Molecular diagnosis of *Hymenolepis diminuta* in human and rats in Babylon Province, Iraq

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Received: July 2022 Accepted: October 2022

Abstract

The current study's objective was to use molecular technique to detect the parasite in samples taken from human and rats in Babylon province. A total of 100 stool samples from human and 40 fecal samples from rats were collected during the beginning of July 2021 to the end of October and analysed by molecular methods. Molecular description of *H. diminuta* was achieved by gene sequence of internal transcribed spacer 1 *(ITS1*). The PCR confirmed the identification of the parasite by electrophoresis as well as DNA sequencing. The result showed that the infection rate in human was (5/100) 5%, while in rats was (11/40) 27.50%, DNA sequencing detected 5 positive samples of human were *H. diminuta* and 5 positive samples from rats were *H. diminuta.* We conclude from the present results that *H. diminuta* is similar in both human and rats, and thus it is a source of infection for human. We recommended to detect *H. diminuta* in other rodents as source of zoonotic infection.

Keywords: *Hymenolepis diminuta,* PCR, Human, Rats, *ITS1*, Sequence analysis

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Introduction

Two of the utmost dominant rodents in the world are the black rat (*Rattus rattus*) and the house mouse (*Mus musculus*) (Goedert *et al.,* 2020). *Hymenolepis diminuta* is parasite predominantly a rodent also infect laboratory rats is distributed globally, but with only a limited cases diagnose as human infections (Karim *et al.,* 2014; Kapczuk *et al.,* 2018; Galoș *et al.,* 2022). One of two adult tapeworms induce human hymenolepiasis a widespread zoonotic infection known as *Hymenolepis diminuta* or *Hymenolepis nana* (Shahnazi *et al.,* 2019). *H. diminuta* in rats is transmitted by arthropod vectors (Majeed and Al-Amery, 2021).

Accidental ingestion of infected insects (intermediate host), who have the larval stage *Cysticercoid* of the parasite in their body cavity, can cause the person become part of the tapeworm's life cycle (Panti-May *et al.,* 2020). The native of *Hymenolepis* to Asia, Eastern and Southern Europe, South and Central America and Africa with socioeconomic and medical relevance (Thompson, 2015; Haq *et al.,* 2015; Cheng *et al.,* 2016; Sharma *et al.,* 2016; Liao *et al.,* 2016). Majeed and Al-Amery, (2021) proved that house mice have vital role in the transfer of parasite to human and that public health must be taken seriously. Some investigators used the *ITS* gene to identify the genotype of parasites (Alobaidii, 2020; Majeed *et al.,* 2020, Ismael and Omer, 2021; Alfatlawi and Alfatlawy, 2022, Hade *et al.,* 2022).

Materials and methods

Fecal sample collection

One hundred stool samples from human and forty fecal samples from rats were collected during the beginning of July 2021 to the end of October from various parts of the *governorate* of Babylon. After collecting the samples, centrifugation was achieved at 3000 RPM for 5 min after being sieved and rinsed with water with distilled water. Before to using processed materials in molecular analysis, they were kept in a -20°C freezer (Yang *et al.,* 2017).

Molecular assay using PCR technique

By using a DNA kit (Add Bio, Korea) the genomic DNA of supposed of *H. diminuta* was extracted. PCR primers (the starting sequences) were formed by (Macnish *et al.,* 2002) to amplify a very conserved sequences inside the 5.8S rRNA and internal transcribed spacer 2 of *Hymenolps* sp. forward and reversed primers:

(5´ - GCGGAAGGATCATTACACGTTC 3', 5´ - GCTCGACTCTTCATCGATCCACG 3', respectively, synthesized by Bioneer, Korea. The PCR strategy has involved initial denaturation at 94°C for 5 m, next 37 cycles of denaturation at 95°C for 1 m, primer annealing at 55°C for 30 sec, and extension at 72°C for 1 m, the last extension was carried at 72°C for 5 m.

DNA sequencing

From the positive PCR samples, 10 samples (5 humans and 5 rats) were selected, and the Amplicons were shipped via DHL in an ice container to Korea Macrogen Company for DNA

sequencing by Sanger sequencing system. Next acquiring of the sequences, Gen-bank accession numbers were obtained by submitting the sequences to NCBI-Gen-Bank.

Phylogenetic analysis

Molecular Evolutionary Genetics Analysis version 10 (Mega X) and multiple sequence alignment analysis based on Clustal W alignment analysis were now used to analyze phylogenetic trees. Using MEGA7 software, this displays the similarities and sequences inside this alignment area (Stecher *et al.*, 2020).

Statistical analyses

The statistical analyses were computer assisted using SPSS program, variables were assessed by Chi-square test (SAS, 2012).

Results

The infection rate in human was 5%, while in rats was 27.50%, this result showed that the infection rate in rats significantly higher than that in human because rats were the natural final host for *Hymenolepis diminuta* (Table 1).

Electrophoresis of the *H. diminuta* PCR products was completed and the bands appeared on the gel 650 -bp for IST_1 gene as shown in (Fig. 1).

Table 1: Rate of infection with *Hymenolepis diminuta* in Human and Rats.

Host	No. of Samples examined	No. Positive	Infection Rate %
Human	100	5	5
Rat	40	11	27.50*

*$p \leq 0.01$

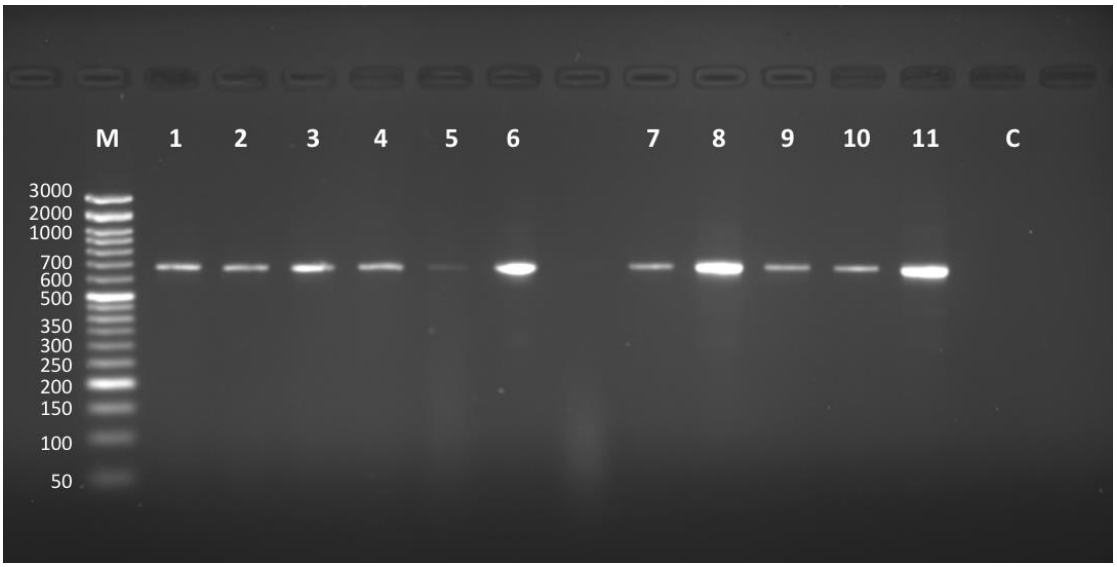


Figure 1: Gel electrophoresis image (agarose 1.5 %) shows the amplicon of *Hymenolepis diminuta* (1-6 in rats and 7-11 in human) represent positive samples within a specific region of (5.8S rRNA and internal transcribed spacer 2) while C is control negative in which similar PCR reaction components were being used except DNA was replaced by H_2O Molecular marker M comes from (Genedirex, Korea).

Sequence analysis

After obtaining accession numbers, a comparison was made of the similarity rates with the global isolates, the similarity rates were between 97.26 and 99.69 % with the global isolates (Table 2).

Phylogenetic tree

Ten isolates of *H. diminuta*, have been checked in the Gen-Bank database under accession no. OP087600 to OP087591. Phylogenetic tree shown there is an identity between the isolates of *H. diminuta* of both human and rats, and it is similar to the *H. diminuta* isolate in Japan with accession number AB494474. and rooted with *Arostrilepis gardneri* (accession number MN019650.1) (Figs. 2 and 3).

As well as through Figure 2, we noticed the identical of sequence of nucleotide in rats and humans.

Table 2: The percentage of homology between local isolates from humans and rats were submitted to gene banks with the accession numbers (OP087591-OP087600) and compared to other global isolates submitted to NCBI-BLAST.

Sample number	Accession number	Homology sequence similarity in NCBI-BLAST (%)				Host
		Identical to	Genbank Accession number	Country	Identity (%)	
1	OP087591	*Hymenolepis diminuta*	KC990408	USA	99.69	Human
2	OP087592	*Hymenolepis diminuta*	AB494474	Japan	98.45	Human
3	OP087593	*Hymenolepis diminuta*	AF461125	Australia	98.14	Human
4	OP087594	*Hymenolepis diminuta*	KC990410	USA	97.26	Human
5	OP087595	*Hymenolepis diminuta*	AB494475	Japan	98.66	Human
6	OP087596	*Hymenolepis diminuta*	LC582812	India	97.9	Rat
7	OP087597	*Hymenolepis diminuta*	KY079339	China	98.66	Rat
8	OP087598	*Hymenolepis diminuta*	KC990404	USA	98.45	Rat
9	OP087599	*Hymenolepis diminuta*	KC990407	USA	99.69	Rat
10	OP087600	*Hymenolepis diminuta*	AB494474	Japan	98.45	Rat

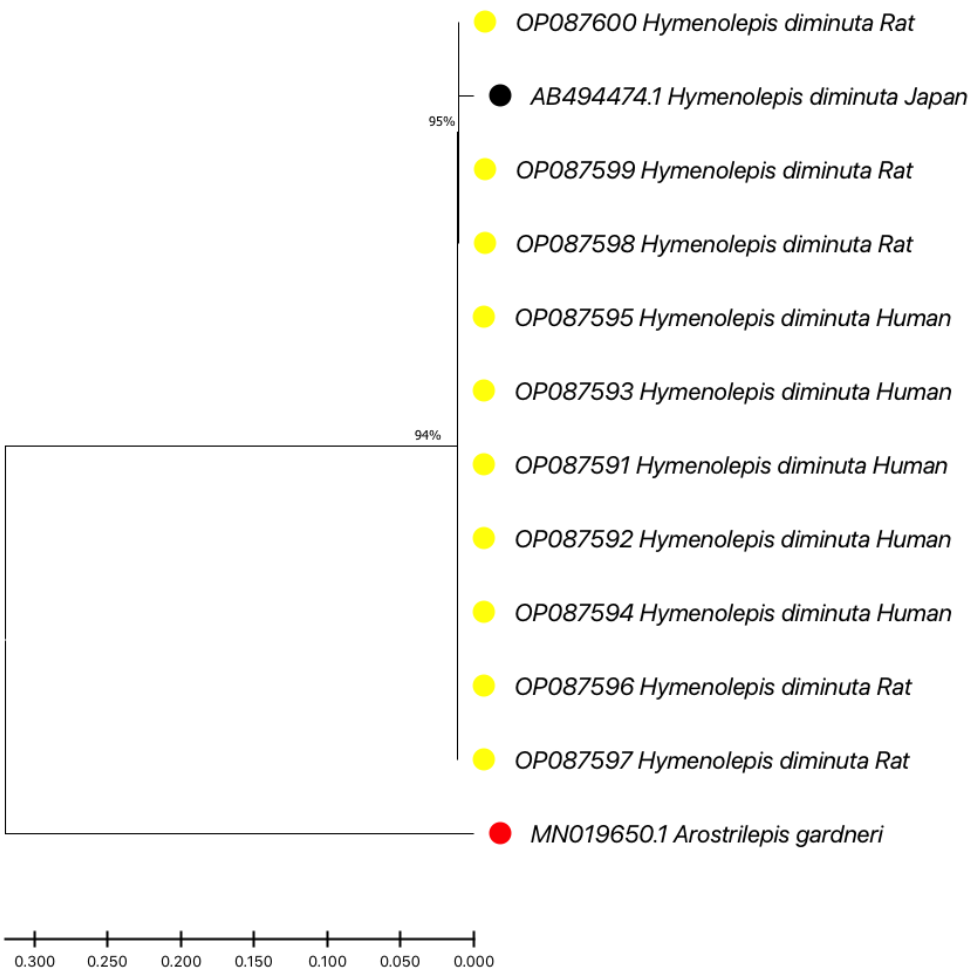
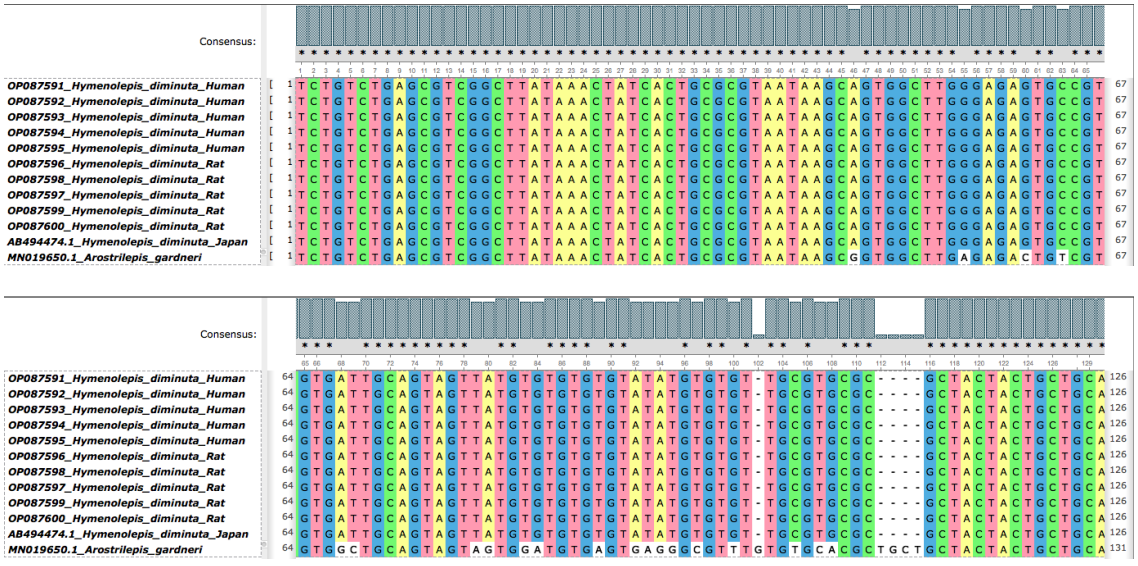


Figure 2: Phylogenetic tree analysis by neighbor-joining methods which shows the genetic similarity of the human and rat isolates targeting (internal transcribed spacer region 1). The obtained accession numbers (OP087591- OP087600) These were being compared with isolate from Japan (accession number AB494474.1) and rooted with *Arostrilepis gardneri* (accession number MN019650.1).



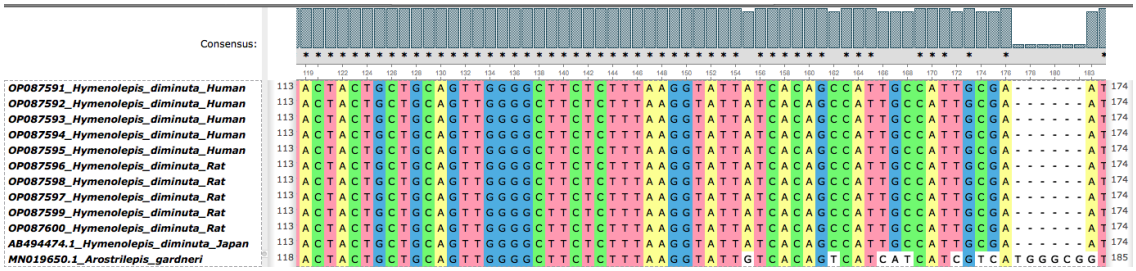


Figure 3: Multiple sequence alignment of the identified sequences with obtained accession numbers (OP087591-OP087600) in comparison to global isolate homologues. Using four different colors to highlight comparable areas.

Discussion.

Two species of *Hymenolepis*, *H. nana* and *H. diminuta*, are often responsible for causing the disease known as human hymenolepiasis, which can occur everywhere in the world (Tena *et al.*, 1998; Singh *et al.*, 2020). In most cases, the shape of the eggs detected during stool examination is used to diagnose and identify these tapeworms in human patients (Nkouawa *et al.*, 2016). However, because of the homologous in the phenotypic and morphological features of various phases of the parasite eggs, identification based on morphology has not proven a reliable approach for identifying the species of tapeworm (Yamasaki *et al.*, 2007).

The existent investigation was done by PCR to identify the *H. diminuta* via fecal specimens of normally human and rats in the Babylon *governorate* and reveal the genetic characterization of these specimens. This parasite is mainly found in mice, but there are studies indicate human infection in several countries, including them Poland, turkey, India, Iraq, Iraq, and Romanian (Kołodziej *et al.*, 2014; Kılınçel *et al.*, 2015; Sethi *et al.*, 2018; Golek *et al.*, 2019; Alomashi, *et al.*, 2021; Galoș *et al.*, 2022) respectively.

The molecular prevalence of *Hymenolepis diminuta* in human was (5%), which higher than that reported by (Ghadirian and Arfaa, 1972) in Iran was 1.1%, and one case reported also in Iran (Mowlavi *et al*. 2008), one case reported in child in Malaysia by (Rohela *et al.*, 2012). This difference in results could be due to difference of area where samples collected (especially in rural) areas and methods of diagnosis (The superior sensitivity of PCR in detecting parasite infection) as well as presence of intermediate host (arthropods) that's paly important role of transmission of infection to human and rodents.

Rats were the most common hosts for *Hymenolepis diminuta* find in this study, the infection rate was 27.50%, the result in this research was in similar with those detected by (Guddissa *et al.*, 2011) whom described the incidence rate of *H. diminuta* (26.79 %) in Ethiopian and (Mazhari *et al*., 2019) who recorded the infection rate of *H. diminuta* (29.50 %) in Iran. Other studies were recorded lower infection rate (2.9%) by (Siti *et al.*, 2012) in Kuala Lumpur, Malaysia, in Iraq (3.17%, 14% and 18%) by (Majeed, 2016; Majeed and Al-Amery, 2021) respectively in Baghdad province.

In our study, the high prevalence rate *H. diminuta* among the black rats might be attributed to the favorable climate and environmental condition in the city, which facilitates the survival of parasite eggs in the environment and spread of zoonotic infection by rodents.

However, findings of our study were in disagreement with those of (Kia *et al.,* 2010; Milazzo *et al.,* 2010) whom reported that higher infection rate of *H. diminuta* was (38.8% and 39.02%) respectively, in Iraq (Amin, 2019) recorded 32.75% in Kurdistan region and (Shubber *et al.,* 2019) recorded 37.5% in Al-Diwaniyah City. This variation of infection rates due to the variation of places and number of samples collection, and availability of arthropods that play an important role in transmission of infection to the rodents were infected eating insects that consequently transferred to human through food and water being contaminated with the feces of infected rodents containing eggs of *H. diminuta.*

The phylogenetic tree convergence of nitrogen bases for *H.diminuta* with the samples globally registered has been noticed: the samples of human of *H. diminuta* in current study were asymptotic to that having the Serial No. KC990408.1 and KC990410.1 registered in USA by (Zhong *et al.,* 2013), the Serial No. AB494474.1 and AB494475.1 registered in Japan by (Okamoto *et al.,* 1997) and the Serial No. AF461125.1 registered in Australia by (Macnish *et al.,* 2002), while the samples of rats of *H. diminuta* in the current study were asymptotic to that having the Serial No. KC990407.1 registered in USA by (Zhong *et al.,* 2013), the Serial No. KY079339.1 registered in China by (Yang *et al.,* 2017), the Serial No. AB494474.1 registered in Japan by (Okamoto *et al.,* 1997), the Serial No. KC990404.1 registered in USA by (Zhong *et al.,* 2013) and the Serial No. LC582812.1 registered in India by (Brar *et al.,* 2021). This dimension of the phylogenetic analysis refers to the difference in the successions of the nitrogen bases between the local sample of human, rats and the one globally registered, this difference might be explained by environmental differences in various places and mutations.

Conclusions and recommendations

We conclude through the evolutionary tree and multiple sequence alignment revealed identical sequence of nucleotide in humans and rats, and thus we can say that black rats are a source of human infection with the parasite *H. diminuta*. We recommended detecting *H. diminuta* in other rodents as source of zoonotic infection.

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