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# Diagnosis of *Ascaris sp*.in Human by Using Molecular Technique

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**ABSTRACT**

*This study aimed to Diagnose of Ascaris sp.in human by using molecular technique. Microscopically looking for Ascaris sp. eggs, human faeces were collected from 120 patients admitted to private clinic in Baghdad, Iraq. The Kato-Katz method (Hell-Tec kit) was used for the parasitological diagnosis. DNA Stool Mini kit (Qiagen) was used to process 200 l of material, although with some adjustments (Proteinase K digestion at 55-60 °C for 4 h). To determine whether typical PCR inhibitors found in faeces were present, human DNA PCR was carried out. The cytb gene and ITS1 (480 bp) were used for molecular detection of this parasite. The genotypes of Ascaris need to be amplified and identified. The most reliable method for diagnosing Ascaris intestinal infections is the detection of parasite eggs in faecal samples using a light microscope. The percentage of Ascaris spp. by microscopical method was 2.5 %. The molecular results showed that cytb gene was showed in 16 samples, while ITS1 gene were showed in 18 samples.*

*In conclusion, the molecular method showed higher sensitivity in diagnosis of Ascaris spp. from human feces when compared with microscopical method.*

## Introduction:

According to (1), about 1.4 billion individuals are infected with *Ascaris lumbricoides* (L.). Millions of pigs are infected with the parasite *Ascaris suum* (2), which causes significant economic losses in several countries due to the same mode of transmission (fecal/oral) (3). Cross-infection has been shown (4, 5), and hence it is crucial to recognise the danger of swine as a possible reservoir of *Ascaris* genotypes that may infect humans despite the lack of physical markers separating the two species. Studies on Ascaris and *Ascaris* genotype identification have focused on the ITS region of nuclear DNA, as well as the mtDNA, , NADH dehydrogenase subunit 1, cytochrome c oxidase subunit 1 as well as microsatellite marker regions (6,7).

*Ascaris* populations in people and pigs were analysed for their ITS1 restriction patterns using PCR-RFLP (8,9). Five human-derived Ascaris ITS1 genotypes were discovered by (10) using SSCP (Single Strand Conformation Polymorphism), indicating that these genotypes are shared across hosts. Using data from a large cohort of *Ascaris* parasites, they looked at how often different genotypes occurred in connection to host species and geographic origin across six endemic locations in China. The findings revealed that human beings were more likely to be carriers of genotype G1, while pigs were more likely to carry genotype G3. The remaining three genotypes were discovered at lower frequency in both hosts. These patterns of dispersal are indicative of a potentially unique host association (11).

This study aimed to Diagnose of *Ascaris sp*.in human by using molecular technique.

**Materials and Methods:**

Microscopically looking for *Ascaris sp*. eggs, human faeces were collected from 120 patients admitted to private clinic in Baghdad, Iraq. The Kato-Katz method (Hell-Tec kit) was used for the parasitological diagnosis, and Calculating the EPG involved multiplying the total number of eggs on the slide by 23 (12).

DNA Stool Mini kit (Qiagen) was used to process 200 l of material, although with some adjustments (Proteinase K digestion at 55-60 °C for 4 h).

To determine whether typical PCR inhibitors found in faeces (13) were present, human DNA PCR was carried out (14). Primers and PCR conditions described by (15) were used to amplify the *Ascaris* cytochrome b (cytb) gene and the ITS1 (480 bp).

**Results and Discussions:**

The most reliable method for diagnosing *Ascaris* intestinal infections is the detection of parasite eggs in faecal samples using a light microscope. The percentage of Ascaris spp. by microscopical method was 2.5 % (Table 1).

**Table 1. Number and Percentage of *Ascaris spp*.**

| Parasite | No. | Percentage |
|---|---|---|
| *Ascaris spp.* | 3 | 2.5% |

*Ascaris lumbricoides* was found to have a very low prevalence (2.2%). (16), (17), and (18) all found similar things in various locations of Iraq. *Ascaris lumbricoides* is a widespread parasitic infection. It is more widespread in communities with inadequate sanitation, such as in Iraq, where human waste is sometimes used as fertiliser and youngsters often relieve themselves directly on the ground.

The molecular results showed that cytb gene were showed in 16 samples (Fig.1), while ITS1 gene were showed in 18 samples (Fig.2).

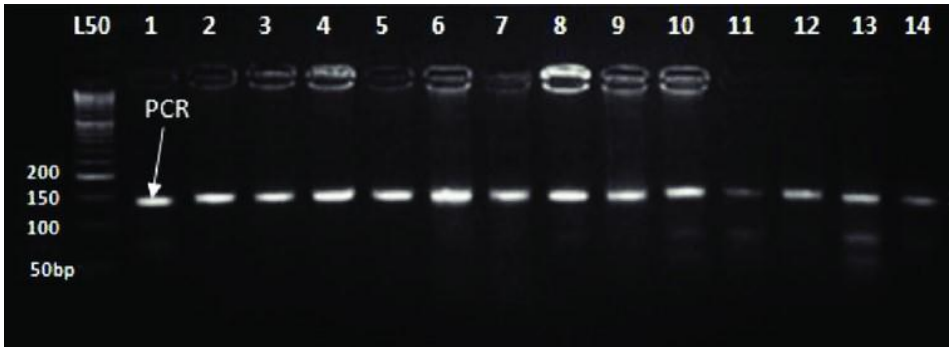


**Figure 1. The cytb-PCR-specific 142-bp band was clearly visible in the electrophoresis results of a 1.8% agarose gel.**

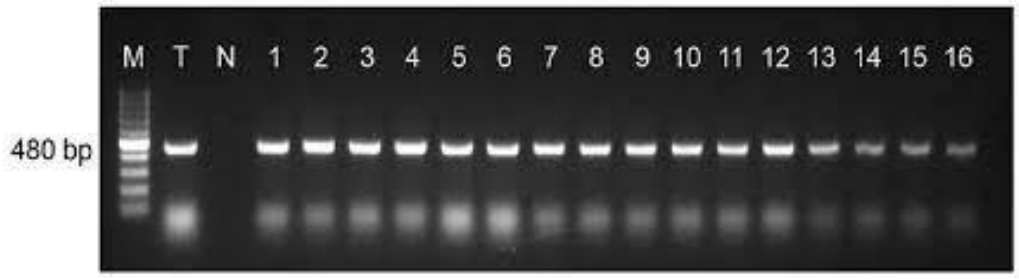


**Figure 2. The ITS1-PCR-specific 480-bp band was clearly visible in the electrophoresis results of a 1.8% agarose gel.**

However, this strategy has its limitations when distinguishing between species. In Latin America, where anthelminthic resistance is rising in pastoral animals (19), almost 8% of all *A. lumbricoides* infections occur (20). In addition, it has been challenging to collect mature worms from people because of the widespread use of anthelmintics. However, there is a dearth of genetic information from Iraq. The newly discovered *Ascaris* genotype was discovered with the use of the newly created molecular diagnostic technology, which allows for *Ascaris* identification from faecal samples.

Real-time quantitative polymerase chain reaction (qPCR) and multiplex tests are two examples of the cutting-edge developments in molecular diagnostics that have resulted in lower prices and more efficiency.

Extraction and amplification of DNA from a single *Ascaris* egg's nuclear first internal transcribed spacer region (ITS1) have been principally improved for use in population genetic investigations (21). By amplifying the DNA from even a single egg, these methods applied to stool samples may allow for very sensitive identification of Ascaris. Techniques like the molecular paleoparasitological hybridization method (22) that can identify very low levels of ancient DNA may increase sensitivity for extremely low infections.

*Ancylostoma sp*., *Necator americanus*, *E. vermicularis*, *Ostertagia ostertagi*, *Oesophagostomum bifurcum*, *Taeniid* worms, as well as other parasites have all been identified through species-specific molecular diagnosis using DNA extracted from faeces (23,24). Studies have used procedures to lyse eggs (ultrasound, microwaving, boiling, freezing), and others have used suspension or flotation to separate eggs from faeces. The eggs of the nematode The *Ascaris* species have a tough chitinous shell, a vitelline membrane, a lipid layer, and a tough outer coat (25).

**Conclusion:**

The molecular method showed higher sensitivity in diagnosis of *Ascaris spp*. from human feces when compared with microscopical method.

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