COMPREHENSIVE STUDY ON PARASITIC CONTAMINATION IN HOUSE FLIES: MOLECULAR DETECTION AND EPIDEMIOLOGICAL SIGNIFICANCE

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ABSTRACT

House flies (*Musca domestica*) are ubiquitous vectors of pathogens, including intestinal parasites, posing significant public health risks. This study aimed to investigate the prevalence of parasitic contamination in house flies collected from various sites in Thi-Qar province, Iraq, using direct microscopic examination and molecular techniques. A total of 500 flies were collected from butchers, fish and chicken shops, garbage sites, vegetable stores, and residential houses. Direct microscopy revealed that 60% (300/500) of flies were contaminated with parasites, while 40% (200/500) were parasite-free. The external surfaces of flies showed higher contamination (41%) compared to internal contents (19%). Molecular detection via conventional PCR targeting the small subunit ribosomal RNA (SSU rRNA) gene was employed for *Entamoeba histolytica*, *Giardiaintestinalis*, *Microsporidium* sp., *Cryptosporidium* sp., *Cyclospora* sp., and *Cystoisospora* sp. The most prevalent parasite was *E. histolytica* (36%), followed by *Microsporidium* sp. (24.7%) and *Cryptosporidium* sp. (17%). This study highlights the role of house flies in transmitting parasitic infections and underscores the need for improved sanitation and vector control measures in urban and periurban environments.

Keywords:

House flies, *Musca domestica*, intestinal parasites, PCR, SSU rRNA, public health, epidemiology

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INTRODUCTION

House flies (*Musca domestica*) are among the most pervasive mechanical vectors of human pathogens, playing a **crucial** role in the transmission of bacterial, viral, and parasitic infections, particularly in regions with inadequate sanitation and waste management systems [1, 2, 3]. Their close association with decaying organic matter, faecal matter, and unsanitary environments makes them efficient carriers of enteric pathogens, including *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium*spp., *Microsporidium* spp., *Cyclospora* spp., and *Cystoisospora* spp., which are responsible for significant morbidity and mortality worldwide, especially in developing countries [4, 5]. *Cryptosporidium parvum* is a widespread opportunistic parasite belonging to the family Apicomplexa that completes its life cycle in a single host and is frequently found in immunocompromised persons [6]. The mechanical transmission of these parasites occurs through the adherence of pathogens to the fly's exoskeleton, regurgitation during feeding, or deposition of contaminated faecal matter on food and surfaces [7]. Epidemiological studies have demonstrated that house flies can harbor multiple parasitic species simultaneously, increasing the risk of co-infections in humans [8]. Traditional diagnostic methods, such as direct microscopy, though widely used, suffer from limitations in sensitivity and specificity, particularly in detecting low parasite loads or differentiating between morphologically similar species [9]. Molecular techniques, and polymerase chain reaction (PCR) in particular, targeting conserved genomic regions such as the small subunit ribosomal RNA (SSU rRNA) gene, have revolutionized parasitic detection by offering higher accuracy, specificity, and the ability to identify species and genotypes [10]. Additionally, zoonotic potential, and evolutionary relationships among parasite strains, which are crucial for understanding transmission dynamics and designing targeted control measures [11]. Despite these advancements, there remains a gap in comprehensive studies integrating microscopy, molecular diagnostics, and phylogenetic analysis to assess the role of house flies in parasitic transmission in urban and periurban settings, particularly in regions like Thi-Qar, Iraq, where environmental contamination and poor sanitation are prevalent [12]. This study aims to bridge this gap by evaluating the prevalence of parasitic contamination in house flies collected from diverse environments, comparing the efficacy of direct microscopy with molecular techniques. The findings will contribute to a deeper understanding of the epidemiological significance of house flies in parasitic disease transmission and inform public health strategies aimed at reducing

fly-borne infections through improved sanitation, waste management, and vector control interventions [13]. **The distribution of infection depends on residence, which was 20.7% for rural and 12.2% for urban [**14].

Materials and Methods

Sample Collection

A total of 500 house flies were collected from five sites in Thi-Qar province, Iraq:

- Butcher shops (n = 91)
- Fish and chicken shops (n = 103)
- Garbage sites (n = 92)
- Residential houses (n = 111)
- Vegetable stores (n = 103)

Flies were captured using sterile nets, stored in 70% ethanol, and transported to the laboratory for analysis.

Collection Methods

The flies were caught directly in collection bottles and transferred to the laboratory for further processing, which consisted of the following steps: placing the flies in the refrigerator for 3 minutes at a temperature of 0º C to inactivate them, then isolating the parasites from the flies, as shown below.

Isolation of intestinal parasites from external surface of *M. domestica* flies

The collected flies were transferred into labelled sterile specimen bottles that carried information such as date and location of the isolate. Flies killed by deep freezing were immersed in 5 ml of normal saline in a sterile test tube, and gently shaken for three minutes. Immediately, the solution was transferred into conical tubes and centrifuged at 3000 rpm for 5 minutes [15].

After discarding the supernatant, smears were prepared from the sediment on glass slides, mixed with one drop of *Lugol's iodine solution*, and examined by light microscope. Slides were prepared in three replicates for each sample to increase the chance of parasite detection. The stages of parasites were identified based on their morphological characteristics as described by other researchers [16, 17].

Isolation of intestinal parasites from the digestive tract of *M. domestica*

The outer surface of the fly samples was initially sterilized by washing them with ethyl alcohol at a concentration of 70% to eliminate external parasites. Then the digestive tract of each fly was dissected out under a microscope using needles and forceps. The smears prepared from the collected internal contents were stained by the modified Ziehl-Neelsen technique and examined using light microscopy to isolate and identify parasites [18].

Direct Microscopic Examination

Each fly was dissected to separate its external surface from its internal content. Samples were stained with iodine solution and examined under a light microscope at 400 × magnification. The microscopic diagnosis of microsporidia typically relies on specific staining methods (Giemsa stain), which allow for the visualization of the standard morphological criteria.

Genomic DNA Extraction

Genomic DNA was extracted from both external and internal fly samples using the **Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan)**, following the manufacturer's instructions. DNA concentration and purity were measured using a **NanoDrop spectrophotometer (Thermo Scientific, UK)**.

PCR Amplification

Conventional PCR was performed to detect *Entamoeba histolytica*, *Giardia intestinalis*, *Micro sporidium* sp., *Cryptosporidium* sp., *Cyclospora* sp., and *Cystoisospora* sp., targeting the small subunit ribosomal RNA (SSU rRNA) gene. Positive samples were confirmative for infections, particularly for *microspoirdia* parasites, testing for the small subunit ribosomal RNA (SSU-rRNA) gene using conventional PCR. The primers used are listed in Table 1.

The PCR reaction was performed in a total volume of

Table 1: Primer sequences and product sizes for PCR detection of intestinal parasites

Parasite	Primer Sequence (5′–3′)	Product Size (bp)	GenBank Accession
Entamoeba histolytica	F: CTGGTTGATCCTGCCAGTATT R: CACCAGACTTGCCCTCCAAT	555	AB282658.1
Giardia intestinalis	F: GAATCAGGGTTCGACTCCGG R: CGTTTACGGCCGGGAATACG	504	U09492.1
Microsporidium sp.	F: GATAGCTCCCACGTCCAAGG R: CTCCTTGTGGTGTAGCTCCG	522	PP625131.1
Cryptosporidium sp.	F: GAGTATGGTCGCAAGGCTGA R: TGTGTACAAAGGGCAGGGAC	530	AY237630.1
Cyclospora sp.	F: AGTTGGATTTCTGCTGTGGTCA R: TCCCTATCTCTAGTCGGCATAGT	423	PQ835031.1
Cystoisospora sp.	F: GCGGTAATTCCAGCTCCAATAG R: GCCCCTAACTTTCGTTCTTGATTA	418	LC377842.1

20 µL, containing 5 µL of DNA template, 2 µL of each forward and reverse primer (10 pmol/µL), 12.5 µL of GoTaq® Green Master Mix, and 3.5 µL of nuclease-free water. Amplification was carried out under the following conditions: an initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 1 minute; with a final extension at 72°C for 5 minutes and an indefinite hold at 4°C.

PCR Product Analysis

PCR products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide, run at 100 V and 80 mA for 1 hour in 1× TBE buffer. A 100 bp DNA ladder was used as a size marker. Gels were visualized under UV light using a transilluminator.

Statistical Analysis

Data were analyzed using SPSS version 26. Chi-square tests were applied to determine associations between parasite prevalence and collection sites, with a significance level of $p < 0.05$.

Results

Prevalence of parasites in house flies

A total of five hundred flies were collected from butchers, fish and chicken shops, garbage of houses, vegetable stores, and house from Thi-Qar province .

The present study showed that 60.0% (300) of flies examined carried a parasite, as shown in Figures 1

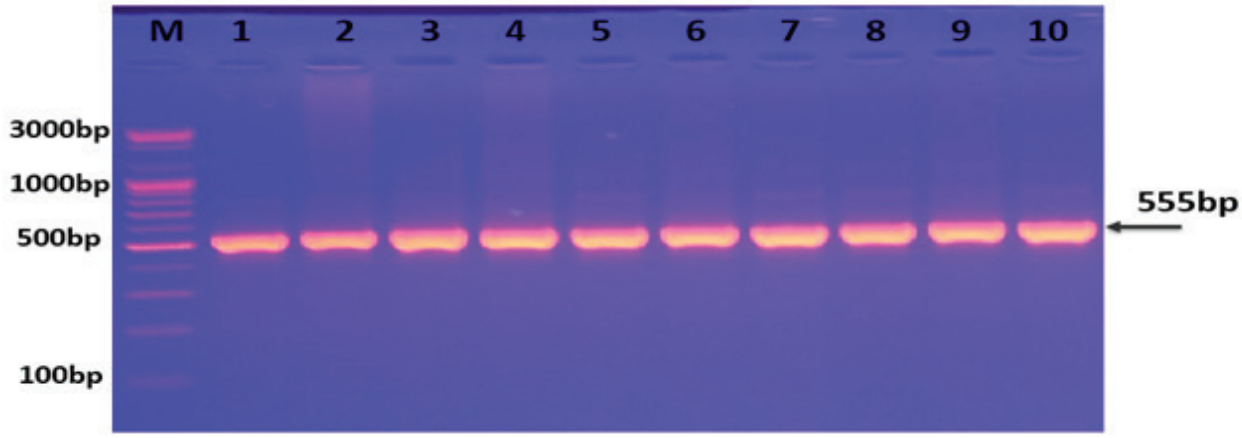

Figure 1: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp)**.** Lanes 1-10**:** some positive for *Entamoeba histolytica* samples with the expected product size of 555 bp.

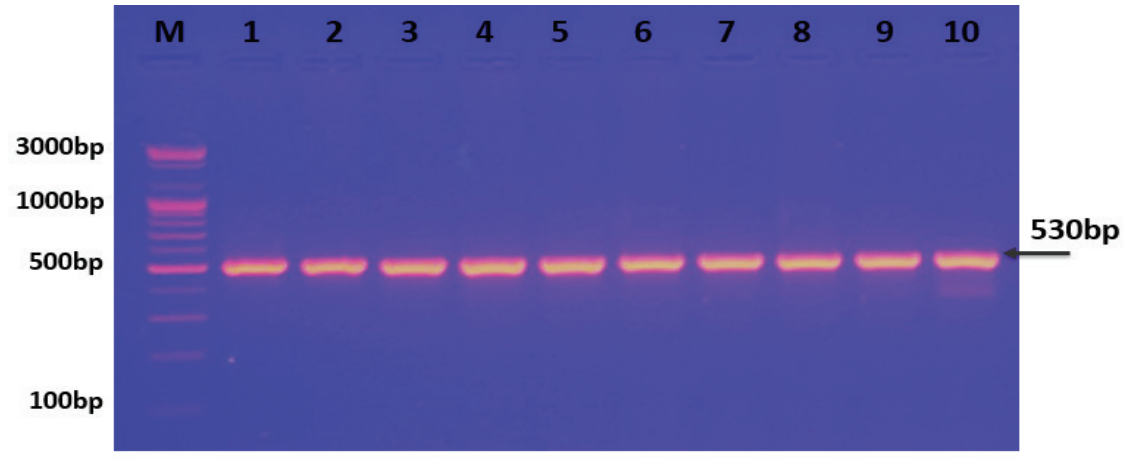

Figure 2: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp). Lanes 1-10: some positive for *Cryptosporidium sp.* samples with the expected product size of 530bp.

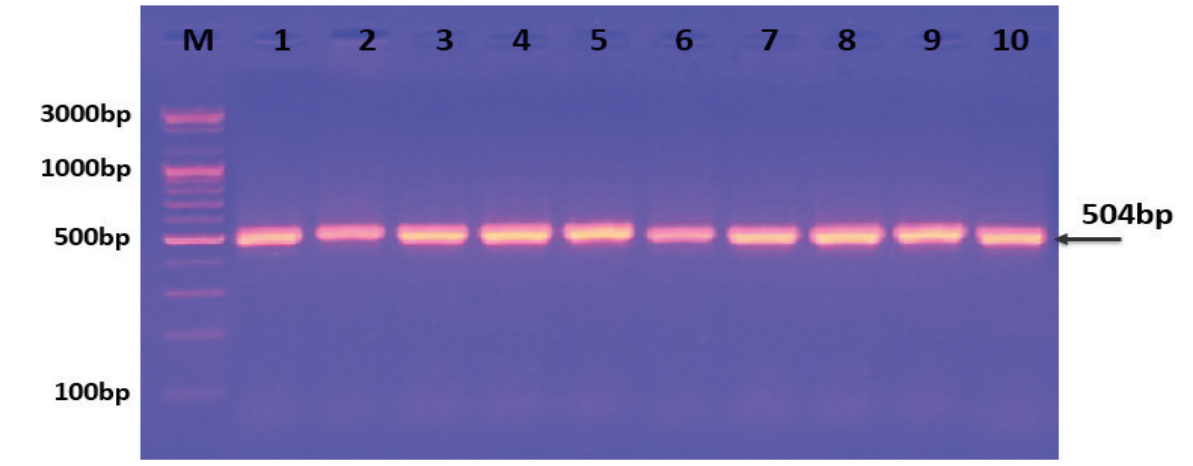

Figure 3: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp)**.** Lanes 1-10**:** some positive for *Giardia intestinalis* samples with the expected product size of 504bp.

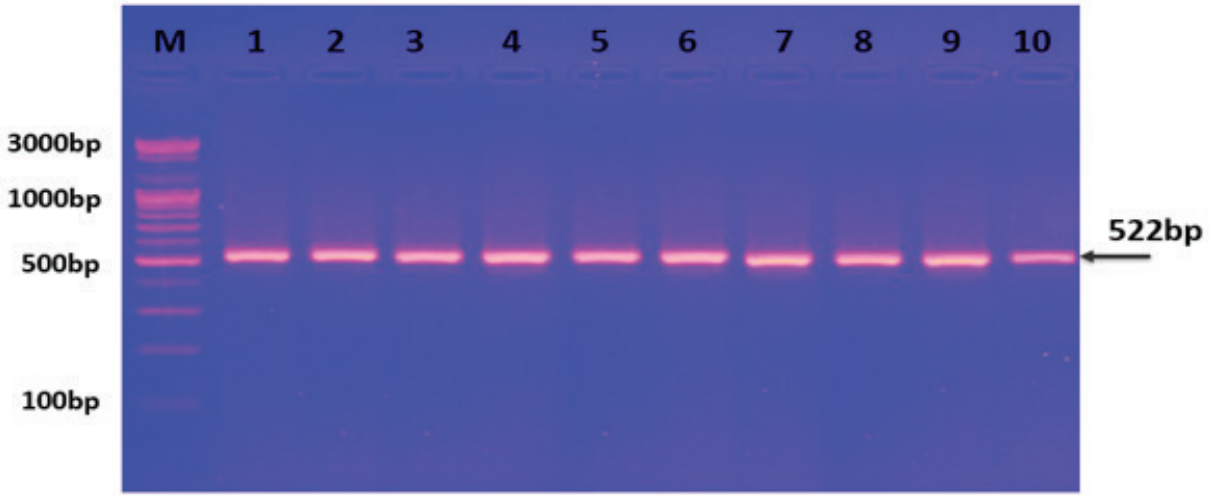

Figure 4: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp)**.** Lanes 1-10**:** some positive for *Microsporidium sp.* samples with the expected product size of 522 bp.

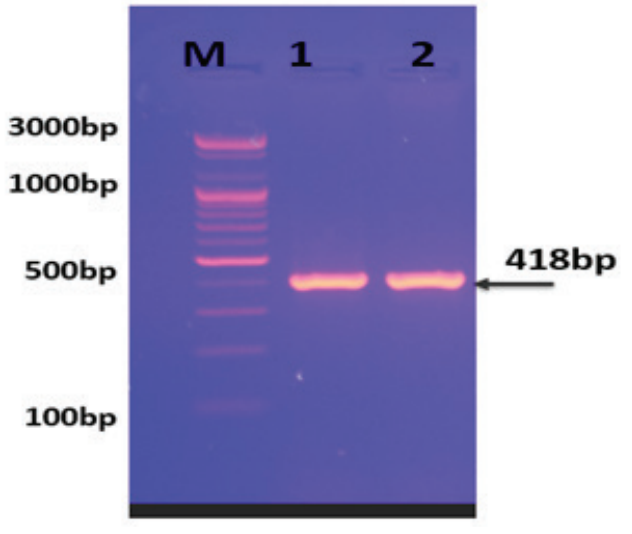

Figure 5: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp)**.** Lanes 1-2**:** some positive for *Cystoisospora sp.* samples with the expected product size of 418 bp.

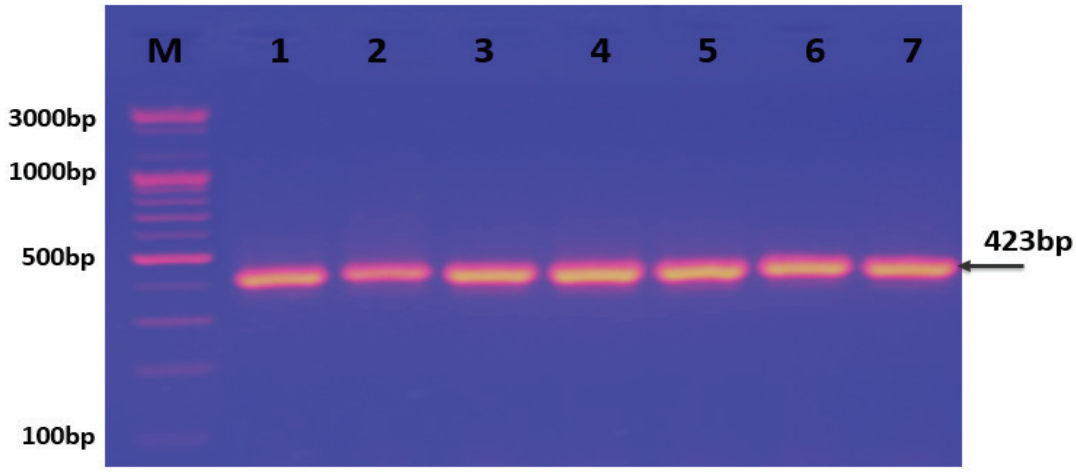

Figure 6: Agarose gel electrophoresis image showing the separation of PCR products from house fly samples examined. Lane M: DNA marker ladder (3000-100bp)**.** Lanes 1-7**:** some positive for *Cyclospora sp*. samples with the expected product size 423bp.

2. Collection of House flies and estimation of parasite infestation

A total of five hundred flies were collected from butchers, fish and chicken shops, garbage of houses , vegetable stores from Nasiriyah distract in Thi-Qar province. The present study showed that 300 (60.0%) of the flies carried a parasite, while 200 (40.0%) were not parasite carriers, as shown in figure (7).

Estimation of parasite infestation of house flies according to the site of isolation

The present study showed that more parasites were isolated from fly external surfaces (41.00%) than from their internal organs (19.00%).

Overall, 40% of the fly specimens examined (16.40% of samples from the external surfaces and 23,60% of samples from the internal organs of flies) were negative for parasites. Chi-squared test conducted showed a statistically significant difference in parasite prevalence between the two isolation sites – flies' external surface and internal organs, 41% vs. 19% (p-value ≤ 0.001, Table 2).

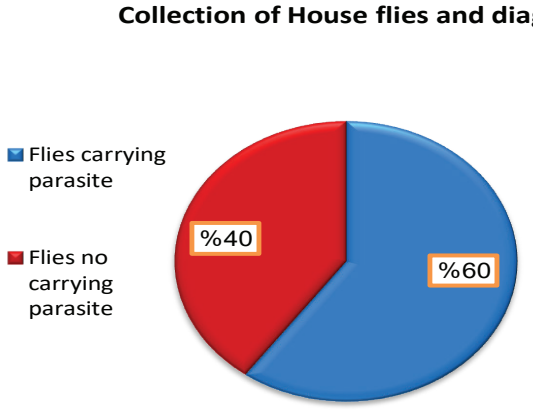

Figure 7: Collection of house flies and carrer state evaluation

Estimation of intestinal parasite infestation of house flies according to place of collection

According to the collection place, flies with the highest number of parasite loads (13.60%) were detected among those collected from house garbage (13.6%), followed by butchers, 12.40% . Of the flies captured in the houses, only 10.40% harboured intestinal parasites, and this group represented the largest number of negative samples (11.80%), as well (Table 3). Thus, the fly collection place, to some extent, determined the parasite carriage rate. This was confirmed by the presence of a statistically significant difference (p-value ≤ 0.001) according to the Chi-squared test applied.

Distribution of isolated intestinal parasite from flies according to study sites (from the external surfaces and digestive tracts)

The present study showed that of the infested flies, a total of six protozoan parasites were isolated (Table 4). The most prevalent parasite in both external surfaces and internal digestive tracts were cysts of *E. histolyt-*

Table 2: Distribution of parasite load in house fly specimens according to the site of isolation

Total		Negative		Positive		Site of isolation
%	No.	%	No.	%	No.	
57.40	287	16.40	82	41.00	205	External surface
42.60	213	23.60	118	19.00	95	Internal organs
100	500	40.00	200	60.00	300	**Total**
Cal X2 = 36.664 df= 1 Sig. = 0.001						

ica (23.67%, n=71 and 12.33%, n=37, respectively), followed by spores of *Microsporidium* spp. **(**16.33%, n=49 and 8.33%, n=25, respectively), and *oocysts of Cryptosporidium* spp. (12.33%, n=37 and 4.67%, n=14, respectively). In our study, the pathogen that was isolated very rarely from internal organs was *Cystoisospora* spp. (0.67%, n=2). Based on the statistical analysis the results obtained were statistically non-significant (p≤ 0.05), (Table 4).

Diagnosis of Isolated parasite from Flies

The present study showed that the most frequently isolated parasite was *E. histolytica* (36%), followed by *Microsporidium* (24.67%) and *Cryptosporidium* (17.00%), while the least frequently isolated parasite was *Cystoisospora* (4.67%), as shown in figure (8).

DISCUSSION

The findings of this study underscore the significant role of house flies (*Musca domestica*) as mechanical vectors of intestinal parasites, with a contamination rate of 60% (300/500) among collected flies, aligning with previous reports from similar regions [19]. The higher prevalence of parasites on external surfaces (41%) compared to internal organs (19%) suggests that flies primarily acquire pathogens through contact with contaminated substrates, supporting the hypothesis that mechanical transmission via surface adherence is a dominant

Table 3: Intestinal parasite carriage rates of house flies according to place of collection

Total		Negative		Positive		Collection Place
%	No.	%	No.	%	No.	
18.4	92	4.80	24	13.60	68	**House garbage**
20.60	103	8.80	44	11.80	59	**Vegetable stores**
20.60	103	8.80	44	11.80	59	**Fish and chicken shops**
22.20	111	11.80	59	10.40	52	**Houses**
18.20	91	5.80	29	12.40	62	**Butchers**
100	500	40.00	200	60.00	300	**Total**
CalX2 = 18.563 df= 4 **Sig. = 0.001**						

Table 4: Isolation rate of intestinal parasites from captured house flies according to sampling sites

Total		Digestive tracts		External surfaces		Direct microscopy Parasites isolated
%	No	%	No	%	No	
36.00	**108**	**12.33**	**37**	**23.67**	**71**	*E. histolytica*
24.67	**74**	**8.33**	**25**	**16.33**	**49**	*Microsporidium* spp.
7.67	**23**	**2.33**	**7**	**5.33**	**16**	*Cyclospora* spp.
17.00	**51**	**4.67**	**14**	**12.33**	**37**	*Cryptosporidium* spp.
10.00	**30**	**3.33**	**10**	**6.67**	**20**	*Giardia lamblia*
4.67	**14**	**0.67**	**2**	**4.00**	**12**	*Cystoisospora* spp.
100	**300**	**31.67**	**95**	**68.33**	**205**	**Total**
CalX2 = 2.917 df= 5 Sig. = 0.713						

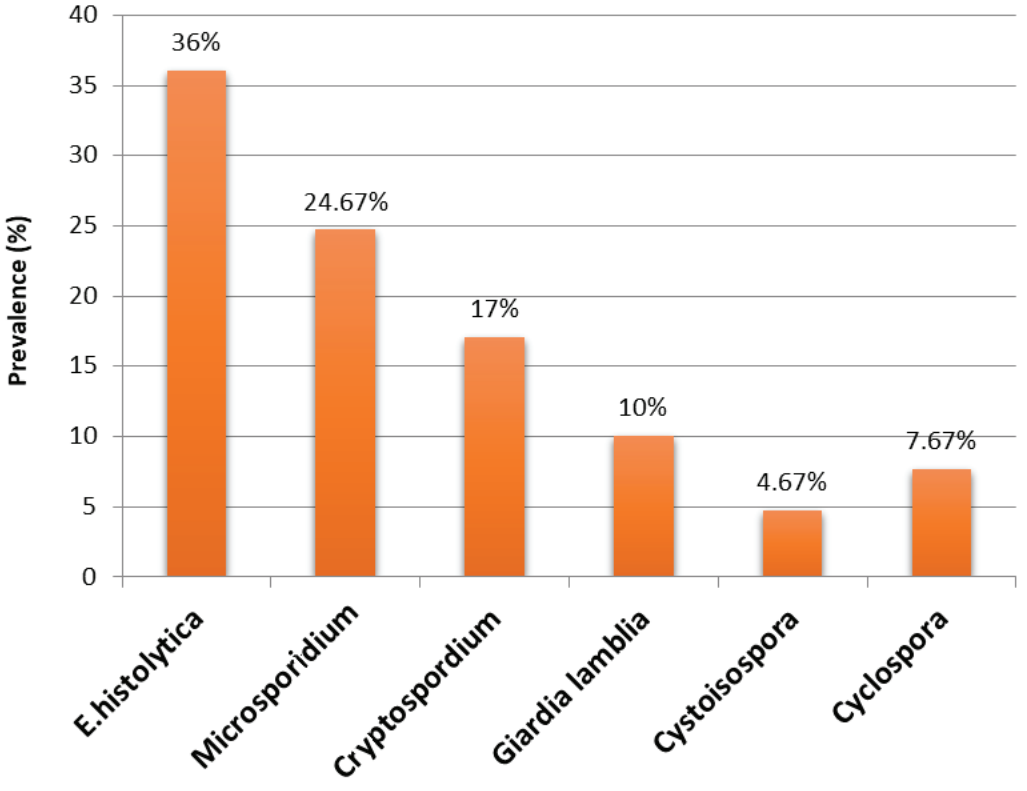

Figure 8: Prevalence of studied parasite species in infested flies.

route [20]. This observation is consistent with studies by Nazni et al. (2005), who reported that flies feeding on faecal matter and decaying organic waste accumulate parasites on their legs and bodies, facilitating passive transfer to human food and water sources [1]. The predominance of *Entamoeba histolytica* (36%) in this study corroborates earlier findings in Iraq, where poor sanitation and limited access to clean water contribute to high rates of amoebiasis [12,21,22]. The detection of *Cryptosporidium* spp. (17%) and *Microsporidium* spp. (24.7%) further highlights the zoonotic potential of fly-borne parasites, as these pathogens are known to infest both humans and animals, with transmission occurring through contaminated environments [9].

Molecular techniques, particularly PCR targeting the SSU rRNA gene, demonstrated superior sensitivity compared to microscopy, detecting low-abundance parasites that were missed by conventional methods [12]. For instance, nested PCR for *Giardia intestinalis* improved detection limits, confirming its utility in epidemiological surveys where false negatives are a concern [11]. These findings align with global trends where molecular tools have redefined parasitic diagnostics, enabling precise identification and tracking of emerging strains [23, 24].

The statistical analysis revealed significant differences in parasite distribution across collection sites ($p<0.001$), with garbage sites and butcher shops showing the highest contamination rates (13.6% and 12.4%, respectively). This pattern reflects the role of unsanitary conditions in facilitating fly breeding and pathogen acquisition [5]. The lower prevalence in residential houses (10.4%) may be attributed to better hygiene practices, though the presence of parasites still indicates ongoing transmission risks [8]. These results emphasize the need for targeted interventions, such as improved waste disposal, insecticide-treated fly traps, and community education on hygiene, to reduce fly populations and associated infections [1]. The study's limitations include the reliance on a single geographic region and the potential underestimation of parasite diversity due to primer specificity. Future research should expand to multiple regions, incorporate metagenomic sequencing for broader pathogen detection, and assess fly resistance to common disinfectants [9]. Despite these limitations, the integration of microscopy, and molecular diagnostics provides a robust framework for understanding fly-borne parasitic transmission and informing evidence-based control strategies [12].

The most commonly isolated parasite was *E. histolytica* (36%), while the least frequently isolated one was *Cystoisospora* sp. (4.67%). This is in agreement with the result of Al-Diwaniya Province, Iraq [17]; Maiduguri metropolis in Nigeria [26] and with the result of Infested Slaughter houses in Khartoum State, Sudan [5]. The majority of the identified intestinal parasites originated from human faeces [27]. This was also observed in Egypt in areas where people defecated in the open [26], The possibility of transmission of parasites remains a cause for concern wherever infestation of parasites in the community is high, particularly in those areas where open field defecation is regularly practiced [15]. These results are consistent with the studies reporting the isolation of parasites from the housefly and indicating a relatively high infectivity rate in the disease vectors. House flies appeared to be a potential mechanical vector of intestinal protozoa in Sebha city and might be in other parts of Libya [28]. Among these studies, almost all parasites were isolated from the body surfaces of the flies. In the study of Eke at al. (2016) 7 parasite species were isolated and identified from *M. domestica* [29].

These results are consistent with previous studies [17] at Al-Diwaniya Province [30], where a higher percentage of parasites from the external surface were isolated, as compared to the contamination in the digestive system of the flies examined.

CONCLUSION

House flies (*Musca domestica*) in Thi-Qar province, Iraq, are significant vectors of a variety of intestinal parasites, with *E. histolytica*, *Microsporidium* spp., and *Cryptosporidium* spp. being the most prevalent. The study underscores the high contamination rates of house flies, particularly on their external surfaces, highlighting the potential risk they pose in spreading parasitic infections.

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