revealed that IgG antibodies against *T. gondii* were present in 66 out of 300 cancer patients, yielding a prevalence rate of 22%. IgM antibodies were detected in 8 out of 300 cancer patients, corresponding to a prevalence rate of 2.7%. Among the different types of cancer, patients with multiple myeloma and uterine cancer exhibited the highest seropositivity for anti-*T. gondii* IgG, with a prevalence rate of 50%. Conversely, the lowest seropositivity for anti-*T. gondii* IgG was observed in ovarian cancer patients, with a prevalence rate of 10%.

The highest seropositivity rate for anti-*T. gondii* IgM was detected in patients with multiple myeloma (12.5%). In contrast, no IgG or IgM antibodies were detected in patients with thyroid, prostate, pancreatic, kidney, brain, or endometrial cancer

Prevalence of Toxoplasma gondii by gender and age

The seropositivity rates for anti-*T. gondii* IgG were 18.5% (n = 30/162) in males and 26.1% (n = 36/138) in females, while the rates for anti-*T. gondii* IgM were 1.9% (n = 3/162) in males and 3.6% (n = 5/138) in females. Among the age groups, the highest seropositivity was observed in patients aged 31–40 years. However, Chi-square tests indicated no significant differences in *T. gondii* infection rates across genders and age groups ($p > 0.05$).

Giardia duodenalis

The prevalence of *G. duodenalis* among different types of cancer was found to be low across various diagnostic methods. The results showed a prevalence of 1.7% (n = 5/300) using direct wet mount microscopy (Figure 3), 2.0% (n = 6/300) with the ImmunoCard STAT *Cryptosporidium*/*Giardia* Rapid Assay Kit, and 0.7% (n = 2/300) by nested PCR (Figure 4).

Among the samples tested using PCR, immunodiagnostic methods, and wet mount microscopy, two samples were positive by all three diagnostic techniques. Additionally, two samples tested positive exclusively by the immunodiagnostic method, while one sample was positive only by the wet mount. Three samples were found to be positive by both the immunodiagnostic method and wet mount microscopy, but negative by PCR. These findings demonstrate partial overlap among the diagnostic tools and highlight variability in sensitivity across methods.

Blastocystis isolates

The study results indicated a high prevalence of

Figure 3. *Giardia duodenalis* cyst in direct wet mount preparation on a light microscope (Olympus CH40/RF200 (Olympus, Tokyo, Japan)) with a ×100 oil immersion lens.

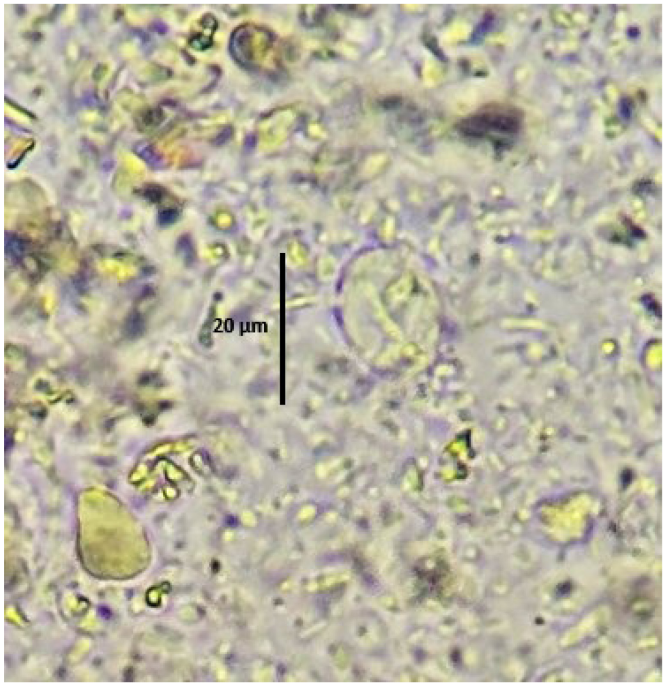
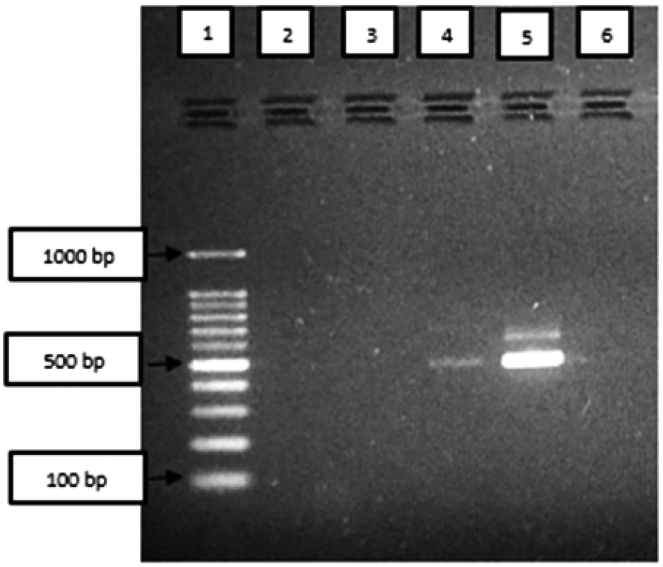


Figure 4. Agarose gel electrophoresis (AGE) image of the PCR products of *Giardia duodenalis* DNA samples. The DNA bands were visualized under UV light after staining with ethidium bromide. Lane 1: DNA ladder. Lanes 4 and 5 showed positive results with fragment sizes approximately 511 bp.



Blastocystis in CRC patients, with a prevalence rate of 31.0% (n = 9/29) using trichrome stain and 48.3% (n = 14/29) using nested PCR (Figure 5). The PCR results showed prevalence rates of 9.3% in males and 8.7% in females, indicating no statistically significant association between the prevalence of *Blastocystis* and either gender or age groups ($p > 0.05$).

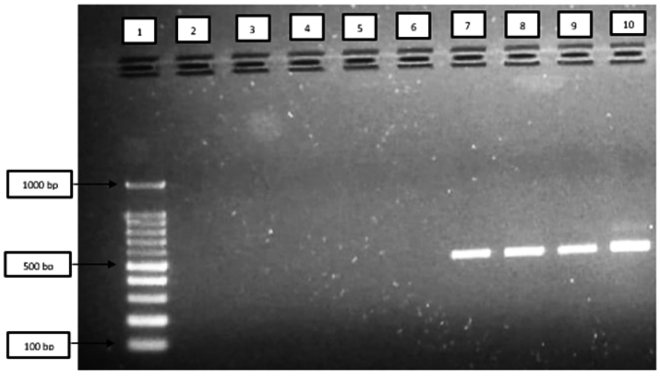
A total of 27 samples tested positive for *Blastocystis* by PCR, while 16 samples were positive using trichrome staining. Of these, 14 samples were positive by both methods, indicating concordant results. Additionally, 13 samples were detected exclusively by PCR, whereas 2 samples were positive only by trichrome stain. These findings highlight the higher sensitivity of PCR and the partial overlap between molecular and microscopic detection methods.

Discussion

Four major parasites—*T. gondii*, *Cryptosporidium*, *G. duodenalis*, and *Blastocystis*—were identified in cancer patients in this study. The seroprevalence of anti-*T. gondii* antibodies was 22.0% for IgG and 2.7% for IgM. A previous study in Jordan reported a higher IgG prevalence (39.5%) but a similar IgM prevalence (2.5%) [10]. Similarly, a study in China found 23.98% IgG and 2.25% IgM seropositivity among cancer inpatients, indicating that cancer patients have a significantly higher *T. gondii* seroprevalence compared to other groups [15].

Variations in *T. gondii* prevalence across studies may be due to differences in sample sizes and population characteristics. The presence of *T. gondii* IgM antibodies indicates recent infection, which is particularly concerning for cancer patients, requiring careful monitoring and management to prevent adverse outcomes.

Figure 5. Agarose gel electrophoresis (AGE) image of the PCR products of *Blastocystis* DNA samples. The DNA bands were visualized under UV light after staining with ethidium bromide. Lane 1: DNA ladder. Lanes 7, 8, 9 and 10 showed positive results with fragment sizes approximately 480 bp.



The current study found a higher prevalence of *T. gondii* in females (26.10%) than in males (18.50%), but the difference was not statistically significant ($p > 0.05$). Previous studies have shown conflicting results, with some reporting higher infection rates in females—possibly due to greater exposure to cat ownership—while others found higher prevalence in males [16]. A systematic review reported significantly higher seroprevalence in males (17.52%) than females (12.35%) [17], and a study in Jordan also found higher rates in males (42.3%) than in females (38%) [10]. These variations suggest complex biological, behavioral, and environmental factors influencing infection, warranting further research.

In this study, the highest anti-*T. gondii* IgG seropositivity was found in patients with multiple myeloma and uterine cancer (50%), while the highest IgM seropositivity was observed in multiple myeloma patients (12.5%). This may be attributed to intensive immunosuppressive treatments recommended for these patients, which weaken their immune system and increases their susceptibility to opportunistic infections and the reactivation of latent *T. gondii* infections [18].

This study did not include IgG avidity testing, which is crucial for distinguishing recent from past *T. gondii* infections. All samples positive for specific IgM were also positive for IgG; however, without avidity assessment, the possibility of false-positive IgM results cannot be excluded, particularly in immunosuppressed patients such as those with multiple myeloma. Additionally, lack of data on blood transfusions precluded evaluation of their potential influence on serological findings. These factors should be considered when interpreting the results, and future studies should incorporate avidity testing and detailed clinical data for more accurate assessment.

In this study, *Cryptosporidium* infection rates among cancer patients were 12.3% by PCR, 11% by immunodiagnostic methods, and 8.3% by modified acid-fast stain; with differences attributed to variations in sensitivity and specificity across detection methods [19,20]. A study conducted among pediatric oncology patients in Jordan reported a higher prevalence (13.8%) compared to non-oncology pediatric patients (5.1%) [11]. Similarly, a study in Iraq found a higher prevalence in cancer patients (17%) than in non-cancer patients (3%) [21]. These findings underscore the increased susceptibility of cancer patients to *Cryptosporidium* infections, emphasizing the need for vigilant monitoring and effective management strategies.

This present study found a higher prevalence of cryptosporidiosis in females (14.5%) than in males (10.5%), though the difference was not statistically significant. Gender-based variations in *Cryptosporidium* prevalence have been reported in different studies, with some, like a study in Cameroon, showing higher infection rates in females (5.36%) than males (3.57%) [22]; while others, such as a study in Jordan, found higher prevalence in males among hemodialysis patients [23]. These findings suggest no clear association between *Cryptosporidium* infection and gender, warranting further research to clarify potential correlations.

A systematic review and meta-analysis found the highest prevalence of *Cryptosporidium* infection in individuals under 18 years [24], aligning with this study's findings of a significantly higher incidence in patients under 20 years. Similarly, a study in Iraq reported higher prevalence in individuals under 20 years and those over 51 years [21]. Increased infection rates among younger individuals may be due to poor hygiene; exposure to contaminated water, surfaces, and soil; and contact with infected animals.

This study found the highest prevalence of *Cryptosporidium* in CRC patients (27.6%), consistent with findings from Iran (42.5%) [25] and China (17.24%) [26]. The increased susceptibility in CRC patients may be due to tumors and associated inflammation compromising the colon's natural protective barriers, making infection more likely.

This study found a low prevalence of giardiasis, with detection rates of 1.7% by wet mount microscopy, 2.0% by an immunodiagnostic method, and 0.7% by PCR. These findings align with previous studies, including a meta-analysis reporting a 6.9% prevalence in cancer patients [27] and an Iranian study identifying *G. duodenalis* as one of the least prevalent infections in this group (2.4%) [28]. While this study found a low prevalence of *G. duodenalis*, some studies have reported higher rates, such as a Brazilian study that detected *G. duodenalis* in 17.3% of cancer patients [29]. The reduced prevalence observed in this study may be attributed to improved public health and hygiene practices following the coronavirus disease 2019 (COVID-19) pandemic, including increased handwashing, enhanced food safety measures, and better water quality awareness. These factors likely contributed to a decline in the transmission of foodborne and waterborne pathogens like *G. duodenalis* and *Cryptosporidium* [30,31].

This study found a higher prevalence of *Blastocystis* in CRC patients, with detection rates of 31.0% by trichrome stain and 48.3% by PCR. These results align with previous studies, such as one in Poland reporting a 5-fold higher risk of blastocystosis in CRC patients (12.15%) compared to controls (2.42%) [32], and another in Uzbekistan showing a prevalence of 80% [33]. These findings suggest an association between *Blastocystis* and CRC, highlighting the importance of routine screening in immunocompromised patients. However, a meta-analysis of 12 studies reported a lower prevalence (5.7%) in CRC patients compared to healthy controls, indicating variability across studies [34]. The high prevalence of *Blastocystis* in CRC patients may be linked to immunosuppression, gut microbiota disruption, and mucosal barrier impairment. Further research is necessary to clarify this association and its clinical significance.

This study found no significant association between *Blastocystis* positivity and age or gender, consistent with findings from North Cyprus and Turkey [35,36], where no statistical differences were observed. These results suggest that age and gender may not be key factors in *Blastocystis* prevalence, with hygiene practices and environmental conditions likely playing a more critical role in infection rates.

The partial overlap in results among PCR, immunodiagnostic assays, and microscopy reflects differences in the sensitivity and specificity of these methods. In the case of *Cryptosporidium*, 23 samples tested positive across all three techniques, while others were detected by only one or two. This discrepancy is likely due to varying detection thresholds, with PCR offering higher sensitivity and the ability to identify low-level infections that may be missed by microscopy or antigen-based tests. Similar patterns were seen for *G. duodenalis* and *Blastocystis*, where PCR identified more positive samples than wet mount or trichrome staining. This highlights the limitations of microscopy, particularly in cases of low parasite load or intermittent shedding. These findings emphasize the importance of using multiple diagnostic tools to improve detection accuracy, reduce false negatives, and more accurately assess true parasite prevalence. They also underscore the need to interpret results in light of each method's analytical strengths and limitations.

Conclusions

Despite advancements in healthcare, parasitic infections remain a significant concern, especially among cancer patients. This study reported the presence of four parasites (*T. gondii*, *Cryptosporidium*, *G. duodenalis*, and *Blastocystis*) among cancer patients at

KHCC. Implementing routine screening, enhancing hygiene education, and providing specialized training for healthcare providers are crucial for managing these infections. Additionally, larger epidemiological studies are needed to inform clinical practices and public health strategies effectively.

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Authors' contributions
All authors contributed to various aspects of this work and have reviewed and approved the manuscript for submission to this journal. NH, study design, supervision of data collection and analysis, manuscript draft; RA, sample collection, molecular and microscopic analysis; HAS, data collection and analysis; MS, data collection and analysis; AR, study conception and design, manuscript preparation.

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Conflict of interest
No conflict of interest is declared.

References
1. Hajare ST, Gobena RK, Chauhan NM, Eriso F (2021) Prevalence of intestinal parasite infections and their associated factors among food handlers working in selected catering establishments from Bule Hora, Ethiopia. BioMed Res Int 2021: 6669742. doi: 10.1155/2021/6669742.
2. Taghipour A, Ghodsian S, Jabbari M, Olfatifar M, Abdoli A, Ghaffarifar F (2021) Global prevalence of intestinal parasitic infections and associated risk factors in pregnant women: a systematic review and meta-analysis. Trans R Soc Trop Med Hyg 115: 457–470. doi: 10.1093/trstmh/traa101.
3. Safdar A, Bodey G, Armstrong D (2011) Infections in patients with cancer: overview. In Safdar A, editor. Principles and Practice of Cancer Infectious Diseases. Totowa, NJ: Humana Press. 3–15. doi: 10.1007/978-1-60761-644-3_1.
4. Ahmed M (2023) Intestinal parasitic infections in 2023. Gastroenterol Res 16: 127–140. doi: 10.14740/gr1622.
5. Salehi Kahyesh R, Alghasi A, Haddadi S, Sharhani A (2020) Intestinal parasites infection in children with cancer in Ahvaz, southwest Iran. Interdiscip Perspect Infect Dis 2020: 8839740. doi: 10.1155/2020/8839740.
6. Garcia LS (2001). Diagnostic medical parasitology. In Truant AL, editor. Manual of Commercial Methods in Clinical Microbiology. 274–305. doi: 10.1128/9781555817961.ch11.
7. Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73: 197–230. doi: 10.1016/S0065-308X(10)73008-6.
8. Wang Z-D, Liu H-H, Ma Z-X, Ma H-Y, Li Z-Y, Yang Z-B, Zhu X-Q, Xu B, Wei F, Liu Q (2017) *Toxoplasma gondii* infection in immunocompromised patients: a systematic review and meta-analysis. Front Microbiol 8: 389. doi: 10.3389/fmicb.2017.00389.
9. Jaran AS (2016) Prevalence and seasonal variation of human intestinal parasites in patients attending hospital with abdominal symptoms in northern Jordan. EMHJ-East Mediterr Health J 22: 756–760. doi: 10.26719/2016.22.10.756.
10. Hijjawi NS, Al-Khreisat MJ, Abuyaman OM, Abdelfattah AM, Rababah EM, Al-Tamimi MA, Al-Hiari MA, Zueter AM (2018) Seroprevalence of Toxoplasma gondii in cancer patients admitted to hospitals of the Royal Medical Services in Jordan. Jordan J Biol Sci 11: 5.
11. Hijjawi N, Zahedi A, Kazaleh M, Ryan U (2017) Prevalence of *Cryptosporidium* species and subtypes in paediatric oncology and non-oncology patients with diarrhoea in Jordan. Infect Genet Evol 55: 127–130. doi: 10.1016/j.meegid.2017.08.033.
12. Gronowski AM, Budelier MM, Campbell SM (2019) Ethics for laboratory medicine. Clin Chem 65: 1497–1507. doi: 10.1373/clinchem.2019.306670.
13. Demeke G, Fenta A, Dilnessa T (2021) Evaluation of wet mount and concentration techniques of stool examination for intestinal parasites identification at Debre Markos Comprehensive Specialized Hospital, Ethiopia. Infect Drug Resist 14: 1357–1362. doi: 10.2147/IDR.S307683.
14. IBM Corp (2023) IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY, USA: IBM Corp.
15. Mao F, Yang Y, Chen Y, Zhang Q, Ding X, Ni B, Xu X, Jin X, Dai Y (2021) Seroprevalence and risk factors of *Toxoplasma gondii* infection among high-risk populations in Jiangsu Province, Eastern China. Front Cell Infect Microbiol 11: 783654. doi: 10.3389/fcimb.2021.783654.
16. Shoukat T, Awan UA, Mahmood T, Afzal MS, Wasif S, Ahmed H, Cao J (2022) Epidemiology of toxoplasmosis among the Pakistani population: a systematic review and meta-analysis. Pathogens 11: 675. doi: 10.3390/pathogens11060675.
17. Bisetegn H, Debash H, Ebrahim H, Mahmood N, Gedefie A, Tilahun M, Alemayehu E, Mohammed O, Feleke DG (2023) Global seroprevalence of *Toxoplasma gondii* infection among patients with mental and neurological disorders: a systematic review and meta-analysis. Health Sci Rep 6: e1319. doi: 10.1002/hsr2.1319.
18. Yıldız S, Esen R, Karaman K, Beyhan YE (2023) Seropositivity of *Toxoplasma gondii* among blood donors and patients with hematologic malignity. East J Med 28: 291–295. doi: 10.5505/ejm.2023.76743.
19. Shams S, Abdul Z, Khan W, Campus U (2016). Differential techniques used for detection of *Cryptosporidium* oocysts in stool specimens. J Parasit Dis 1: 1–12.
20. Mirhashemi ME, Zintl A, Grant T, Lucy FE, Mulcahy G, De Waal T (2015) Comparison of diagnostic techniques for the detection of *Cryptosporidium* oocysts in animal samples. Exp Parasitol 151: 14–20. doi: 10.1016/j.exppara.2015.01.018.
21. Nasir KA, Hama AA, Ali SI (2020) Prevalence of cryptosporidiosis among cancer patients in Sulaimani province/Iraq. Int J Psychosoc Rehabil 24: 9.
22. Tombang AN, Ambe NF, Bobga TP, Nkfusai CN, Collins NM, Ngwa SB, Diengou NH, Cumber SN (2019) Prevalence and risk factors associated with cryptosporidiosis among children

within the ages 0–5 years attending the Limbe regional hospital, southwest region, Cameroon. BMC Public Health 19: 1–10. doi: 10.1186/s12889-019-7484-8.
23. Zueter AM, Hijjawi NS, Hamadeneh KN, Al-Sheyab MM, Hatamleh AM (2019) Cryptosporidiosis among hemodialysis patients in Jordan: first preliminary screening surveillance. Trop Med Infect Dis 4: 131. doi: 10.3390/tropicalmed4040131.
24. Dong S, Yang Y, Wang Y, Yang D, Yang Y, Shi Y, Li C, Li L, Chen Y, Jiang Q (2020) Prevalence of *Cryptosporidium* infection in the global population: a systematic review and meta-analysis. Acta Parasitol 65: 882–889. doi: 10.2478/s11686-020-00230-1.
25. Ghanadi K, Khalaf AK, Jafrasteh A, Anbari K, Mahmoudvand H (2022) High prevalence of *Cryptosporidium* infection in Iranian patients suffering from colorectal cancer. Parasite Epidemiol Control 19: e00271. doi: 10.1016/j.parepi.2022.e00271.
26. Zhang N, Yu X, Zhang H, Cui L, Li X, Zhang X, Gong P, Li J, Li Z, Wang X, Li X, Li T, Liu X, Yu Y, Zhang X (2020) Prevalence and genotyping of *Cryptosporidium parvum* in gastrointestinal cancer patients. J Cancer 11: 3334–3339. doi: 10.7150/jca.42393.
27. Mahdavi F, Sadrebazzaz A, Chahardehi AM, Badali R, Omidian M, Hassanipour S, Asghari A (2022) Global epidemiology of *Giardia duodenalis* infection in cancer patients: a systematic review and meta-analysis. Int Health 14: 5–17. doi: 10.1093/inthealth/ihab026.
28. Esteghamati A, Khanaliha K, Bokharaei-Salim F, Sayyahfar S, Ghaderipour M (2019) Prevalence of intestinal parasitic infection in cancer, organ transplant and primary immunodeficiency patients in Tehran, Iran. Asian Pac J Cancer Prev APJCP 20: 495. doi: 10.31557/APJCP.2019.20.2.495.
29. Jeske ST, Macedo MRP, Bianchi T, Leon Í, Pinheiro NB, Borsuk S, Villela M (2022) Molecular characterization of *Giardia lamblia* and risk factors for giardiasis among immunocompromised patients in southern Brazil. Braz J Biol 82: e265055. doi: 10.1590/1519-6984.265055.
30. Akil L, Ahmad HA (2023) Socioeconomic impacts of COVID-19 pandemic on foodborne illnesses in the United States. Eur J Environ Public Health 7: 2. doi: 10.29333/ejeph/12585.
31. Cissé G (2019) Food-borne and water-borne diseases under climate change in low-and middle-income countries: further efforts needed for reducing environmental health exposure risks. Acta Trop 194: 181–188. doi: 10.1016/j.actatropica.2019.03.012.
32. Sulżyc-Bielicka V, Kołodziejczyk L, Adamska M, Skotarczak B, Jaczewska S, Safranow K, Bielicki P, Kładny J, Bielicki D (2021) Colorectal cancer and *Blastocystis* sp. infection. Parasit Vectors 14: 1–9. doi: 10.1186/s13071-021-04681-x.
33. Toychiev A, Abdujapparov S, Imamov A, Navruzov B, Davis N, Badalova N, Osipova S (2018) Intestinal helminths and protozoan infections in patients with colorectal cancer: prevalence and possible association with cancer pathogenesis. Parasitol Res 117: 3715–3723. doi: 10.1007/s00436-018-6070-9.
34. Beghini F, Pasolli E, Truong TD, Putignani L, Cacciò SM, Segata N (2017) Large-scale comparative metagenomics of *Blastocystis*, a common member of the human gut microbiome. ISME J 11: 2848–2863. doi: 10.1038/ismej.2017.139.
35. Beyhan YE, Yilmaz H, Cengiz ZT, Ekici A (2015) Clinical significance and prevalence of Blastocystis hominis in Van, Turkey. Saudi Med J 36: 1118. doi: 10.15537/smj.2015.9.12444.
36. Seyer A, Karasartova D, Ruh E, Güreser AS, Turgal E, Imir T, Taylan-Ozkan A (2017) Epidemiology and prevalence of *Blastocystis* spp. in North Cyprus. Am J Trop Med Hyg 96: 1164. doi: 10.4269/ajtmh.16-0706.