



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(12): 433-439

© 2025 VET

www.veterinarypaper.com

Received: 18-10-2025

Accepted: 22-11-2025

Safaa M Kareem

Department of Parasitology,
College Veterinary Medicine,
Al-Qasim Green University,
Babylon 51013, Iraq

Akeel Mohammed Al-Musawi

Department of Parasitology,
College Veterinary Medicine,
Al-Qasim Green University,
Babylon 51013, Iraq

Molecular detection of some intestinal protozoa isolation from farm soil

Safaa M Kareem and Akeel Mohammed Al-Musawi

DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i12g.2859>

Abstract

Intestinal protozoan parasites are globally distributed and are related with both humans and animals. They are transmitted to humans by the consumption of food and beverages infected with parasite cysts and oocysts. The current study registered the percentage Intestinal protozoan in Hilla city microscopic and molecular diagnosis from different area of Babylon between (April of 2024 to April of 2025). collected (200) farm soil samples, the prevalence of intestinal protozoa forms the soil samples by the molecular detection show *Giardia intestinalis* recorded 71/200 (35.5%), *Cryptosporidium spp* was 63/200 (31.5%) and *Entamoeba histolytica* recorded 46/200 (23%). The phylogenetic analysis identified five sequences related with the *G. intestinalis* A and B (PX289656, PX289657, PX289658, PX289659, and PX289660). Furthermore, the phylogenetic analysis revealed five *Cryptosporidium spp* sequences (PX279584, PX279585, PX279586, PX279587, and PX279588). Lastly, five *Entamoeba histolytica* sequences were identified (PX247889, PX247890, PX247891, PX247892 and PX247893).

Keywords: *Giardia intestinalis*, *Entamoeba histolytica*, *Cryptosporidium spp*, Iraq, food, water, soil, molecular detection

Introduction

Single-celled eukaryotic microorganisms are intestinal protozoan parasites. The fecal-oral pathway is the means by which intestinal protozoa are spread. As a result, they mostly impact underprivileged groups, particularly children, who lack access to safe water, better sanitation, and proper hygiene (Paul, 2024) ^[14]. According to Ghazy *et al.* (2025) ^[8], these infections may be asymptomatic or result in diarrhoea, malnourishment, stomach discomfort, and stunted growth and development. Soil-transmitted parasites constitute the biggest category of parasites, encompassing intestinal protozoa that inhabit the soil during their developmental stages (Blackburn *et al.*, 2024) ^[3]. Soil contamination with cysts and oocysts represents the primary risk factor for zoonotic parasite infections (Kihara *et al.*, 2025) ^[9]. According to Zeng *et al.*, (2024) ^[22], the estimated worldwide burden of enteric protozoa in 2010 was nearly 3 million Disability Adjusted Life Years. However, these are approximations based on national and regional statistics that are lacking. Furthermore, sensitive molecular diagnostic methods have not been employed in the majority of surveillance investigations (Mejia *et al.*, 2024) ^[11]. As of now, there has been no study on soil protozoan networks, and elucidating the "dark box" of connections within the soil food web continues to pose a significant problem in soil biology. Pathogenic protozoa can contaminate soil via the faeces of sick humans and animals (Rossi *et al.*, 2024) ^[17]. Protozoans are able to launch infectious stages in the form of oocysts and cysts, which are released into the environment by the feces of sick hosts and are resistant to disinfectants and other environmental agents. As a result, they may endure for extended periods of time under a variety of circumstances and are extensively present in the environment, water, and food. The World Health Organisation (WHO) identified several protozoan parasites that are frequently spread through tainted food and water, including *Giardia intestinalis*, *Entamoeba histolytica* and *Cryptosporidium spp.*, as serious public health concerns in 1987 because they primarily affect children in developing nations and cause malabsorption (Marhoon and Al-Aredhi, 2025) ^[10].

Corresponding Author:

Safaa M Kareem

Department of Parasitology,
College Veterinary Medicine,
Al-Qasim Green University,
Babylon 51013, Iraq

These microorganisms cause IPI (intestinal parasites infections) which inhibits children's growth due to chronic diarrhea (Namkuna, 2024) [13]. The purpose of this study is to use molecular and microscopic detection to examine the prevalence of certain intestinal diseases that have been isolated from the soil of Hilla City, Iraq.

Materials and Methods

Collection of Samples

200 farm soil samples were taken at different Babylonian locations between April 2024 and April 2025 using the procedures outlined below. Five-gram soil samples from farms are put into sterilized acrylic containers with the label "area date". They then travel to the Department of Parasitology at Al-Qasim Green Uni. College of Vet. Med. for molecular examinations.

Laboratory Examination

DNA extraction

In accordance with the manufacturer's instructions, DNA was extracted using the fecal lysis method with proteinase K (DNA extraction kit, Korea, Bioneer). Before being used in PCR amplification, the extracted genomic DNA (gDNA) was measured using a NanoDrop spectrophotometer (Thermo, United States) and kept at -20 °C. (Alseady and Kawan, 2019) [2].

Molecular diagnosis of *Giardia intestinalis* and *Cryptosporidium* spp by using Nested PCR (nPCR)

In order to isolate *Cryptosporidium* spp. from cow faecal samples, the nested PCR method was based on the 18S ribosomal rRNA gene. Procedure by Paul *et al.* (2009) were the faecal samples subjected to DNA extraction using an AccuPrep® stool DNA Extraction Kit (Bioneer, Korea). The forward (5-AGACGGTAGGGTATTGGCCT-3) and reverse (5-TACGAATGCCCCCAACTGTC-3) primer pairs from a Maxime™ PCR PreMix Kit (i-Taq) were used to prepare the primary PCR master mixes, which were then put into a PCR thermocycler (MyGene, Bioneer, Korea). The same procedure was used to prepare the secondary PCR master mix (forward, 5-ATTGGAGGGCAAGTCTGGTG -3; and reverse, 5-TACGAATGCCCCCAACTGTC; -3), but they were placed in separate MyGene PCR thermocyclers (Bioneer, Korea). For *Giardia intestinalis*, the exact same first PCR master mix was completed using the forward (5'-CCAA GAAGGCTAAGCGTGC-3' and reverse (5'-GGTCAAGAGCTTACAACACG-3' Insert primer pairs from a Maxime™ PCR PreMix Kit (i-Taq) and placed into a PCR Thermocycler (MyGene, Bioneer, Korea). The secondary PCR master mix was taken from the forward (5'-GCACAGAACGTGTATCTGG-3' and reverse (5'-CTCTGCTCATTGGTCTCGC-3') inserts from a Maxime™ PCR PreMix Kit (i-Taq) in the same way using a MyGene PCR Thermocycler (Bioneer, Korea).

Molecular diagnosis *Entamoeba* spp

We used particular primers for *Entamoeba* species in our PCR experiment. The National Center for Biotechnology Information (NCBI) established national guidelines, which were followed in the construction of the 18S rRNA gene primers. Next, the *Entamoeba* spp. RL2 partial 18S rRNA gene (GenBank: FR: 686362.1) was sequenced and used online using primers 3 plus (Bioneer) and the NCBI GenBank database for determination. 590 bp of the 18S rRNA gene were amplified using the primers 18S rRNA-F

(ATTGGAGGGCAAGTCTGGTG) and 18S rRNA-R (CATACTCCC CCTGAAGTCCA). Consequently, the Bioneer AccuPower®PCR Premix Kit was incorporated in the PCR mix (EL-Hamid *et al.*, 2021) [6].

Statistical Analysis

Microsoft Excel 2010 and the Statistical Package for Social Science (SPSS) for Windows program version 27 were used for statistical analysis. Using the chi-square test, the differences between the groups were assessed. P-values less than 0.05 were utilized as the significance level in all statistical analyses (Zhao *et al.*, 2022) [23].

Results

The Table 1, presents the molecular detection results of intestinal protozoa in a total of 200 agricultural soil samples. *Giardia intestinalis* was the most frequently detected parasite, identified in 71 samples (35.5% of the total samples). *Cryptosporidium* spp. were detected in 63 samples (31.5%), reflecting their strong environmental resistance. *Entamoeba histolytica* showed the lowest prevalence, with 46 positive samples (23%). Overall, these results demonstrate that molecular techniques provide a more sensitive assessment of protozoan contamination in agricultural soils.

Table 1: Molecular detection rates of intestinal protozoa in 200 soil samples

Test	<i>Giardia intestinalis</i>	<i>Entamoeba histolytica</i>	<i>Cryptosporidium</i> spp
Molecular	71 35.5%	46 23%	63 31.5%

Nested PCR

Farm soil samples were used to identify *G. intestinalis* assemblages using amplified gene products from nested PCR using primers targeting the *tpi* gene. Agarose gel electrophoresis was used for verification. Figure 1 shows a single 535 bp band for *G. intestinalis* assemblage A, while Figure 2 shows a single 470 bp band for *G. intestinalis* assemblage B.



Fig 1: Agarose gel electrophoresis image showing the polymerase chain reaction (PCR) amplification products of the *TPI* gene from *Giardia intestinalis* assemblage A detected in soil samples. (M) DNA marker ladder (100 bp-2000 bp), with the expected PCR product size of 535 bp.



Fig 2: The analysis of polymerase chain reaction products for the *TPI* gene in *Giardia intestinalis* assemblage B from soil sample was demonstrated using agarose gel electrophoresis. Marker ladder (2000-100 bp, 470 bp PCR result)



Fig 3: Agarose gel electrophoresis shows a nested PCR product analysis of the small subunit ribosomal RNA gene in *Cryptosporidium* spp. isolated from agricultural soil samples. M: Marker 2000-100 bp; lane 1-12 showing several positive *Cryptosporidium* spp. samples with a nested PCR product size of 318 bp.



Fig 4: PCR analysis of the 18S ribosomal RNA gene of *Entamoeba* species isolated from agricultural soil samples, visualized using agarose gel electrophoresis. The lane labeled “M” (marker) shows the DNA ladder ranging from 2000 to 100 base pairs, while the positive PCR product for the 18S rRNA gene appears at approximately 590 base pairs.

Phylogenetic analysis *G. intestinalis* assemblages A and B

Five isolate sequences (PX289656, PX289657, PX289658, PX289659, and PX289660) from *G. intestinalis* assemblages A and B farm soil samples the isolates of *G. intestinalis* assemblage A and B were identified using the phylogenetic

tree. As shown in Figures 5, the isolates resembled *G. intestinalis* assemblage A from Iranian stool samples (accession No. LC329330) and *G. intestinalis* assemblage B from Iranian stool samples (Accession No KY444789).

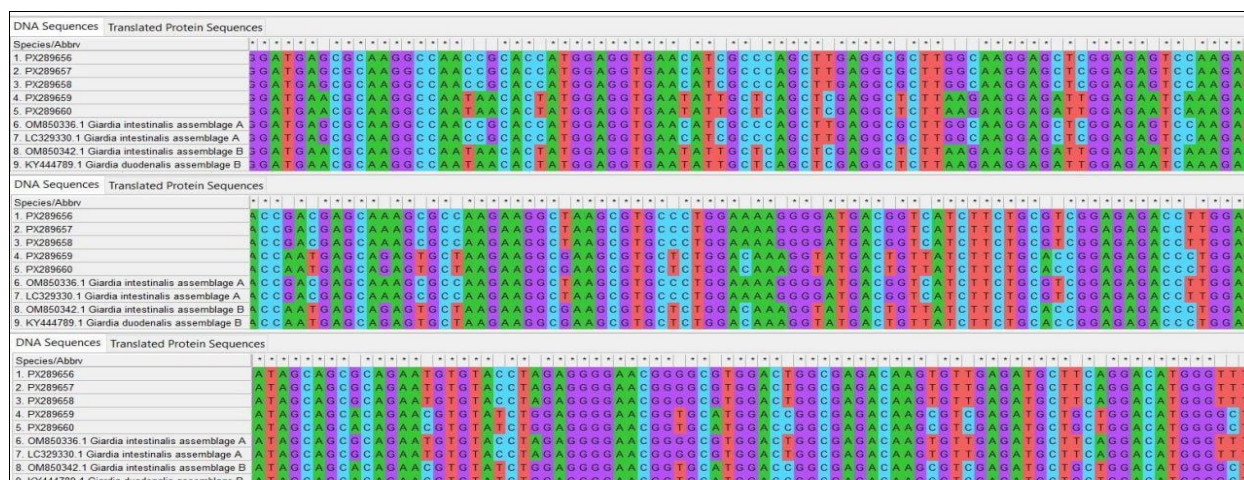


Fig 5: The figure shows a multiple sequence alignment of the *tpi* gene between local *Giardia intestinalis* IQ-soil isolates and reference genotypes from GenBank (NCBI), generated using Clustal W in MEGA 6.0. Identical nucleotides are marked with (*), and variations including substitutions and mutations across the gene are indicated

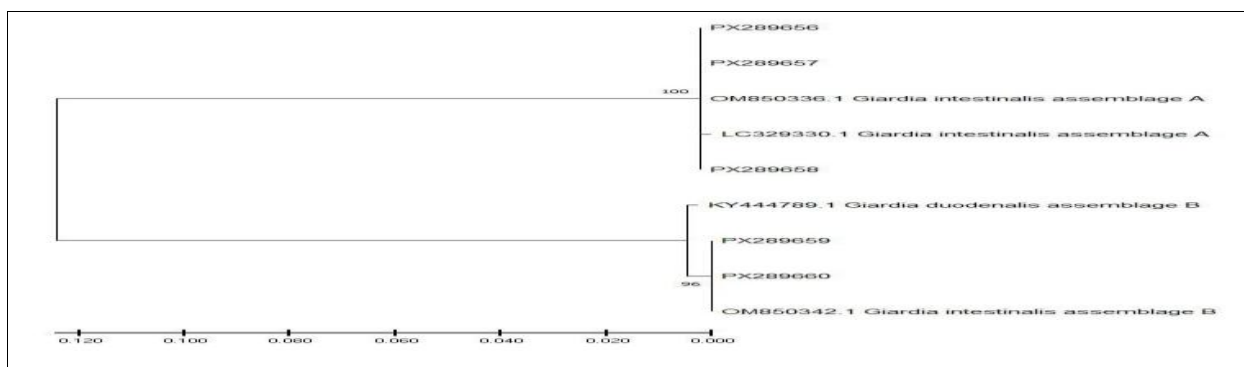


Fig 6: Using UPGMA in MEGA 6.0, a phylogenetic tree based on the partial TPI gene of *Giardia intestinalis* soil isolates was created. The isolates (PX289656-PX289660) exhibited genetic distances of 0.015-0.050% and strongly grouped with Assemblage A (LC329330.1) and Assemblage B from Iran (KY444789)

Phylogenetic analysis *Cryptosporidium* spp

Five sequences each belonging *Cryptosporidium* spp (PX279584, PX279585, PX279586, PX279587 and PX279588). The phylogenetic tree revealed the identity

between the isolates of *Cryptosporidium* spp. The isolates were similar to (Accession no: MF671873.1) as highlighted in Figures 7.

DNA Sequences	Translated Protein Sequences
Species/Abbrev	
1. PX279584	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
2. PX279585	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
3. PX279586	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
4. PX279587	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
5. PX279588	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
6. MF074687.1 <i>Cryptosporidium</i> parvum	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
7. KJ531688.1 <i>Cryptosporidium</i> andersoni	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCGT
8. KP334135.1 <i>Cryptosporidium</i> bovis	GGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTAAATTTTCGT

DNA Sequences	Translated Protein Sequences
Species/Abbrev	
1. PX279584	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
2. PX279585	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
3. PX279586	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
4. PX279587	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
5. PX279588	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
6. MF074687.1 <i>Cryptosporidium</i> parvum	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
7. KJ531688.1 <i>Cryptosporidium</i> andersoni	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA
8. KP334135.1 <i>Cryptosporidium</i> bovis	AGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAAATACCCAATCCTAATACAGGGAGGTAGTGACAAGA

DNA Sequences	Translated Protein Sequences
Species/Abbrev	
1. PX279584	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
2. PX279585	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
3. PX279586	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
4. PX279587	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
5. PX279588	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
6. MF074687.1 <i>Cryptosporidium</i> parvum	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
7. KJ531688.1 <i>Cryptosporidium</i> andersoni	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC
8. KP334135.1 <i>Cryptosporidium</i> bovis	AATTGGAATGAGTTAAGTATAAACCCTTTTCAAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCAGC

Fig 7: Shows representative partial sequence of the small subunit rRNA gene from local soil isolates of *Cryptosporidium* spp. for purposes of identifying genotypes. IQ farm soil isolates were compared to NCBI GenBank database *Cryptosporidium* spp. genotypes. The alignment of multiple sequences was performed using ClustalW in MEGA version 6.0.

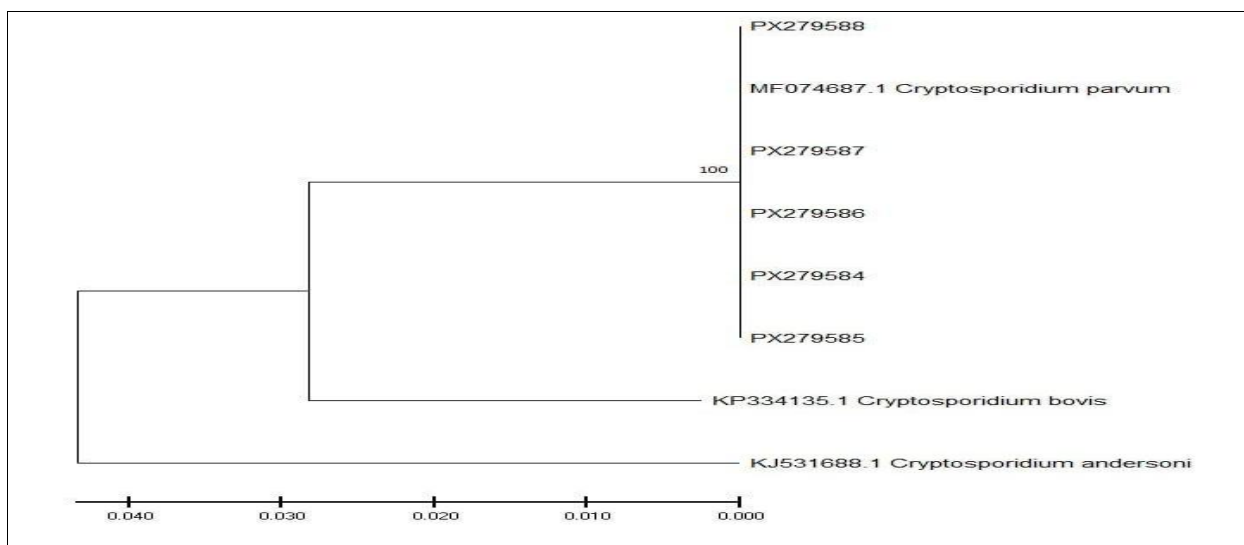


Fig 8: The partial small subunit rRNA gene was used for phylogenetic analysis of local farm soil isolates of *Cryptosporidium* spp. Under the UPGMA model in MEGA 6.0, evolutionary distances were computed using the Maximum Composite Likelihood technique. With a total genetic variation of 0.01-0.04%, the isolates (PX279584-PX279588) closely grouped with the NCBI-BLAST *Cryptosporidium* parvum isolate MF671873.1.

Phylogenetic analysis *Entamoeba histolytica*

Five sequences each belonging *Entamoeba histolytica* Phylogenetic analysis sequences each belonging (PX247889, PX247890, PX247891, PX247892 and PX247893). The

phylogenetic tree revealed the identity between the isolates of *Entamoeba histolytica*. The isolates were similar to (accession No ON724165.1) and (accession No. OM268856) for *Entamoeba dispar* as highlighted in Figures-9.

DNA Sequences Translated Protein Sequences	
Species/Abbrev	
1. PX247889	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
2. PX247890	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
3. PX247891	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
4. PX247892	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
5. PX247893	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
6. ON724165.1 <i>Entamoeba histolytica</i> (Iraq)	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
7. OM268853.1 <i>Entamoeba histolytica</i> (Iraq)	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
8. MK332025.1 <i>Entamoeba histolytica</i> (China)	TTTCACTCAACTGTGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
9. OM268859.1 <i>Entamoeba dispar</i> (Iraq)	TTTCACTCAACTGGGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA
10. MK559465.1 <i>Entamoeba dispar</i> (China)	TTTCACTCAACTGGGTCCATTAAATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCCTAACTATA

DNA Sequences Translated Protein Sequences	
Species/Abbrev	
1. PX247889	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
2. PX247890	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
3. PX247891	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
4. PX247892	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
5. PX247893	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
6. ON724165.1 <i>Entamoeba histolytica</i> (Iraq)	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
7. OM268853.1 <i>Entamoeba histolytica</i> (Iraq)	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
8. MK332025.1 <i>Entamoeba histolytica</i> (China)	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
9. OM268859.1 <i>Entamoeba dispar</i> (Iraq)	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA
10. MK559465.1 <i>Entamoeba dispar</i> (China)	TAAAAGGAACAATTGGGGTGATTCAGAAAATAACGGGAGAGGTGAAAATCCATGATCGCTATAAGATGCACGAGAGCGAAA

DNA Sequences Translated Protein Sequences	
Species/Abbrev	
1. PX247889	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
2. PX247890	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
3. PX247891	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
4. PX247892	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
5. PX247893	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
6. ON724165.1 <i>Entamoeba histolytica</i> (Iraq)	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
7. OM268853.1 <i>Entamoeba histolytica</i> (Iraq)	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
8. MK332025.1 <i>Entamoeba histolytica</i> (China)	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
9. OM268859.1 <i>Entamoeba dispar</i> (Iraq)	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA
10. MK559465.1 <i>Entamoeba dispar</i> (China)	AAGGTGTTTAAAGCAAACATTATGTTAATGAATATTCAGCATGGGACAATGCTGAGGGGATGTCAATAAGACATTTTCA

Fig 9: Segment of the small subunit rRNA gene from local *Entamoeba* spp. isolates taken from soil to use in genetic identification analysis. The IQ farm soil isolates were compared to *Entamoeba* spp. genotypes obtained from the NCBI GenBank database. A multiple sequence alignment was performed using ClustalW in MEGA version 6.0.

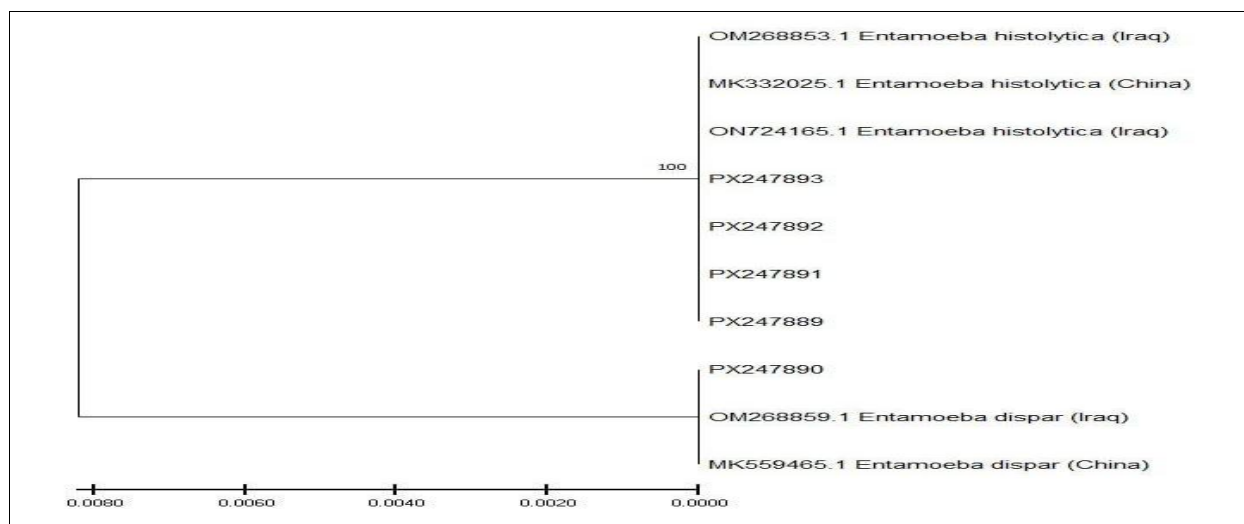


Fig 10: Phylogenetic tree of *Entamoeba* spp. from agricultural soil based on partial 18S rRNA gene sequences, using the UPGMA method in MEGA 6.0 program. Isolation (PX247889-PX247893) are closely related to *E. histolytica* (ON724165.1) and *E. dispar* (OM268856) isolates with a total genetic variation of 0.01-0.02%.

Discussion

The present study provides molecular evidence of widespread contamination of agricultural soils with intestinal protozoa, highlighting the potential public health risks associated with soil-human transmission routes. Using sensitive PCR-based methods, *Giardia intestinalis* was identified as the most prevalent protozoan, followed by *Cryptosporidium* spp. *Entamoeba histolytica* appears to be a parasite found in environmental samples and has been shown to have low levels of detection with traditional visual methods, whereas molecular diagnostic tests are capable of identifying low levels of parasitic organisms as well as any non-viable stages of the parasite that traditional microscopy cannot detect. The

high rate of detection (35.5%) of *G. intestinalis* in soil samples validates the well-studied ability of the parasite to persist in the environment and its potential to affect both humans and animals through zoonotic routes. Cysts of *Giardia* spp. may remain dormant in moist soil for several months or more and are widespread in agricultural areas due to the potential for contamination by organic materials and the use of irrigation water. The identification of both assemblages A and B in soil samples is significant because they have been shown to be responsible for human illness associated with zoonotic transmission; this suggests that humans and animals could contaminate the environment with *Giardialyticus* by direct contact with each other and through the environment.

The close genetic relationship between local samples and samples reported from other human stool samples obtained from countries nearby helps substantiate this possible method of transmission. Detection rates of the *Cryptosporidium* genus were observed to be 31.5% of the samples indicating an ability to withstand environmental pressures, given the environmental pressure placed on the organisms. *Cryptosporidium* spp. oocysts were previously discovered to have the ability to survive in the soil and in water supplies for extended periods of time as a result of their ability to endure drying, fluctuations in temperature, and the effects of ordinary chemical disinfectants (Checkley *et al.* 2015) [5]. The phylogenetic analysis indicated that the isolates clustered with *Cryptosporidium parvum*, an important zoonotic pathogen that is often at the center of causes of human crypto outbreaks. Thus, it is plausible to consider agricultural fields to serve as sources of *Cryptosporidium* transmission, especially in regions with large livestock populations and where the practice of applying manure directly to fields remains common (Zahedi & Ryan 2020) [21]. The majority portion (23%) of all detected infectious agents was *E. histolytica*, which is a low-level organism; however, its detection still has some clinical significance based on its ability to cause symptomatic disease. The genetic relationship between the organisms identified at the time of testing was determined through the use of molecular analysis. The fact that *E. histolytica* and *E. dispar* (the non-pathogenic, morphologically identical organism) were closely related, and the use of PCR methods for determining the organism's identity would not have been feasible with microscopy techniques alone are significant advantages for using PCR-based molecular methods (Tachibana *et al.*, 2012) [19]. *Entamoeba* spp. detected in agricultural soils indicates the existence of fecal contamination and unsatisfactory sanitation in the affected areas. Phylogenetic analysis of the three different protozoans all had low levels of genetic diversity, which indicates that the organisms form a tight cluster with similar reference strains. The findings point to stable patterns of transmission and potential common sources of contamination. The data emphasize the importance of incorporating molecular surveillance into standard protocols for environmental monitoring. To minimize protozoan contamination in agricultural soils, there is a need to improve methods for managing irrigation water, the use of animal manure, and sanitation practices. Therefore, it's crucial reduce the chances of transmitting protozoans from food production to humans through improved management of these aspects (Robertson *et al.*, 2014) [16].

Acknowledgments

The authors would like to thank Al-Qasim Green University's College of Veterinary Medicine for its laboratory facilities and technical assistance.

Funding: The authors no receive funding

Conflict of interest: No conflict of interest

Financial Support

Not available

Reference

1. Al-Difaie RS. Molecular study to detect genotyping of *Giardia lamblia* from human and cattle feces in Al-Qadisiya Governorate, Iraq. *Ibn Al-Haitham J Pure Appl*

- Sci. 2016;29(3):1-13.
2. Alseady HH, Kawan MH. Prevalence and molecular identification of *Cryptosporidium* spp. in cattle in Baghdad province, Iraq. *Iraqi J Vet Sci.* 2019;33(2):389-94.
3. Blackburn CC, Yan SM, McCormick D, Herrera LN, Iordanov RB, Bailey MD, *et al.* Parasitic contamination of soil in the Southern United States. *Am J Trop Med Hyg.* 2024;111(3):506.
<https://doi.org/10.1016/j.molbiopara.2008.04.006>
4. Cacciò SM, Ryan U. Molecular epidemiology of giardiasis. *Mol Biochem Parasitol.* 2008;160(2):75-80.
5. Checkley W, White AC, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, *et al.* A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *Lancet Infect Dis.* 2015;15(1):85-94.
[https://doi.org/10.1016/S1473-3099\(14\)70772-8](https://doi.org/10.1016/S1473-3099(14)70772-8)
6. El-Hamid ABD, Aiat A, Din SEMI, Azzam MI, Amer AS, Ghobashy MA. Distribution of enteric protozoa in the River Nile drains, Egypt using field and remote sensing studies. *J Egypt Soc Parasitol.* 2021;51(1):153-162.
7. Feng Y, Xiao L. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in humans. *Clin Microbiol Rev.* 2017;30(1):67-112.
<https://doi.org/10.1128/CMR.00019-16>
8. Ghazy RM, Alshaikhi SA, Assiri HAH, Almozaini AA, Alhazmi AF, Elhasaneen HEM, *et al.* Tropical diseases and the gastrointestinal tract: An overlooked connection. *Front Trop Dis.* 2025;6:1612952.
9. Kihara R, Mbugua A, Githigia SM, Kamuti NM, Munde BM. Prevalence and risk factors associated with zoonotic gastrointestinal parasites of dogs in Kitui Central Sub-County, Kitui, Kenya. *PAMJ One Health.* 2025;17(8).
10. Marhoon IA, Al-Aredhi HS. The effect of intestinal protozoan infections on hematological parameters and micronutrients in colorectal cancer patients. *J Parasit Dis.* 2025:1-7.
11. Mejia R, Slatko B, Almazan C, Cimino R, Krolewiecki A, Duran NM, *et al.* Molecular testing of environmental samples as a potential source to estimate parasite infection. *Trop Med Infect Dis.* 2024;9(10):226.
12. Mo Z, Quan J, Xu B, Yu H, Li J, Luo X, *et al.* A One Health study on phylogenetics and risk of pathogenic intestinal parasites at a ranch in Inner Mongolia. *One Health.* 2024;19:100912.
13. Namkuna HN. Prevalence and factors associated with gastrointestinal parasitic infestation among children presenting with acute diarrhea at Dodoma Regional Referral Hospital, Tanzania [Master's Thesis]. Dodoma: University of Dodoma; 2024.
14. Paul J. Gastrointestinal tract infections. In: Disease causing microbes. Cham: Springer International Publishing; 2024, p. 149-215.
15. Paul S, Chandra D, Tewari AK, Banerjee PS, Ray DD, Raina OK, Rao JR. Prevalence of *Cryptosporidium andersoni*: a molecular epidemiological survey among cattle in India. *Vet Parasitol.* 2009;161(1-2):31-5.
16. Robertson LJ, Sprong H, Ortega YR, van der Giessen JWB, Fayer R. Impacts of globalisation on foodborne parasites. *Trends Parasitol.* 2014;30(1):37-52.
<https://doi.org/10.1016/j.pt.2013.09.005>
17. Rossi F, Santonicola S, Amadoro C, Marino L, Colavita G. Food and drinking water as sources of pathogenic

- protozoans: An update. Appl Sci. 2024;14(12):5339.
18. Ryan U, Paparini A, Monis P, Hijjawi N. It's official- *Cryptosporidium* is a genus with multiple species. Trends Parasitol. 2019;35(7):503-7.
<https://doi.org/10.1016/j.pt.2019.04.012>
 19. Tachibana H, Yanagi T, Akatsuka A, Kobayashi S, Kanbara H, Takeuchi T. Differentiation of pathogenic *Entamoeba histolytica* and non-pathogenic *Entamoeba dispar* by PCR. J Clin Microbiol. 2012;50(9):3125-30.
<https://doi.org/10.1128/JCM.01253-12>
 20. World Health Organization. Guidelines on sanitation and health. Geneva: World Health Organization; 2017.
 21. Zahedi A, Ryan U. *Cryptosporidium*-an update with an emphasis on foodborne and waterborne transmission. Res Vet Sci. 2020;132:500-512.
<https://doi.org/10.1016/j.rvsc.2020.08.015>
 22. Zeng J, Nakanishi T, Itoh S. Two-year monitoring of microbiological water quality in small water supply systems: implications for microbial risk management. Environ Manage. 2024;74(2):256-267.
 23. Zhao ZY, Li MH, Lyu C, Meng XZ, Qin YF, Yang XB, et al. Prevalence of *Giardia duodenalis* among dogs in China from 2001 to 2021: A systematic review and meta-analysis. Foodborne Pathog Dis. 2022;19:179-91.

How to Cite This Article

Kareem SM, Musawi AM AL. Molecular detection of some intestinal protozoa isolation from farm soil. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(12):433-439.

Creative Commons (CC) License

This is an open-access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.