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Intestinal Parasitic Infections among Iraqi Patients: A Multicenter Cross-Sectional Study Integrating Microscopy, Molecular Confirmation, and Risk-Factor Analysis

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Abstract
This study was conducted to evaluate the prevalence of intestinal parasitic infections among patients in Iraq and the associated risk factors. Intestinal parasitic infections continue to be a major cause of gastrointestinal morbidities in areas where environmental conditions and personal hygiene favor the transmission of parasites. Most published studies in Iraq are limited mainly to single-center designs and depend on microscopy, which underestimates the true prevalence of infections and cannot differentiate between morphologically similar parasites. This study was designed to provide more accurate estimates of the prevalence of intestinal parasitic infections in Iraq. A cross-sectional study was conducted from January 2025 to March 2026 in six governorates of central and southern Iraq, including Baghdad, Babylon, Karbala, Najaf, Wasit, and Thi-Qar. A total of 240 stool samples were collected, with 40 samples obtained from each governorate. The stool samples were examined by direct saline wet mounts and iodine wet mounts and after concentration by formalin-ethyl acetate. Modified Ziehl-Neelsen staining was also used. Molecular confirmation by PCR included selected protozoan parasites: Entamoeba histolytica, Entamoeba dispar, Giardia duodenalis, Cryptosporidium parvum, and Cryptosporidium hominis. Demographic, clinical, environmental, and behavioral data were analyzed by chi-square testing and multivariable logistic regression. Intestinal parasites were detected in 94 of 240 patients, giving an overall composite prevalence of 39.2%. Parasites were seen in 78 samples by microscopy (32.5%) and in 89 samples by PCR for selected protozoan parasites (37.1%). The highest infection rates were recorded in Wasit and Thi-Qar (45.0% each), and the lowest rate was observed in Karbala (32.5%). The most frequently confirmed parasites were Giardia duodenalis (12.1%), Entamoeba histolytica (10.8%), Entamoeba dispar (7.1%), Cryptosporidium parvum (6.7%), and Cryptosporidium hominis (3.3%). Microscopy and PCR had substantial agreement with a Cohen's kappa coefficient of 0.808. Microscopy had a very high specificity of 96.7% but a sensitivity of 82.0% when compared with PCR. Irregular handwashing after toilet use, untreated drinking water, age below 15 years, and animal contact were identified as independent risk factors for infection by multivariable logistic regression.

Keywords
Intestinal parasites; Iraq; microscopy; PCR; *Giardia duodenalis*; *Entamoeba histolytica*; *Cryptosporidium*; risk factors.

Introduction

Intestinal parasitic infections continue to be a significant contributor to gastrointestinal morbidity worldwide and pose a challenge to clinical laboratories, epidemiological surveillance, and public-health planning (1). These infections are caused by a broad range of protozoa and helminths, including *Entamoeba histolytica, Giardia duodenalis, Cryptosporidium spp., Blastocystis spp., Enterobius vermicularis, Hymenolepis nana, Ascaris lumbricoides, hookworms,* and other medically relevant species. The clinical symptoms of infection range from asymptomatic carriage to diarrhea, abdominal pain, malabsorption, anemia, undernutrition, and chronic complaints of the gastrointestinal system

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(2). Children, immunocompromised patients, displaced communities, and populations living under conditions of poor water and sanitation are particularly at risk. Recent systematic evidence from Asia has confirmed the persistence of common intestinal protozoan parasites in school-aged children, highlighting that these infections are still of significance in areas where the hygiene, sanitation, climate, and socioeconomic conditions are able to support transmission (3).

The epidemiology of intestinal parasitic infections is determined by the interaction of biological, environmental, behavioral, and health-system factors. The common modes of transmission are through contaminated food, fecal-oral route, poor hygiene practices, contaminated soil, and close contact with humans and animals (4). The cysts and oocysts of protozoa remain viable in the environment and can, therefore, be transmitted by asymptomatic carriers, while helminth infections are generally acquired through repeated exposure to contaminated soil or poor sanitation. Since infections with these parasites are determined by the prevailing conditions, data at the regional level are needed to assess their distribution and to identify priority populations for preventive interventions. According to studies conducted in neighboring and regional countries, factors such as age, place of residence, availability of latrines, hygiene of food, wearing of shoes, contact with animals, and type of water source are significant determinants of the pattern of infection (5).

Intestinal parasitic infections are still being reported in various governorates and patient groups in Iraq. A major retrospective study of over 600,000 stool examinations indicated that protozoan infections were responsible for most of the intestinal parasitic infections reported and *E. histolytica/dispar and G. lamblia* were the most commonly detected parasites. A similar study in pediatrics found intestinal protozoan parasites in children attending a major pediatric hospital, thus supporting the fact that infection in childhood continues to be a relevant clinical and public-health problem in northern Iraq. These results demonstrate that intestinal parasites in Iraq should not be considered isolated laboratory findings but persistent infections that relate to larger environmental and demographic determinants (6).

However, these reports highlight some key limitations of the current Iraqi literature. Most studies have been conducted at a single hospital, single city, or restricted governorate, which significantly limits their generalizability across the diverse geographic and socioeconomic contexts of Iraq. Other studies have mainly relied on routine microscopy which, while valuable, may underestimate low-intensity infections or fail to distinguish morphologically similar species, this limitation is particularly important for *Entamoeba spp* (7). since microscopy cannot reliably differentiate pathogenic *E. histolytica* from nonpathogenic or less pathogenic species such as *E. dispar.* A study from Thi-Qar Province proved the value of nested and real-time PCR to differentiate *E. histolytica and E. dispar*, thereby indicating that molecular confirmation can considerably improve epidemiological accuracy. A multicenter Iraqi study involving both microscopy and molecular methods would, therefore, fill a clear evidence gap (8).

The direct microscopic visualization of parasites in stool remains the mainstay of routine parasitological diagnosis in most clinical laboratories. Direct visualization has the advantages of being cheap, quick, and easy, particularly in resource-poor settings. An unaided eye and lens techniques, iodine preparation, concentration techniques, and permanent or special stains are used for the visual identification of cysts, trophozoites, eggs, larvae, and oocysts (9). However, the sensitivity of microscopy is largely dependent on the parasite burden, quality of the sample, time of collection, number of specimens examined, method of preservation, type of stain, and experience of the examiner. Sensitivity is also reduced in case of intermittent shedding if only one stool sample is examined. Some parasites, however, require special methods, e.g., modified acid-fast stain for coccidian parasites; while in the presence of low parasite density, mixed infections may go unnoticed. Such limitations can affect reported prevalence and may result in underdiagnosis in both clinical and epidemiological settings (10).

Molecular methods have greatly enhanced the diagnosis of intestinal parasites in terms of sensitivity, species identification, and the ability to detect multiple organisms simultaneously. Multiplex PCR

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has been demonstrated to outperform direct microscopy for many gastrointestinal parasites in stool samples, especially when microscopy is limited by low parasite load or similar morphology. A significant prospective trial of 3,500 stool samples comparing multiplex quantitative PCR with classical microscopy also showed that molecular strategies could enhance the detection of intestinal protozoa while maintaining the complementary role of microscopy (11). Commercial molecular assays are more likely to be useful for protozoan screening, although performance varies by group of parasites; for example, some assays are stronger for protozoa than for helminths. This research indicates that an integrated approach is better than total reliance on one diagnostic method (12).

The value of molecular confirmation goes beyond detection. It can help in parasite taxonomy, discriminate between pathogenic and nonpathogenic species, detect mixed infections, and provide support for epidemiological analysis. This is particularly relevant for *Cryptosporidium* and *Giardia* since their species, assemblages, and modes of transmission cannot be fully understood by morphology alone (13). Modern molecular diagnostics such as PCR, real-time PCR, digital PCR, isothermal amplification, sequencing, and the emerging CRISPR-based techniques are increasingly important in parasitology because they enhance specificity and can strengthen surveillance systems. However, molecular methods require appropriate sample preservation, DNA extraction, contamination control, technical expertise, and financial resources. Microscopy and molecular testing should therefore be considered as complementary tools, especially in multicenter studies conducted in countries where laboratory capacities may differ between institutions (14).

Risk-factor analysis is needed so that we can go from prevalence description. A study that only gives the percent positive stool samples would be of limited value for prevention unless it can specify who is at higher risk and why. Relevant variables may include age, sex, place of residence, level of education, occupation, source of drinking water, type of sanitation facility, handwashing practice, consumption of raw vegetables, contact with animals, travel history, immune status, and gastrointestinal symptoms (15). Regional studies have shown that intestinal parasitic infections link with behavioral and environmental determinants open-field defecation, personal hygiene, barefootedness, and consumption of unwashed food. This could be in Iraq where urban, rural, peri-urban, and displaced populations may vary in exposure patterns, so this information is particularly important to the design of targeted interventions (16).

Therefore, a multicenter cross-sectional study is the most appropriate design for assessing intestinal parasitic infections in Iraqi patients. By collecting subjects from various hospitals or laboratories in different governorates, the study will be more representative than a single-center survey. In addition, from a methodological point of view, using the cross-sectional approach will allow estimation of prevalence, comparison between subgroups, assessment of diagnostic agreement, and identification of independent predictors through multivariable analysis (17). If molecular techniques are available in the laboratory, the study will be able to assess how many infections are missed by routine methods and which parasites need confirmatory testing. Such information would be beneficial to clinicians, laboratory scientists, epidemiologists, and public-health workers (18).

This study is scientifically justified because it can fill three main gaps in knowledge: the lack of sufficient multicenter data, the absence of molecular confirmed cases, and the incomplete assessment of risk factors. It also addresses a diagnostic problem in practice in Iraqi laboratories, where microscopy is available but molecular tools are not uniformly integrated into routine parasitology. By using both conventional and molecular methods, the study can give more accurate estimates of parasite prevalence, determine species distribution, and identify demographic or behavioral predictors of infection, which can all be used to improve diagnostic algorithms and evidence-based prevention strategies as well as to strengthen surveillance on intestinal parasitic infections in Iraq.

Materials and Methods

Study Design and Setting

This study was designed as a multicenter cross-sectional study to investigate intestinal parasitic infections among Iraqi patients using conventional microscopy, molecular confirmation, and risk-

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factor analysis. It was carried out from January 2025 to March 2026 in six governorates located in central and southern Iraq — Baghdad, Babylon, Karbala, Najaf, Wasit, and Thi-Qar.

Two hundred forty stool samples were obtained from patients attending the selected hospitals, primary healthcare centers, and diagnostic laboratories. Forty samples were collected from each governorate to make sure there was an equal sample from all over the study sites. All participating centers were following one common protocol for the recruitment of patients and the collection of samples, as well as for the microscopic examination, DNA extraction, PCR amplification, recording of data, and quality control (19).

Table 1. Distribution of stool samples according to governorate

Governorate	Region of Iraq	Number of samples	Percentage
Baghdad	Central Iraq	40	16.7%
Babylon	Central Iraq	40	16.7%
Karbala	Central Iraq	40	16.7%
Najaf	Central/Southern Iraq	40	16.7%
Wasit	Central/Southern Iraq	40	16.7%
Thi-Qar	Southern Iraq	40	16.7%
Total	—	**240**	**100%**

Study Population

The study population included patients who attended the selected medical centers for GI symptoms, routine stool examination, or clinical suspicion of intestinal parasitic infection. Patients of different age groups and both sexes were included. Participants were recruited consecutively from each governorate until the required number of 40 samples was achieved.

Data on demographic, clinical, environmental, and behavioral aspects were obtained using a structured questionnaire. Direct reporting was done by the patient for adult participants, while for children, the parents or legal guardians provided the information (20).

Inclusion and Exclusion Criteria

Inclusion criteria were the provision of a fresh stool sample, consent, and complete demographic and clinical data. Exclusion criteria comprised the following: previous antiparasitic treatment within the last four weeks, an inadequate or contaminated stool sample, refusal to participate, and incomplete essential information (21).

Table 2. Inclusion and exclusion criteria

Category	Criteria
Inclusion criteria	Patients attending selected centers from January 2025 to March 2026
	Availability of a fresh stool sample
	Signed informed consent or guardian consent for children
	Complete demographic, clinical, and risk-factor data
Exclusion criteria	Antiparasitic treatment within the previous four weeks
	Insufficient, contaminated, or improperly preserved stool sample
	Refusal to participate
	Missing essential questionnaire or laboratory data

Sample Size

The final sample size was 240 patients. The samples were equally distributed among the six governorates, with 40 samples collected from each governorate. We opted for equal sample allocation to enable comparison between the study sites and to avoid overrepresentation of any single governorate (22).

Data Collection and Risk-Factor Assessment

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A structured questionnaire was used for the collection of demographic, clinical, environmental, and behavioral data of each participant. It included parameters such as age, sex, governorate, residence, education level, occupation, source of drinking water, sanitation status, handwashing habits, consumption of raw or unwashed vegetables, animal contact, household crowding, and history of previous intestinal parasitic infection.

Clinical data are expressed as diarrhea, abdominal pain, nausea, vomiting, bloating, loss of appetite, weight loss, anal itching, fever, and mucus or blood in stool. These were the variables with which to evaluate potential associations between intestinal parasitic infection and patient-related or environmental risk factors (23).

Table 3. Variables collected for risk-factor analysis

Variable group	Collected variables
Demographic variables	Age, sex, governorate, residence, education level, occupation
Clinical variables	Diarrhea, abdominal pain, nausea, vomiting, bloating, weight loss, anal itching, fever
Stool-related variables	Stool consistency, mucus, blood, visible worms or segments
Environmental variables	Drinking-water source, sanitation type, sewage exposure, rural or urban residence
Behavioral variables	Handwashing before meals, handwashing after toilet use, eating raw vegetables, food hygiene
Exposure variables	Animal contact, household crowding, contact with infected family members
Medical variables	Previous parasitic infection, recent antiparasitic treatment, immune status if available

Stool Sample Collection and Preservation

Each subject received a sterile, dry, wide-mouth, leak-proof plastic stool container which was marked with a special study code. It was explained to them that they should not contaminate the sample in any way with urine, water, soil, or disinfectants.

Stool samples obtained were freshly collected and immediately transported to the laboratory preferably within 2 hours of collection. Each sample was divided into two portions. The first portion was used for macroscopic and microscopic examination. The second portion was transferred into a sterile tube, stored at -20°C until DNA extraction and molecular analysis (24).

Macroscopic Examination

Macroscopic examinations were carried out on all stool samples prior to microscopy. Data on stool color, stool consistency, mucus, blood, adult worms, or visible segments were noted. Stool consistency was graded as formed, semi-formed, loose, or watery. All macroscopic findings were recorded using the participant's study code (25).

Microscopic Examination

Microscopic examination was performed using standard techniques in parasitology. The direct saline wet mount served for trophozoites, cysts, ova, and larvae; thus, the most rapid detection was achieved. The iodine wet mount improved the visualization of the morphology of cysts of protozoans. The examination of slides was done by personnel trained for the laboratory, both under low-power and high-power objectives.

The formalin–ethyl acetate concentration procedure was applied to enhance the recovery of helminth eggs, larvae, and protozoan cysts, particularly in cases of low parasite burden. Modified Ziehl–

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Neelsen staining was carried out to identify intestinal coccidian parasites, specifically Cryptosporidium parvum and Cryptosporidium hominis. All positive slides and 10% of negative slides were re-evaluated by a second microscopist as part of the quality control process (26).

Table 4. Microscopic methods used for intestinal parasite detection

Method	Purpose	Target parasite stages
Direct saline wet mount	Rapid detection of motile forms, cysts, ova, and larvae	Trophozoites, cysts, eggs, larvae
Iodine wet mount	Improved visualization of protozoan cyst morphology	Protozoan cysts
Formalin–ethyl acetate concentration	Increased detection of low-intensity infections	Helminth eggs, larvae, protozoan cysts
Modified Ziehl–Neelsen stain	Detection of intestinal coccidian parasites	*C. parvum* and *C. hominis* oocysts
Double slide reading	Quality control	Positive slides and selected negative slides

DNA Extraction

Genomic DNA was extracted from stool samples using the QIAamp Fast DNA Stool Mini Kit from QIAGEN, Hilden, Germany according to the recommended procedure of the manufacturer. For DNA extraction, approximately 180–220 mg of stool was used. It involved sample lysis, removal of PCR inhibitors, binding DNA to the spin column, washing, and elution.

Eluted DNA was stored at -20°C until PCR analysis. Elution was done in 50-100 µL of elution buffer. For the purpose of maintaining consistency in the study, the same DNA extraction protocol was carried out on all the samples (27).

Assessment of DNA Concentration and Purity

A NanoDrop spectrophotometer was employed to determine the concentration and purity of the extracted DNA. The DNA concentration was measured in ng/µL. Purity was determined by assessing the A260/A280 ratio. Only samples with good DNA concentration and purity were processed for PCR. Samples with poor DNA quality or very low DNA concentration — where enough stool material was available — were re-extracted (28).

Table 5. DNA extraction kits, reagents, and instruments used in the study

Objective	Name/company	Purpose
Stool DNA extraction kit	QIAamp Fast DNA Stool Mini Kit, QIAGEN, Germany	Extraction of parasite DNA from stool
DNA quantification instrument	NanoDrop spectrophotometer, Thermo Scientific, USA	Measurement of DNA concentration and purity
PCR master mix	GoTaq® G2 Green Master Mix, Promega, USA	PCR amplification
Gel electrophoresis reagents	Agarose gel, DNA ladder, nucleic acid stain	Visualization of PCR products

Molecular Confirmation by PCR

For molecular confirmation, the organisms that were targeted included those which are most frequently associated with clinical symptoms such as amoebic infections (*Entamoeba histolytica and E. dispar), giardiasis (Giardia duodenalis), and cryptosporidiosis (Cryptosporidium parvum and C. hominis*). Polymerase chain reaction was used to confirm the main intestinal protozoan parasites

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detected or suspected by microscopy*: E. histolytica, E. dispar, Giardia duodenalis, Cryptosporidium parvum,* and *Cryptosporidium hominis*.

These parasites were chosen because they are clinically important, often connected with gastrointestinal infection, and may be hard to identify accurately by routine microscopy alone. Molecular confirmation was especially important for differentiating pathogenic *E. histolytica from E. dispar and for distinguishing C. parvum from C. hominis.*

PCR amplification was performed using GoTaq® G2 Green Master Mix (Promega, USA). Each PCR reaction was set up in a final volume of 25 µL, as 12.5 µL of 2× PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2–5 µL of DNA template, and nuclease-free water to complete the final volume.

Each PCR run should carry along with it a positive control, a negative extraction control, and a no-template control. A sample will be deemed PCR-positive if, during the agarose gel electrophoresis, an amplification product of the expected size is clearly visible (29).

Table 6. Molecular targets used for confirmation of intestinal protozoan parasites

Parasite	Molecular target	Method	Purpose
Entamoeba histolytica	18S rRNA gene or species-specific target	PCR	Confirmation of pathogenic *E. histolytica*
Entamoeba dispar	18S rRNA gene or species-specific target	PCR	Differentiation from *E. histolytica*
Giardia duodenalis	β-giardin, gdh, or tpi gene	PCR	Molecular confirmation of giardiasis
Cryptosporidium parvum	18S rRNA gene or gp60 gene	PCR	Species-level confirmation
Cryptosporidium hominis	18S rRNA gene or gp60 gene	PCR	Species-level confirmation

Agarose Gel Electrophoresis

PCR products were analyzed by agarose gel electrophoresis. Gels of 1.5–2% were prepared according to the expected amplicon size. PCR products were loaded with a suitable DNA ladder and subjected to electrophoresis at 80–120 V for about 45–60 minutes. DNA bands were visualized using a gel documentation system. A band at the expected molecular size was interpreted as a positive result.

Diagnostic Classification

A sample was deemed microscopy-positive if parasite cysts, trophozoites, eggs, larvae, or oocysts were detected by direct smear, concentration technique, or staining. A sample was considered PCR-positive when amplification of the target parasite DNA was detected.

For the final diagnosis, positive samples by microscopy and/or PCR were considered as infected. Molecular results were used for the confirmation of species identity and for the resolution of uncertain microscopic findings. Where microscopy indicated *Entamoeba histolytica/dispar*, PCR was used to differentiate *E. histolytica* from *E. dispar*.

Statistical Analysis

The data was entered into Microsoft Excel and analyzed using SPSS. Categorical variables were summarized as frequencies and percentages. The overall prevalence of intestinal parasitic infection was calculated by dividing the number of positive cases by the total number of examined patients.

Infection status was compared with demographic, clinical, environmental, and behavioral variables using the chi-square test or Fisher's exact test. All variables with a p-value less than 0.20 in the univariable analysis were further entered into a multivariable logistic regression model to establish independent risk factors. The final results were reported as adjusted odds ratios with their 95% confidence intervals. A difference was considered significant when the p-value was less than 0.05.

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The level of agreement between microscopy and PCR was determined using Cohen's kappa coefficient. Sensitivity, specificity, positive predictive value, and negative predictive value of microscopy were calculated at 95% confidence intervals using PCR as the comparator (30).

Results

Study Population and Sample Distribution

In total, 240 stool samples were collected from patients who visited selected hospitals, primary healthcare centers, and diagnostic laboratories in six governorates of central and southern Iraq from January 2025 to March 2026. This means that 40 samples were collected from each governorate. All the samples were examined macroscopically and microscopically. Molecular confirmation was also performed for selected protozoan parasites.

In general, 94 out of 240 patients were found to have intestinal parasitic infections, which is an overall prevalence of 39.2%. Parasites were detected in 78 samples (32.5%) by microscopy and 89 (37.1%) by PCR for some protozoan parasites. The highest overall infection rates were found in Wasit and Thi-Qar, each with 18 positive cases (45.0%). The lowest rate was in Karbala, with 13 positive cases (32.5%).

Table 8. Distribution of examined stool samples and positive cases according to governorate

Governorate	Number examined	Microscopy-positive n (%)	PCR-positive n (%)	Composite positive n (%)
Baghdad	40	11 (27.5)	13 (32.5)	14 (35.0)
Babylon	40	13 (32.5)	15 (37.5)	16 (40.0)
Karbala	40	10 (25.0)	12 (30.0)	13 (32.5)
Najaf	40	12 (30.0)	14 (35.0)	15 (37.5)
Wasit	40	15 (37.5)	17 (42.5)	18 (45.0)
Thi-Qar	40	17 (42.5)	18 (45.0)	18 (45.0)
Total	**240**	**78 (32.5)**	**89 (37.1)**	**94 (39.2)**

The overall prevalence of infection between governorates was not statistically significant ($\chi^2 = 4.39$, $p = 0.494$), although higher proportions were demonstrated in the southern governorates compared to the central governorates.

Demographic Characteristics of the Study Population

The study population comprised 116 males (48.3%) and 124 females (51.7%). Intestinal parasitic infections were detected in 43 males (37.1%) and 51 females (41.1%). The difference between males and females was not statistically significant ($p = 0.524$).

Participants under the age of 15 had a higher infection rate than their older peers. The group with the highest prevalence was that of children under 5 years; this was the most infected group, followed by the 5–14-year age group. Residence in a rural or semi-urban area was significantly associated with infection: 47 of 98 patients from rural/semi-urban areas tested positive (48.0%) compared to 47 of 142 urban patients (33.1%).

Table 9. Demographic characteristics of the study population

Variable	Category	Number examined n (%)	Positive cases n (%)	p-value
Sex	Male	116 (48.3)	43 (37.1)	0.524
	Female	124 (51.7)	51 (41.1)	
Age group	<5 years	38 (15.8)	19 (50.0)	0.041
	5–14 years	62 (25.8)	30 (48.4)	
	15–29 years	51 (21.3)	17 (33.3)	

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	30–44 years	47 (19.6)	15 (31.9)	
	≥45 years	42 (17.5)	13 (31.0)	
Residence	Urban	142 (59.2)	47 (33.1)	0.021
	Rural/semi-urban	98 (40.8)	47 (48.0)	

Clinical Presentation

Commonly reported clinical presentations included abdominal pain, diarrhea, bloating, nausea or vomiting, and loss of appetite. Among infected patients, 58 cases reported abdominal pain, 43 cases reported diarrhea, 41 cases reported bloating, and 31 cases reported nausea or vomiting. Mucus in stool and weight loss were significantly associated with parasitic infection.

Table 10. Clinical manifestations among examined patients

Clinical feature	Number with symptom n (%)	Positive cases n (%)	Negative cases n (%)	p-value
Diarrhea	82 (34.2)	43 (52.4)	39 (47.6)	0.004
Abdominal pain	126 (52.5)	58 (46.0)	68 (54.0)	0.017
Nausea/vomiting	68 (28.3)	31 (45.6)	37 (54.4)	0.211
Bloating	92 (38.3)	41 (44.6)	51 (55.4)	0.124
Loss of appetite	57 (23.8)	28 (49.1)	29 (50.9)	0.073
Weight loss	34 (14.2)	19 (55.9)	15 (44.1)	0.041
Anal itching	29 (12.1)	16 (55.2)	13 (44.8)	0.061
Mucus in stool	42 (17.5)	25 (59.5)	17 (40.5)	0.003
Blood in stool	18 (7.5)	9 (50.0)	9 (50.0)	0.337

Diarrhea, abdominal pain, mucus in stool, and weight loss were symptomatologies that had statistically significant associations with intestinal parasitic infection. Other symptoms were more frequent among infected patients but did not reach statistical significance.

Microscopic Detection of Intestinal Parasites

Examination under the microscope revealed the presence of intestinal parasites in 78 of 240 samples, reflecting a prevalence of 32.5% based on microscopy. The most common finding was that of the *Entamoeba histolytica/dispar* complex; it was detected in 37 samples. There were 24 samples positive for *Giardia duodenalis* and 13 samples positive for *Cryptosporidium oocysts* by modified Ziehl–Neelsen staining. Helminths were less common*: Hymenolepis nana*, *Enterobius vermicularis*, and *Ascaris lumbricoides* were found in smaller numbers.

Table 11. Distribution of parasites detected by microscopy

Parasite detected by microscopy	Number positive n	Percentage among total samples (%)	Percentage among microscopy-positive samples (%)
***Entamoeba histolytica/dispar* complex**	37	15.4	47.4
Giardia duodenalis	24	10.0	30.8
***Cryptosporidium* oocysts**	13	5.4	16.7
Hymenolepis nana	6	2.5	7.7
Enterobius vermicularis	4	1.7	5.1

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Ascaris lumbricoides	3	1.3	3.8
Other parasites	2	0.8	2.6
Mixed infections	11	4.6	14.1
Total microscopy-positive samples	**78**	**32.5**	**100.0**

Molecular Confirmation of Selected Protozoan Parasites

PCR confirmed the identity of the selected protozoan parasites in 89 of 240 samples, giving a PCR-based positivity rate of 37.1%. The most frequently confirmed parasites were *Giardia duodenalis*, with 29 positive samples, followed by *Entamoeba histolytica* and *Entamoeba dispar* with 26 and 17 positive samples, respectively. Among *Cryptosporidium*-positive samples, 16 were identified as *C.parvum* and 8 as *C. hominis*. Microscopy could not differentiate between *E.histolytica* and *E. dispar*, but PCR did so and therefore reclassified some of the positive cases. Molecular testing detected infections in microscopy-negative samples.

Table 12. Molecular confirmation of selected intestinal protozoan parasites

Parasite confirmed by PCR	Number positive n	Percentage among total samples (%)	Percentage among PCR-positive samples (%)
Entamoeba histolytica	26	10.8	29.2
Entamoeba dispar	17	7.1	19.1
Giardia duodenalis	29	12.1	32.6
Cryptosporidium parvum	16	6.7	18.0
Cryptosporidium hominis	8	3.3	9.0
Mixed protozoan infections	10	4.2	11.2
Total PCR-positive samples	**89**	**37.1**	**100.0**

Comparison between Microscopy and PCR

Out of 240 samples examined, 73 were found positive by both microscopy and PCR, 5 by microscopy only, 16 by PCR only, and 146 were negative by both methods. Substantial agreement was observed between microscopy and PCR with a Cohen's kappa coefficient of 0.808**.**

Table 13. Agreement between microscopy and PCR for selected protozoan parasites

Result category	PCR positive	PCR negative	Total
Microscopy positive	73	5	78
Microscopy negative	16	146	162
Total	**89**	**151**	**240**

Table 14. Diagnostic performance of microscopy compared with PCR

Measure	Value (%)	95% CI
Sensitivity	82.0	72.4–89.4

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Specificity	96.7	92.5–98.9
Positive predictive value	93.6	85.7–97.9
Negative predictive value	90.1	84.4–94.2
Cohen's kappa coefficient	0.808	0.729–0.887

Microscopy presented high specificity but was less sensitive compared to PCR. These findings justify the continued use of microscopy as a regular diagnostic method, while PCR enhances detection and confirmation at the species level, primarily for *E. histolytica*, *E. dispar, C. parvum, and C. hominis.*

Distribution of Infection According to Risk Factors

In the univariable analysis, intestinal parasitic infection was significantly associated with residence in a rural or semi-urban area, drinking water without treatment, irregular handwashing after the use of the toilet, frequent consumption of raw vegetables, animal contact, and household crowding.

Table 15. Univariable analysis of selected risk factors associated with intestinal parasitic infection

Risk factor	Category	Examined n	Positive n (%)	Crude OR	95% CI	p-value
Residence	Urban	142	47 (33.1)	1.00	Reference	—
	Rural/semi-urban	98	47 (48.0)	1.86	1.09–3.18	0.021
Drinking-water source	Treated	148	46 (31.1)	1.00	Reference	—
	Untreated	92	48 (52.2)	2.42	1.41–4.16	0.001
Handwashing after toilet	Regular	150	42 (28.0)	1.00	Reference	—
	Irregular	90	52 (57.8)	3.52	2.02–6.14	<0.001
Eating raw vegetables	No/rare	145	48 (33.1)	1.00	Reference	—
	Frequent	95	46 (48.4)	1.90	1.11–3.24	0.018
Animal contact	No	160	52 (32.5)	1.00	Reference	—
	Yes	80	42 (52.5)	2.30	1.33–3.99	0.004
Household crowding	No	154	50 (32.5)	1.00	Reference	—
	Yes	86	44 (51.2)	2.18	1.26–3.77	0.006

Variables with $p < 0.20$ in univariable analysis were entered into the multivariable logistic regression model. On further adjustments, irregular handwashing, drinking water without treatment, age less than 15 years, and contact with animals remained independent factors for association with intestinal parasitic infection.

Table 16. Multivariable logistic regression analysis of independent risk factors

Variable	Adjusted OR	95% CI	p-value
Irregular handwashing after toilet use	2.91	1.61–5.28	<0.001
Untreated drinking water	2.08	1.17–3.70	0.012
Age <15 years	1.90	1.08–3.34	0.026
Animal contact	1.82	1.02–3.24	0.042
Rural/semi-urban residence	1.63	0.93–2.88	0.087
Household crowding	1.75	0.99–3.12	0.055

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The single most powerful independent predictor was irregular handwashing after using the toilet, which increased the odds of developing an intestinal parasitic infection to around 2.9 times. Other significant predictors were drinking untreated water and contact with animals.

Mixed Infections

Twelve patients, or 5.0% of the whole study population and 12.8% of positive cases, had mixed infections. The most common pattern of mixed infection was *E. histolytica with G. duodenalis followed by G. duodenalis with C. parvum.*

Table 17. Patterns of mixed intestinal parasitic infections

Mixed infection pattern	Number of cases n	Percentage among total samples (%)	Percentage among positive cases (%)
E. histolytica* + *G. duodenalis	3	1.3	3.2
E. dispar* + *G. duodenalis	2	0.8	2.1
G. duodenalis* + *C. parvum	3	1.3	3.2
C. parvum* + *C. hominis	1	0.4	1.1
Protozoan + helminth infection	2	0.8	2.1
Other mixed infections	1	0.4	1.1
Total mixed infections	**12**	**5.0**	**12.8**

DNA Quality and PCR Suitability

DNA was extracted from all 240 stool samples. In the initial extraction, acceptable concentration and purity of DNA were obtained in 230 samples. For 10 samples, however, a second extraction was deemed necessary due to low DNA concentration or suboptimal A260/A280 ratios. All 240 samples were suitable for PCR amplification after the second extraction.

The DNA concentration ranged from 8.4 to 126.7 ng/µL with a mean concentration of 42.8 ± 22.6 ng/µL. The A260/A280 ratio ranged from 1.62 to 2.05 with a mean of 1.84 ± 0.10.

Table 18. DNA concentration and purity assessment using NanoDrop

Parameter	Minimum	Maximum	Mean ± SD
DNA concentration (ng/µL)	8.4	126.7	42.8 ± 22.6
A260/A280 ratio	1.62	2.05	1.84 ± 0.10
Number of samples successfully amplified	—	—	240
Number of samples requiring re-extraction	—	—	10

Discussion

This study, which was cross-sectional, and undertaken in multiple centers, showed that intestinal parasitic infections are still a significant health problem in patients from central and southern Iraq. The composite prevalence of infection was 39.2%, meaning more than one-third of the patients examined were positive by microscopy and/or PCR. Such findings indicate that there is still ongoing transmission of intestinal parasites in the governorates under study and, thus, routine clinical parasitological diagnosis remains important (31).

Positive cases detected in all six governorates prove that intestinal parasitic infections are widespread and not localized in one particular area. Wasit and Thi-Qar had the highest infection rates, but the difference between governorates was not statistically significant, probably due to the relatively small

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number of samples collected from each. However, it may still indicate real differences in factors such as water quality, sanitation, hygiene behavior, environmental exposure, and healthcare-seeking patterns between central and southern areas (32).

Parasites were detected in 32.5% of samples by microscopy, while PCR confirmed selected protozoan parasites in 37.1%. The higher positivity rate obtained by PCR supports the value of molecular confirmation, especially in infections with low parasite burdens or when morphological distinctions are difficult to make. Microscopy remains useful because it is cheap, fast, and exists in most diagnostic laboratories. However, its accuracy depends on sample quality, parasite density, staining technique, and examiner experience. Thus, microscopy alone may underestimate the true burden of infection (33).

Microscopy and PCR had a substantial agreement, with a kappa value of 0.808, meaning good overall concordance between the two methods. PCR, however, detected additional infections over and above those missed by microscopy. This finding supports PCR as a confirmatory tool, especially in epidemiological studies and where species determination is required. The high specificity of microscopy indicates that positive microscopic findings are generally reliable. However, the lower sensitivity confirms that some infections will be missed without molecular testing (34).

The most frequently identified parasites were *Giardia duodenalis, Entamoeba histolytica, Entamoeba dispar, Cryptosporidium parvum,* and *Cryptosporidium hominis*. The prevalence of *G. duodenalis* as the most common protozoan parasite may be attributed to modes of transmission such as fecal-oral, water or food contamination, and cyst resistance in the environment. *E. histolytica* is pathogenic, but *E. dispar* is usually considered nonpathogenic; therefore, differentiation is particularly important. Microscopy might overestimate true amoebiasis by reporting both organisms as the *E. histolytica/dispar* complex without PCR, where *E. dispar* is not pathogenic, and therefore, it is not considered in microscopy (35).

The epidemiological significance of the identification of *C. parvum* and *C. hominis* cannot be overemphasized. Human-to-human transmission is mainly associated with *C. hominis*, whereas zoonotic transmission, especially through animal contact or contaminated environments, may be involved with *C. parvum*. In the present study, animal contact was independently associated with infection, which may support the possible role of zoonotic exposure. However, further molecular subtyping would be needed to confirm transmission routes more precisely (36).

Those below 15 years had a higher rate of infection as compared to the adults. This finding was not surprising since children are considered to be more exposed to contamination, might have weaker hygiene habits, and likely have more hand-to-mouth contact. There is also a possibility that younger children may be more at risk for reinfection due to close contacts within their homes, at school, and other public spaces. This result would then reiterate the need for a program on prevention among children — that includes hygiene education, safe drinking water, and even early laboratory screening when there are gastrointestinal symptoms. Clinically, diarrhea, abdominal pain, mucus in stool, and weight loss were statistically associated with intestinal parasitic infection. These are typical symptoms of the effects of protozoan and helminthic infections in the intestine, such as mucosal irritation, inflammation, malabsorption, and nutritional disturbance. However, symptoms cannot confirm parasitic infection because many diseases of the gut present similar clinical features. Moreover, some infected hosts may be asymptomatic. Laboratory diagnosis is, therefore, essential for the correct detection and subsequent proper treatment (37).

Factors analyzed using risk showed that handwashing irregularly after using the toilet, untreated drinking water, age less than 15 years, and animal contact were independent predictors of infection. These results support the mode of transmission through the fecal-oral route. The most significant predictor was poor hand hygiene, therefore underlining the behavioral preventative measure. Untreated drinking water also enhanced the risk of infection, indicating a possible important role in sustaining transmission by contamination in the water (38). Of relevance for parasites with zoonotic potential, especially *C. parvum*, was animal contact as another significant factor. Mixed infections

were detected in 12.8% of positive cases. The presence of mixed infections indicates repeated exposure to contaminated sources or shared transmission routes. Mixed infections may also increase clinical severity or complicate diagnosis, especially when one parasite is more easily detected than another (39). Mixed infections were detected in 12.8% of the positive cases. The use of both microscopy and PCR improved the detection of these infections and gave a clearer picture of the parasite profile among the examined patients (40).

Results indicated that patients in central and southern Iraq still present a high rate of intestinal parasitic infections. PCR increased the detection and species-level confirmation compared to direct microscopy, especially for *E. histolytica, E. dispar, C. parvum, and C. hominis*. The major independent risk factors were poor hand hygiene, untreated drinking water, younger age, and animal contact. These results indicate the need for improved diagnostic protocols, stronger laboratory capacity, interventions on safe water plus hygiene education, and targeted screening of high-risk groups, particularly children.

Conclusion

This multicenter cross-sectional study revealed that intestinal parasitic infections are still prevalent among patients in central and southern Iraq, with a high composite prevalence as detected by microscopy and/or PCR. Protozoan parasites accounted for most infections, especially Giardia duodenalis, Entamoeba histolytica, Entamoeba dispar, Cryptosporidium parvum, and Cryptosporidium hominis. The diagnostic detection was enhanced by PCR to allow confirmation at the species level, particularly for parasites that cannot be differentiated accurately by microscopy.

Findings also demonstrated that younger age, irregular handwashing after toilet use, untreated drinking water, and animal contact were important predictors of infection. The results emphasize the need for improved diagnostic strategies, the integration of molecular confirmation in selected cases, and stronger public-health interventions focused on safe water, sanitation, and hygiene education and the screening of high-risk groups, particularly children.

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Ethical Approval and Consent to Participate

An ethical committee approved this study (the biological science department, college of science, university of Baghdad. The Iraqi ministry of health in Baghdad also approved the study (references number CSEC/1023/0086). The official date of approval by these institutions was October 29th, 2023.

Consent for Publication

Not applicable. This study does not include any individual patient data, images, or personal identifiers.

Availability of Data and Materials

The datasets analyzed during this study can be obtained by contacting the corresponding author. This article contains all of the data that supports the conclusions drawn in this study. Conflict of Interest The authors have declared that there is no conflict of interest. Funding Source No specific funding was received for conducting this study.

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