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Molecular Detection of Hymenolepis Nana from Patients in Al-Shatrah Hospital

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Annotation: This study was carried out at in Thi-Qar Province, College of Science Department of Pathological analysis .The samples were collected from Patients in Al-Shatrah hospital 65 Samples was collected from patients suffering from diarrhea and abdominal pain in different age from male and female . The sample were collected in a clean plastic containers and labeled it with writing the number, age and sex and then transported in refrigerated bag than kept cool until the sample exanimated. Microscopically examination include and also used molecular detection by DNA extraction and PCR amplification. The result was positive for only 37 sample show Hymenolepis nana in all methods that used.

Keywords: Hymenolepis nana, Microscopically, PCR method.

Introduction:

Hymenolepiasis is recognized as the most prevalent intestinal tapeworm infection in humans and is often referred to as the dwarf tapeworm infection. It is considered endemic in regions such as Asia, Africa, and parts of southern and eastern Europe (1,2). The infection predominantly affects children and, in many cases, does not produce noticeable symptoms. However, when the parasitic load is high, individuals may experience clinical signs including diarrhea, abdominal discomfort, nausea, and loss of appetite; in severe cases, the condition may lead to bloody diarrhea (3,4). This

parasite present worldwide and is a prevalent cestode parasite in the phylum Platyhelminths. It infects various domestic and wild animals and human specially in children (5). Transmission of the infection to human mainly by the fecal-oral route via ingestion of contaminated food,water with feces.Also happens through accidental ingestion of rat fleas infected with cysticercoids (6,7). The identification of eggs in human stool samples is essential for diagnosing parasitic infections, as it aids in recognizing key morphological features. Furthermore, molecular techniques like polymerase chain reaction (PCR) have enhanced the efficiency of parasite detection by providing fast and straightforward methods (8). The microscopic examination of the stool in human is considered the highest diagnostic method to detect Hymenolepis eggs, there are another methods used to diagnose this parasite for example genetically or PCR techniques (9). The primary aim of this study was to isolate and identify Hymenolepis nana eggs using both microscopic examination and the PCR technique within Thi-Qar Province.

Methodology:

Sample collection:

65 Samples was collected from patients suffering from diarrhea and abdominal pain in different age from male and female .The sample were collected in a clean plastic containers and labeled it with writing the number ,age and sex and then transported in refrigerated bag than kept cool until the sample exanimated. Theses sample diagnosis microscopy by direct wet-mount according to (10).

DNA Extraction and PCR Amplification

To isolate the genomic DNA from stool specimens, the QIAamp DNA Stool Mini Kit (QIAGEN, Germany) was utilized following the manufacturer's instructions. The amplification process targeted Hymenolepis nana using designated primers:

- ✓ Forward primer (ITS2F): 5′ GTGAATCGCAGACTGCTTTG 3′
- ✓ Reverse primer (ITS2R): 5′ CTGAGGTCAGGTCTTCCATAC 3′

The DNA amplification workflow was executed in 20 microliter reactions utilizing a commercial Taq polymerase-based premix (Master Mix RED; Amplicon, Copenhagen, Denmark) combined with template DNA, bidirectional oligonucleotide primers (0.1 µM each), and molecular-grade aqueous solvent.

Thermal cycling parameters were initiated with a 5-minute primary strand dissociation phase at 95°C to ensure complete genomic template separation. Subsequently, 35 amplification rounds were executed, each comprising three temperature-regulated intervals:

1. Template Melting: 95°C (20 sec) for double-stranded DNA destabilization

2. Primer-Target Hybridization: 59°C (20 sec) for sequence-specific primer-template binding

3. Polymerization: 72°C (30 sec) for enzymatic DNA strand synthesis

A terminal 5-minute incubation at 72°C was incorporated to finalize incomplete amplicons.

Following PCR, the amplified DNA fragments (amplicons) were analyzed via gel electrophoresis. A 2% agarose gel, prepared with TBE buffer (containing Tris, boric acid, and EDTA), was used to separate the DNA by size. After staining with a fluorescent dye, distinct DNA bands were observed under UV light to confirm successful amplification.

Statistical analysis:

Statistical analysis is often used to analyze quantitative data, and provides methods for data description, simple inference for continuous and categorical data. The procedure involves the collection of data leading to test of the relationship between two statistical data sets. In this study all data are presented as frequency and persentage. We used SPSS (version 26) and the dependent

t-test (two-tailed) and independent t-test (two-tailed) for variables that had a normally distributed distribution. For variables that did not have a normally distributed distribution, we used the Mann-Whitney U test, the Wilcoxon test, and the Chi-square test. $P < 0.05$ was seen as statistically significant.

Ethical approval:

The study was approved by the human ethics committee of Dept. of Path. Analysis/ College of Science, University of Thi-Qar, Iraq, Everyone who took part in the study was told about it and asked to sign a consent form. The patient was also guaranteed that his information would be kept private.

Results

In the present study show 18 sample was positive from 45 sample of total patients To diagnosis of *Hymenolepis nana* egg used microscopy and PCR techniques.

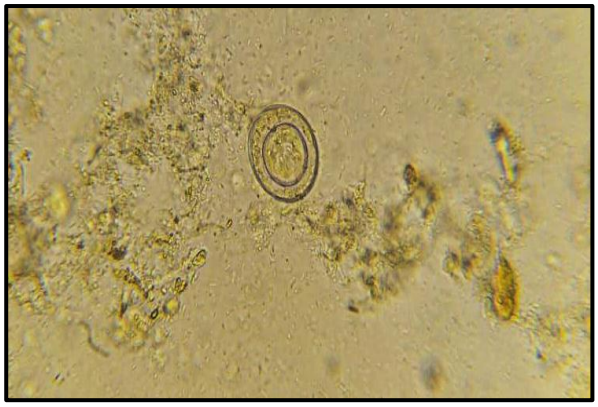

Figure 1: *H.nana* egg of in stool examination (10x).

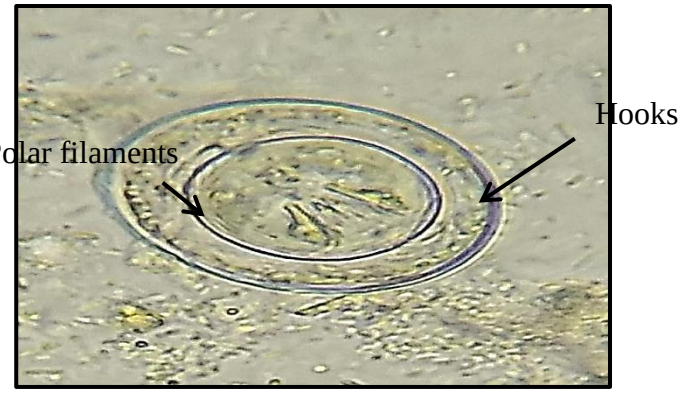

Figure 2: *H.nana* egg of in stool examination (100 x).

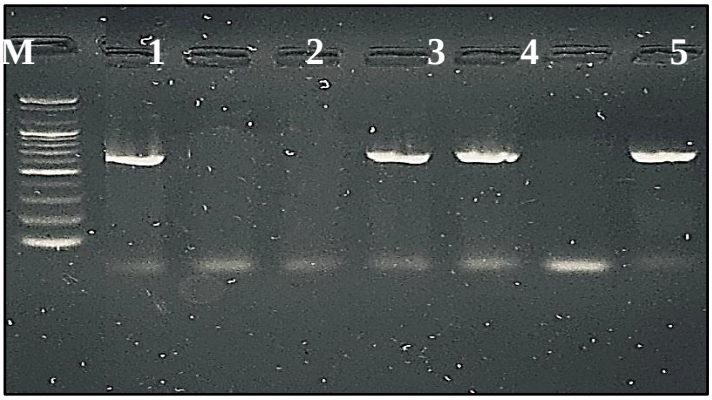

Figure 3: PCR product of *H.nana* (629 bp) (1,4,5,7 Positive sample and 2,3,6 Negative sample). In 1.5% agarose at 70 volts for45 minutes.

Discussion

Hymenolepiasis is regarded as a significant public health issue in numerous countries around the world (11). In Iraq, limited research has addressed *Hymenolepis nana*, with some studies conducted in Erbil (12,13), Sulaimani (14), and Baghdad (15). In the current study, stool samples were obtained from individuals experiencing diarrhea, as *H. nana* is known to be linked with diarrhea and other gastrointestinal disturbances, especially among children (16,17). Molecular techniques are considered the most accurate approach for detecting *Hymenolepis* species, complementing traditional morphological methods that rely on identifying adult worms or eggs (18,19). In this study, genomic DNA was examined using specific primers targeting *Hymenolepis nana*: the forward primer ITS2F (5′ GTGAATCGCAGACTGCTTTG 3′) and the reverse primer ITS2R (5′ CTGAGGTCAGGTCTTCCATAC 3′), following a methodology consistent with that of a previously published investigation (20). Clinically, *Hymenolepis nana* infection is commonly associated with a range of symptoms, including headaches, fatigue, reduced appetite, nausea, stomach discomfort, and most notably, diarrhea (21). The current findings revealed that diarrhea was the most commonly reported gastrointestinal symptom among the studied individuals. This observation aligns with outcomes from earlier studies conducted in countries like India and Mexico, as well as in other regions (22,23). The climatic and ecological conditions habitat characteristics, abundance of rodents, and host vulnerability to infection might influence the prevalence of hymenolepiasis, Also the difference in the prevalence rates of hymenolepiasis could also be attributed mainly to the level of hygienic (24). This research aligns with many studies conducted both in Iraq and globally regarding the diagnosis of *Hymenolepis nana* through morphological examination using a microscope. For instance, a study in Babylon province demonstrated the microscopic identification of *H. nana* in children (25). Beyond traditional diagnostic techniques, numerous studies have employed genetic methods to detect this parasite. To assess genetic variability in *H. nana* populations, several molecular markers have been applied, including the mitochondrial *cytochrome c oxidase subunit I (COX1)* gene and the nuclear ribosomal DNA (rDNA) regions spanning the *internal transcribed spacers (ITS1 and ITS2)* (26, 27).

Conclusion:

The examination by morphological observation through microscopy can be used as an initial diagnosis of Hymenolepis nana; however, molecular techniques are needed for the final identification of the species. In diagnosing this parasite, it is necessary to employ PCR-sequencing techniques and study the phylogeny of H. nana, characterizing the genetic differences within its population not only at the individual gene level but also at the whole genome level.

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