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Microscopic and Molecular Diagnosis of *Ascaris lumbricoides* in Human of Babylon Governorate

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Abstract **Original Research Article**

Human ascariasis is caused by the soil-transmitted helminth (STH) *Ascaris lumbricoides.* The disease found all across the world, with particularly high incidence in tropical and subtropical regions, as well as Iraq. In the current investigation, 200 stool samples were collected between January 2024 and January 2025 from four distinct in the province of Babylon in order to isolate and identify *Ascaris lumbricoides.* 200 stool samples for traditional diagnosis by (salt floatation, Kato-Katz technique) and 100 samples for Molecular Diagnosis. The prevalence rate was 13% (26/200), 21% (21/100) by traditional and Molecular Diagnosis respectively, according to sex was higher in males (28%) than females (14%) and the age groups 5-10 recorded the highly in male and female (50%), (42.8) , the area of study recorded heights in Al –Kifl was 28% (7/25), seasonal show Summer had the greatest infection rate (32%) and winter had the lowest (12%), Ten sequences analysis each belonging of *Ascaris lumbricoides* Phylogenetic tree (PX418260 ,PX418261, PX418262, PX418263, PX418264, PX418265, PX418266, PX418267, PX418268 and PX418269).

Keywords: Soil-transmitted helminth, molecular detection, Kato-Katz technique, *Ascaris lumbricoides*, iraq.

INTRODUCTION

Ascariasis, caused by the large intestinal roundworm Ascaris lumbricoides, is still the most common soil-transmitted helminth (STH) (Pal *et al.,* 2025). Infection worldwide, affecting an estimated one billion people, particularly in tropical and subtropical countries with inadequate sanitation (Badri *et al.,* 2025). The parasite is spread by the consumption of infective eggs present in contaminated soil, food, or water (Bowman, 2021). Upon ingestion, larvae hatch in the gut, move through the liver and lungs, and subsequently return to the small intestine to grow into adult worms (Devi, 2022). Children are especially affected by ascariasis because of their growing immune systems and increasing exposure to polluted settings (Fauziah *et al.,* 2022). Heavy infections can result in serious morbidity, such as intestinal blockage, malnourishment, development retardation, and respiratory symptoms during larval migration, whereas small worm loads might be asymptomatic (Holland *et al.,* 2022). Ascariasis is a neglected tropical disease that has a substantial global impact, with millions of individuals afflicted annually, according to the World Health Organisation (WHO) (Debbarma *et al.,* 2023). While current molecular approaches offer improved sensitivity and specificity, microscopic identification of distinctive eggs in stool samples is still the standard method for diagnosing *Ascaris lumbricoides* infection (Miswan *et al.,* 2022). Improved sanitation, safe food handling, health education and recurring deworming programs in endemic areas are essential for effective prevention (Khan *et al.,* 2025). The purpose of this study is to investigate the rate of *Ascaris lumbricoides* infection among the population of the Babylon province, based on both microscopic analyses and molecular diagnostics. The primary focus of this research is to compare the precision and sensitivity of conventional parasitological approaches as opposed to novel molecular techniques, which will further increase the probability of early identification, enhance the level of epidemiological knowledge and strengthen the preventive measures for ascending species in the area.

MATERIALS AND METHODS

Stool samples collection

A total of 200 stool samples were collected between January 2024 and January 2025 from various areas of Babylon hospital container with the date of age, sex, and region. Afterward, the stool samples were sent to the Department of Parasitology, at Al-Qasim Green University, College of Veterinary Medicine for both molecular and microscopy analyses.

Sample examined Methods

The abundance of Ascaris lumbicoides eggs was determined and detected using microscopy. The eggs were obtained from the Kato-Katz process (Taravider *et al.,* 2020). This technique is used to quantify thick fecal smears for the detection of intestinal worms resulting from environmentally acquired parasites, such as Ascaris lumbicoides (Lutz *et al.,* 2025). The flotation technique was used to isolate the eggs from the sample, providing high morphological variation (shape, dimensions, appearance), which is essential for assessing genetic variation (Rahmat *et al.,* 2023).

Flotation methods

The solution Zinc Sulphate using to investigate eggs of parasite to (Kareem and Kawan, 2020).

DNA extraction

The method for extracting DNA from faeces was based on the use of Proteinase K, as specified by the manufacturer using the Stool DNA Extraction Kit from Bioneer. The obtained gDNA was quantified using a Thermo NanoDrop spectrophotometer and stored at minus 20 degrees Celsius until needed for PCR amplification (Alseadyand Kawan, 2019).

Molecular diagnosis

Specific primers for *Ascaris lumbricoides* were utilized in this PCR experiment. The 5.8S rRNA partial sequence gene primers were created using national guidelines. ITS1 F (5′-CTTGAACCGGGTAAAAGTCG-3′) and ITS1 R (5′-ATGTGTCTGCAATTCGCACT-3′) primers were used to amplify about 500 bp of the ribosomal internal transcribed spacer (ITS) region (Dos Santos *et al.,* 2022).

Statistical Analysis

Statistical Package for Social Science (SPSS) version 27 for Windows software were used for statistical analysis. The chisquare test was used to evaluate group differences. The P-value below the 0.05 threshold was taken into account in all of these statistical analyses (Zhao *et al.,* 2022).

RESULTS

The purpose of this study comparative between Molecular diagnosis and traditional diagnosis by (salt floatation, Kato-Katz technique) the prevalence of *Ascaris lumbricoides* forms the stool samples.200 samples for traditional diagnosis and 100 samples was diagnostic by Molecular technique. The results show 20%(21/100), 13% (26/200) by Molecular diagnosis and traditional diagnosis respectively.

Table 1: Comparative study between Molecular diagnosis and traditional diagnosis and infected rate of *Ascaris lumbricoides*

Test	Number of stool samples	Number of Positive	Percentage %
Molecular	100	21	21%
Microscopic	200	26	13%
X^2	2.52		
P-value	0.11		

There was no statistically significant difference (P > 0.05).

The prevalence of *Ascaris lumbricoides* infection according to sex was higher in males (28%) than females (14%) however the age groups 5-10 recorded the highly in male and female (50%) , (42.8) and >15 age groups show lowest rate of infection in both sex (male and female) 23.07% and 14.2% respectively the difference was not statistically significant (P > 0.05). table. 2

Table 2: Molecular detection of *Ascaris lumbricoides* accoeding to sex and age groups

Age groups	Male positive %		Female positive %	
5–10	7	50%	3	42.8%
10-15	4	30,7%	3	42.8%
>15	3	23.07%	1	14.2%
Total	14	28%	7	14%
X^2	2.95			
P-value	0.086			

No statistically significant (P > 0.05).

The rate of infection by Molecular diagnosis the *Ascaris lumbricoides* according to the area of study recorded heights in Al-Kifl was 28% (7/25) and lowest rate in Al-Hilla city and Al-Musayyab 16% (4/25) respectively**.** There is no statistically significant difference in the positivity rates among the studied areas (p > 0.05). The variation in prevalence across locations is likely due to random variation rather than a true area-related effect table (3).

Table 3: Molecular detection of *Ascaris lumbricoides* according to geographic area

Area	No of samples	No of positive %	Percentage %
Al-Hilla city	25	4	16%
Al-Musayyab	25	4	16%
Al -Kifl	25	7	28%
Al- Qasim	25	6	24%
Total	100	21	21%
X^2	1.63		
P-value	0.65		

No statistically significant difference (p > 0.05).

The prevalence of *A. lumbricoides* does not alter statistically significantly across the four seasons (p > 0.05). Summer had the greatest infection rate (32%) and winter had the lowest (12%), but these differences were not statistically significant and might be explained by chance. table 4

Table 4: Molecular detection of *A. lumbricoides* according to season.

Season	No of samples	No of positive %	Percentage %
Summer	25	8	32%
Autumn	25	5	20%
Winter	25	3	12%
Spring	25	5	20%
Total	100	21	21%
X^2	3.24		
P-value	0.36		

No statistically significant difference (p > 0.05).

Phylogenetic sequences analysis *Ascaris lumbricoides*

Ten sequences analysis each belonging of *Ascaris lumbricoides* Phylogenetic tree (PX418260, PX418261, PX418262, PX418263, PX418264, PX418265, PX418266, PX418267, PX418268 and PX418269). The phylogenetic tree revealed the identity between the isolates of *Ascaris lumbricoides* similar to *Ascaris lumbricoides* from Thailand (accession No. MF358959.1) and (accession No HQ721819.1) from China as highlighted in Figure 1.

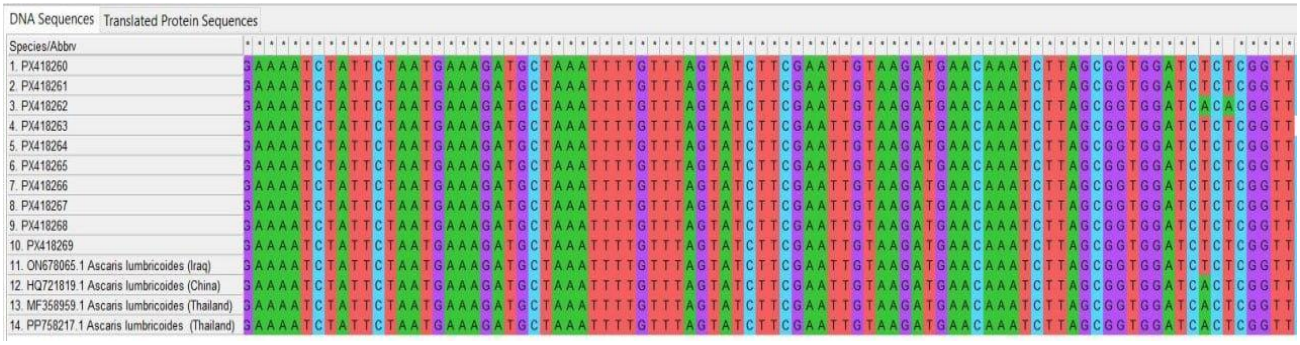

Figure 1. The 5.8S rRNA partial sequence gene in *Ascaris lumbricoides* stool isolates from the area. analysis of genetic identification.IQ-stool isolates and Genbank *Ascaris lumbricoides* genotype isolates from the National Center for Biotechnology Information. The multiple alignment analysis was performed using the Clustal W alignment tool in Molecular Evolutionary Genetics Analysis 6.0 version.

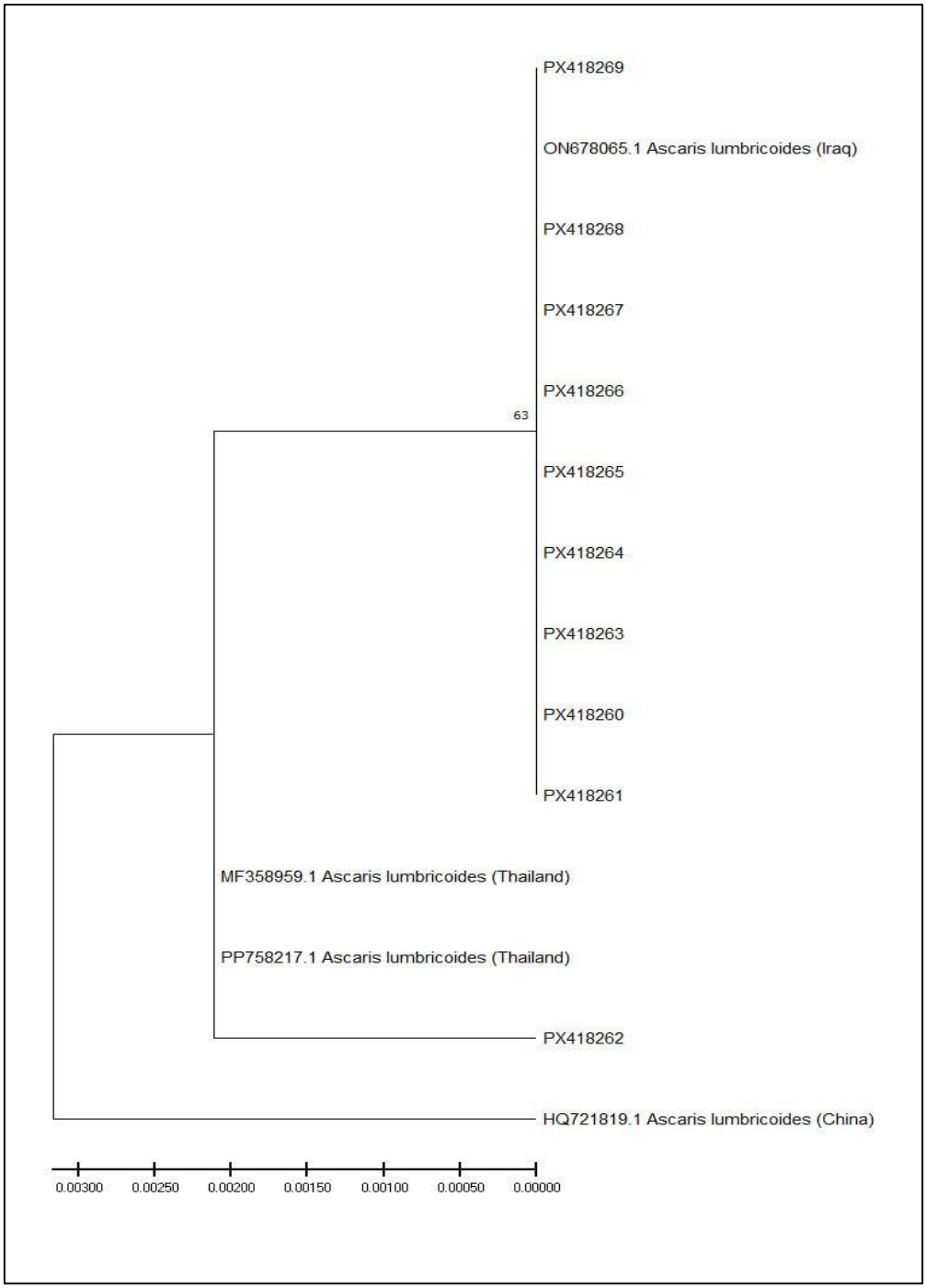

Figure 2: Phylogenetic tree analysis based on the partial sequence of 5.8S rRNA partial sequence gene in *Ascaris lumbricoides* stool isolates from the area. analysis of genetic identification

DISCUSSION

Ascariasis continues to be a serious public health concern in many developing nations, including Iraq, due to inadequate sanitation, poor hygiene habits, and favorable environmental circumstances for parasite transmission. The current study compares conventional microscopic techniques and molecular methods for diagnosing Ascaris lumbricoides in Babylon governorate, Iraq, and assesses the impact of sex, age, geographic area, and seasonality on infection rates. The total incidence of A. lumbricoides revealed by molecular approaches (21%) was greater than that found by classical microscopic techniques (13%). Although the difference was not statistically significant ($P > 0.05$), this data validates earlier findings that show that molecular diagnostics are often more sensitive than microscopy, particularly in situations of low-intensity infections or intermittent egg shedding (Miswan *et al.,* 2022; Lotz *et al.,* 2025). Microscopic techniques like Kato-Katz or flotation are low cost and commonly performed in endemic regions; their sensitivity may vary based on egg density, sample quality and user experience. PCR

methods targeting the ITS region can detect eggs at all densities with great accuracy, helping explain the high rates seen in our study. We also found the prevalence of males to be 28 percent versus only 14 percent for females, although this difference was not statistically significant. Other research has concluded that males may have higher infection rates due to their involvement in outdoor activities or farming, and thus are more exposed to soil contaminants, and due to having lower levels of personal hygiene (Fawzia et al. 2022; Khan et al. 2025). A lack of statistical significance indicates that both genders are at equal risk within this population due to extensive environmental pollution present within the study area; therefore, further investigations are required to determine whether environmental pollution is the only cause of disease transmission. Age-based studies are indicative of the highest incidence of Ascaris lumbricoides infections, with males (50%) and females (42.8%), between ages 5 and 10 experiencing the highest number of infections and individuals over age 15 reporting the least number of infections, which is supported by similar reports from around the world on the prevalence of Ascaris lumbricoides in school-age children due in part to exposure to soil and dirt through recreational play, as well as lack of good hygiene habits (Holland *et al.,* 2022; Debbarma *et al.,* 2023). Older age groups had lower rates of infection with the parasite, likely caused by greater immunity, more awareness of hygiene, or less exposure. However, the lack of a statistical significance shows that age is not the only variable that affects the risk of infection in the Babylon governorate. Additionally, the geographical distribution shows that the highest infection rate was found in the district of Al-Kifl (28%) while the lowest was found in Al-Hilla city and Al-Musayyab (16%) and while these results did not yield a statistically significant difference, the discrepancies can be explained by differences in sanitation practices, population density, types of available water and socioeconomic conditions. Areas with greater numbers of homeless people and more individuals depending on untreated water and traditional farming methods (e.g., squattering) likely have much more environmental contamination with Ascaris eggs than do other areas. This type of increased environment contamination with Ascaris eggs has also been reported from other places where these parasites are endemic (Badri *et al.,* 2025). As there are no statistically significant differences found, Ascaris is likely to be transmitted in all districts within Al-Najaf. When comparing the seasonal survey results, a higher prevalence of infection was found in summer (32 percent) than in winter (12 percent). Although the seasonal increase of infections in summer months is not statistically significant, physiologically, increased egg maturation and survival time can cause an increase in infections during this period. Increased human contact with soil due to living conditions in summer months, combined with water shortages, may be the cause of increased seasonal transmission of disease. This seasonal phenomenon is evident in tropical and subtropical climates, although the extent of seasonal variability depends on the climate change and local cultural behaviors. Genetic and molecular analyses of ten Ascaris (Ascaris lumbricoides) isolates demonstrated a close genetic relationship between isolates from Thailand and China. The data show the Ascaris populations contain high levels of genetic similarity across large areas worldwide, therefore all contain conserved genotypes. Genetic identity demonstrated by the data correlates with previous molecular studies, including those using ITS gene sequences and rRNA analysis. Improvements in diagnostic accuracy and identification of local isolates have occurred due to the use of molecular methods to identify these pathogens.

CONCLUSION

Molecular diagnostic methods are more accurate than conventional microscopy for identifying Ascaris lumbricoides; however, both of these methods remain useful complementary tools when used together in epidemiological studies. The incidence rate of ascariasis is higher among children than adults, with some degree of seasonal and geographical variation, although this variation is not statistically significant. Therefore, there is significant evidence to support the need for an integrated approach (e.g., implementing regular deworming programs, improving sanitation, implementing health education programs and using molecular technologies to monitor activity) for improving control of ascariasis in all areas where the disease is endemic (i.e., including Babylon).

Conflict of Interest

There are no conflicts of interest

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REFERENCES

- Anderson, C. E., Hanif, S., Hernandez, J., Crider, Y., Lepech, M., Billington, S. L., ... & Benjamin-Chung, J. (2025). Investigating cement-based surfaces as a sustainable flooring solution to improve Ascaris Suum egg removal and inactivation in low-resource settings. *PLoS neglected tropical diseases*, *19*(10), e0012919.
- Badri, M., Olfatifar, M., Gharibi, Z., Pal, M., Hatam-Nahavandi, K., Asghari, A., and Eslahi, A. V. (2025). A systematic review and meta-analysis on the global prevalence of helminthic parasites among schoolchildren: a public health concern. *BMC Public Health*, *25*(1), 2852.

- Bowman, D. D. (2021). Ascaris and Toxocara as foodborne and waterborne pathogens. *Research in Veterinary Science*, *135*, 1-7.
- Debbarma, S., Talukdar, J., Maurya, P., Barkalita, L. M., & Brahma, A. (2023). Neglected parasitic infections: history to current status. *Parasitic Infections: Immune Responses and Therapeutics*, 156-175.
- Devi, U. (2022). Ascariasis. In *Textbook of Parasitic Zoonoses* (pp. 469-477). Singapore: Springer Nature Singapore.
- Dos Santos, T. R., Furtado, L. F. V., de Carvalho Araujo, A., da Silva Medeiros, C., Germano, P. H. V., de Oliveira, V. N. G. M., & Rabelo, E. M. L. (2022). Development of allele-specific PCR methodology (AS-PCR) to screening A. lumbricoides and A. suum. *Parasitology Research*, *121*(8), 2389-2397.
- Fauziah, N., Aviani, J. K., Agrianfanny, Y. N., & Fatimah, S. N. (2022). Intestinal parasitic infection and nutritional status in children under five years old: a systematic review. *Tropical medicine and infectious disease*, *7*(11), 371.
- Holland, C., Sepidarkish, M., Deslyper, G., Abdollahi, A., Valizadeh, S., Mollalo, A., ... & Rostami, A. (2022). Global prevalence of Ascaris infection in humans (2010–2021): a systematic review and meta-analysis. *Infectious Diseases of Poverty*, *11*(1), 113.
- Khan, A. U., Hussain, S., Khan, M., Said, A., Bibi, A., Khan, T., ... & Afshan, K. (2025). Effectiveness of Anthelmintic Therapy and Determinants of Ascaris lumbricoides Infection among School-Aged Children: A Community-Based Cross-Sectional Study in Rural Khyber Pakhtunkhwa, Pakistan. *Acta Parasitologica*, *70*(4), 1-14.
- Lotz, C. N., Mrimi, E. C., Schneeberger, P. H., Ali, S. M., Hattendorf, J., & Keiser, J. (2025). Performance of real-time polymerase chain reaction and Kato-Katz for diagnosing soil-transmitted helminth infections and evaluating treatment efficacy of emodepside in randomized controlled trials. *PLOS Neglected Tropical Diseases*, *19*(2), e0012872.
- Miswan, N., Singham, G. V., and Othman, N. (2022). Advantages and limitations of microscopy and molecular detections for diagnosis of soil-transmitted helminths: an overview. *Helminthologia*, *59*(4), 321.
- Pal, M., Rebuma, T., Desalegn, M., and Baidya, S. (2025). Ascariasis: An Important Soil Transmitted Helminthiasis of Public Health Significance. *Indian Journal of Veterinary Public Health| Volume*, *11*(1), 1.
- Rahmat, S. M., Panggabean, M., Depari, A. A., Putra, T. R. I., & Endalif, D. (2023). Diagnostic Test of Blood Eosinophil Level as a Marker of Ascaris lumbricoides Infection. *Indonesian Journal of Tropical and Infectious Disease*, *11*(2), 85.
- Tarafder, M. R., Carabin, H., Joseph, L., Balolong Jr, E., Olveda, R., & McGarvey, S. T. (2010). Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, Ascaris lumbricoides and Trichuris trichiura infections in humans in the absence of a 'gold standard'. *International journal for parasitology*, *40*(4), 399-404.