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Morphological and Molecular Study of *Hymenolepis* Sp. From House Rats in Baghdad/Iraq through Mitochondrial Cox1 and ITS1 Gene PCR Analysis

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Abstract

Rodents are popular companion animals and they can be reservoirs of many parasites of zoonotic pathogens. The occurrence of *Hymenolepis nana* and *H. diminuta* in rodents from variety cities from Baghdad/Iraq Totally, 100 fecal samples were collected from Rats. The fecal samples were examined by direct and different fecal diagnostic techniques. The total prevalence of parasitic infection was 25(25%), *H. diminuta* were detected in 18(18%) while *H. nana* were detected in 7(7%). Higher prevalence 11(40.74%) were found in Abu Ghraib area with significant differences ($p \leq 0.01$). Our results revealed differences in the infection rates of Rat Hymenolepiasis according to sex. The present study showed relatively male was higher prevalence than female. The reported of Rat Hymenolepiasis increased in September than the other month which decreased in December and no infection in November. Ten samples with positive PCR products in any of the DNA regions include *Hymenolepis* sp mitochondrial cox1 gene, *Hymenolepis nana* 18S rRNA-ITS1 gene and *Hymenolepis diminuta* 18S rRNA-ITS1 gene gave profiles characteristic of *H. nana* and *H. diminuta*. The results imply the risk of zoonotic transmission of Hymenolepiasis in Baghdad. Particular attention should be given to hygiene level maintained and disposal rodents for avoid parasite transmission to humans.

Keyword: prevalence, *Hymenolepis nana*, *hymenolepis diminuta*, Rat, Molecular diagnosis

Introduction

Hymenolepiasis is one of a largely rodent-borne group of parasitic diseases caused by cestodes belonging to the family of cyclophyllidae tapeworms, Hymenolepididae, and is included in the list of neglected zoonotic helminthoses (Thompson 2015). Rodents are the main definitive hosts of both *Hymenolepis nana* and *H. diminuta,* which are zoonotic and known as the dwarf and rat tapeworms, respectively (Steinmann *et al*., 2012). *H. nana* is the most common cestode infecting humans, whereas *H. diminuta* causes occasional human infections (Soares Magalhães *et al*., 2013). Both cestodes can be differentiated by the morphology. Scolex of *H. nana* consists of four suckers and a retractable rostellum armed with hooks, whereas the scolex of *H. dimunita* has four suckers similar to that of *H. nana* and is unarmed. *H. nana* and *H. diminuta* are two commonly occurring cestodes causing hymenolepiasis in rodents and humans especially young children (Sood *et al*., 2018).

H. nana and *H. diminuta* have been detected in brown rats in many countries and areas. *H. nana* has been found in the Netherlands; 3.3% (1/30) in farms and 4.1% (2/49) in rural environments Franssen et al. (2016); 8.8% (10/112) in Brazil Simões et al. (2016). *H. diminuta* has been found in the Netherlands; 50% (15/30) in farms, 10.2% (5/49) in rural environments, and 10.5% (4/38) in suburban environments Franssen et al. (2016); 6.3% (2/32) in Taiwan Tung *et al*. (2013) and Goswami *et al*., (2011) who diagnosed positive for spontaneous *Hymenolepis diminuta* infection was78 (19.23%) in adult laboratory and wild rats investigated for parasitic diseases. Majeed. (2016) who mention that the prevalence rate of *Hymenolepis nana* 4(6.34%), while *Hymenolepis diminuta* 2(3.17%) in Baghdad/ Iraq.

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In the diagnosis of Hymenolepiasis and differentiation of causative species, eggs recovered from host feces usually play an important role for identifying their morphological features (Nkouawa et al., 2016). However, PCR-based molecular techniques not only increase detection rates of parasites, but also provide the accurate species differentiation and their genetic characterizations, currently; the first and second internal transcribed spacer regions (ITS1 and ITS2) of nuclear ribosomal RNA gene can be helpful for resolving remarkable taxonomic issues and discriminating closely related genera and species, Meanwhile, mitochondrial (mt) genome sequences have been proven to be useful and reliable genetic markers for population genetics and systematic studies (Sharma et al., 2016 and Shahnazi *et al* 2019). The aim of this study was to estimate the prevalence of *Hymenolepis nana* and *H. diminuta* parasites in Rat and to evaluate its risk on Human in Baghdad governorate/ Iraq.

Material and method

Samples collection

One hundred rats are trap from some districts in Baghdad city include (Bakeries, Grain storage factory and farm in Abu Ghraib area, Al-Amiriya area including veterinary medicine/ university Baghdad , Al-Sha'ab area and Haifa Street area). All samples of rat is then bringing to laboratory and examined 5grams of fecal samples immediately the following morning. Fecal sample examined (about 7 to18 samples/ month) in parasites laboratory for detecting eggs and worms (*Hymanolepis* spp.). The samples are dividing into two parts: first part for microscopically examination and second part for DNA extraction.

Dissection and collection of parasites:

Trapped rats is kill humanely by anesthesia (9:1, ketamine and xylasine) per 100 gm. rat body weight after were caught from tail and interperitoneal injection as accorded by **(**Struck *et al*., 2011**).** Stool samples fixed in formalin were processed using the formalinethyl acetate concentration technique to detect eggs, Cestodes were collected directly from the intestine and kept in separate plastic containers, and the samples were transported to Parasitology Laboratory located at the College of Veterinary Medicine, University of Baghdad for examination (Al- Zubaidei and Kawan 2020). So the permanent slides were prepared and stained with acetocarmine acide, dehydrated in different grades of alcohols, cleared in xylene and mounted in Canada balsam. After those cestodes were morphologically identified under microscope using the taxonomic keys described previously (Palmer *et al*., 2011).

Diagnosis by use conventional PCR:-

DNA extraction

PCR technique was performed for detection *Hymenolepis* species based mitochondrial cox1 gene and specific detection *Hymenolepis nana* and *Hymenolepis diminuta* based 18S rRNA-ITS1 gene from Rat stool samples. This method was carried out according to method described by (Kandil *et al*., 2010). Worm DNA from stool samples were extracted by using genomic DNA from worm tissue samples were extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions. Also Cestode tissue samples were fixed and preserved in 70–100% ethanol and DNA extracted using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions.

Amplification was performed using specific primers *Hymenolepis sp.* mitochondrial cox1 gene: F:5`ACTTCATTGCTTTTGCTTTTTAGA3`R:5`TGCTGTCATAAATGAACCAACAGT3` with PCR product size ~700bp (Kandil *et al*., 2010). *Hymenolepis nana* 18S rRNA-ITS1 gene: F:5`GTTAGGCCTGCATGTTGTGC3`R:5`TGTGTGCCAGCTTGTGTGTA3`with PCR product

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size 535bp (This study Genbank:AF461124.1) and *Hymenolepis diminuta* 18S rRNA-ITS1 gene: F: 5` TGCAGCCAACTGCTGTGATA3`R: 5`GGGGGAGCATGTGTCAAAGT3` PCR product size 361bp. PCR master mix preparation by DNA template 5-50ng with Volume 5µL, Cox1 gene Forward primer (10pmol) with 1µL, Cox1 gene Reverse primer (10pmol) with 1µL and PCR water 13µL. then placed in standard Maxime PCR PreMix that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, stabilizer, and tracking dye). Then, all the PCR tubes were transferred into Exispin vortex centrifuge at 3000rpm for 3 min. Then placed in PCR Thermocycler by using conventional PCR thermocycler system at step: Initial Denaturation 95ºC at 5min for 1 repeat, Denaturation 95 ºC at 30sec, Annealing 58 ºC at 30sec, Extension 72 ºC at 2min for 35 cycle and Final extension 72 ºC at 5min for 1 repeat.

DNA sequencing and phylogenetic analysis

DNA sequencing and phylogenetic analysis PCR products of cox1 were purified and sequenced using both forward and reverse complements by Genetic Analyzer. A multiple sequence alignment was generated for the samples using the ClustalW (Thompson *et al*., 1994). A BLAST search was performed for each sequence to locate related sequences. The Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat *Hymenolepis diminuta* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis diminuta* isolates (MW454851.1 , MW454848.1, MW454845.1, and MW454844.1) were showed closed related to NCBI-BLAST *Hymenolepis diminuta* Iran isolates at total genetic changes (0.4-0.010%) (Figure 8).So the Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat *Hymenolepis nana* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis nana* isolates (MW454842.1) were showed closed related to NCBI-BLAST Hymenolepis nana Egypt isolate. The *Hymenolepis nana* the local *Hymenolepis nana* isolates (MW454843.1, MW454846.1, MW454847.1, and MW454850.1) showed closed related to NCBI-BLAST *Hymenolepis nana* Japan isolate. Whereas, the local *Hymenolepis nana* isolates (MW454849.1) showed new variant isolate at total genetic changes (0.025-0.0050%) (Figure 9).

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Result

Microscopic Examination

The present study revealed that the rat were infected with two species of cestodes (egg and worm), namely *H. nana* and *H. diminuta***.** Eggs of *H. nana* (Fig. 1) are oval or subspehrical and smaller, ranging 44–54 (length) × 38–44 µm (width). On the inner membrane are two poles, from which 4 to 8 polar filaments spread out between the two membranes. The oncosphere has six hooks.

The morphological feature of *Hymenolepis nana* (fig. 2) are characterized by smallest cestode, scolex of *H. nana* has four suckers, and an armed rostellum with a single circle of 20 to 30

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hooks that is clearly visible. Genital pores are unilateral. *H. nana* contains both male and female reproductive structures in each proglottid, This contains three testicles and one ovary (Fig. 3).

The tapeworm's egg of *H. diminuta* (Fig.4) is round, ringing from 70×80 (length) × 60-70 µm (width) in diameter with hexacanth embyreo and has no polar filaments in the inner shell. *Hymenolepis diminuta* are characterized by small in size but larger then *H. nana*. The scolex of has no hooks (unarmed) but have four suckers (fig. 5).

The prevalence of the *H. nana* and *H. diminuta* in the examined fecal samples of all rats was 25(25%) in microscopy examination were infected with Hymenolepiasis of four regions in Baghdad city. Also 18(18%) were infected with *Hymenolepis diminuta* and 7(7%) were infected with *Hymenolepis nana.* Higher prevalence 11(40.74%) were found in Abu Ghraib area with significant differences ($p \leq 0.01$) while lower prevalence 3(12%) was recorded in Al-Sha'ab area. While *H. diminuta* is the highest 7(26.92%) in Abu Ghraib with significant differences ($p \leq 0.01$) and *H. nana* was 3(13.63%) in Haifa Street with significant differences ($p \leq 0.05$) (Table1).

The results of this study revealed also differences in the distribution of parasites between sexes. Out of 67 male examined, 19(28.35%) were positive with significant differences ($p \leq 0.05$), while on the contrary, out of 33 female examined, 6(18.18%) were positive with *hymenolepis* spp. Infection number of *H. diminuta* 14(20.89%) and *H. nana* 5(7.46%) in male were higher prevalence than female (Table 2).

The prevalence of *H. nana* and *H. diminuta* according to months was also appeared between the examined rats. The results of this study revealed high prevalence rate 10(62. 5%) at September with significance differences ($p \leq 0.01$) than the other month which decreased in December 1(9.09%) and no infection in November. (Table3).

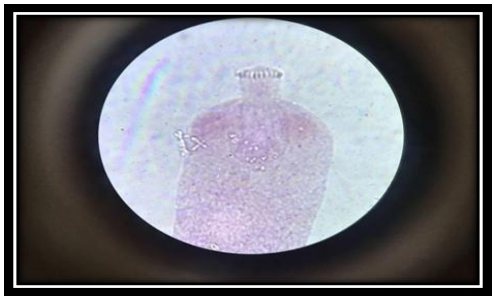

Figure 2: Scolex of *H.nana* staining with carmine stain X4

Figure 1: Egg of *H.nana* X40

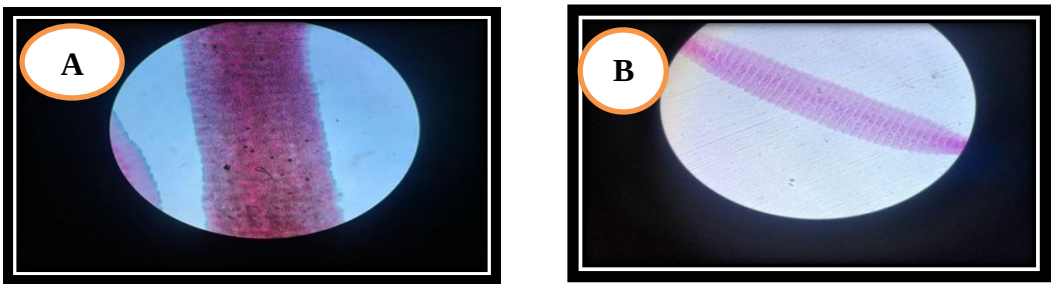

Figure 3: (A) Gravid and (B) mature segment of *Hymenolepis spp.* staining with carmine stain X4.

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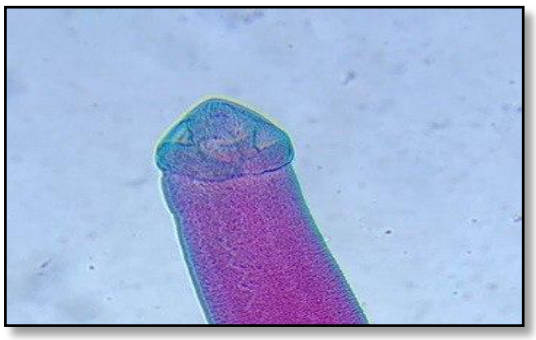

Figure 5:Scolex of *H.diminuta* staining with carmine stain X10

Figure 4:Egg of *H.diminuta* X40

Table (1): Prevalence of rats Hymenolepiasis according to areas of study

Area	Examination No.	Infection No.	Percentage (%)	Infection No. of *H. nana* and Percentage (%)	Infection No. of *H. diminuta* Percentage (%)
Abu Ghraib	27	11	40.74%	3(11.11%)	8(29.62%)
Al-Ameriya	26	8	30.76%	1(3.84%)	7(26.92%)
Al-Sha'ab	25	3	12%	0(0.00%)	3(12%)
Haifa Street	22	3	13.63%	3(13.63%)	0(0.00%)
Total no.	100	25	25%	7(7%)	18(18%)
Chi-Square (χ^2)	--	--	7.39 **	5.02 *	8.44 **

*** (P≤0.05), ** (P≤0.01).**

Table (2). Prevalence of rats Hymenolepiasis according to sex

Sex	Examination No.	Infection No.	Percentage (%)	Infection No. of *H.nana* and Percentage (%)	Infection No. of *H.diminuta* and Percentage (%)
Male	67	19	28.35%	5(7.46%)	14(20.89%)
Female	33	6	18.18%	2(6.06%)	4(12.12%)
Total	100	25	25%	7(7%)	18(18%)
Chi-Square (χ^2)	--	--	4.87 *	0.673 NS	4.06 *

*** (P≤0.05).**

Table (3): Prevalence of rats Hymenolepiasis according to months

Months	Examination No.	Infection No.	Percentage (%)	Infection No. of *H.nana* and Percentage (%)	Infection No. of *H.diminuta* and Percentage (%)
December	11	1	9.09%	0(0.00%)	1(9.09%)

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January	**8**	**1**	**12.5%**	**0(0.00%)**	**1(12.5%)**
February	**9**	**1**	**11.11%**	**1(11.11%)**	**0(0.00%)**
June	**7**	**1**	**14.28%**	**0(0.00%)**	**1(14.28%)**
July	**10**	**2**	**20%**	**2(20%)**	**0(0.00%)**
August	**13**	**5**	**38.46%**	**0(0.00%)**	**5(38.46%)**
September	**16**	**10**	**62. 5%**	**2(12.5%)**	**8(50%)**
October	**18**	**4**	**22.22%**	**1(5.55%)**	**3(16.66%)**
November	**8**	**0**	**0(0.00%)**	**0(0.00%)**	**0(0.00%)**
Total	**100**	**25**	**25%**	**7(7%)**	**18(18%)**
Chi-Square (χ^2)	**--**	**--**	**11.38 ****	**8.63 ****	**11.52 ****

** ($P \leq 0.01$).

Molecular analysis

Amplification of cox1 and 18S rRNA-ITS1 gene in *H. nana* and *H. diminuta* from rat

It was done in order to detect the presence of *hymenolepis* genes, and identify the specific genus and species, which using PCR. Then detect the presence of cox1 gene (700bp) region for 100 random isolated the sample. Agarose gel electrophoresis image that showed the PCR product analysis of mitochondrial cytochrome c oxidase subunit 1(cox1) gene in *Hymenolepis* sp. from Rats feces samples. (Figure: 6) Also agarose gel electrophoresis image that showed the Duplex PCR product analysis of 18S rRNA-ITS1 gene in *Hymenolepis nana* and *Hymenolepis diminuta* from Rat stool samples. Where, the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-20) were showed some positive PCR *Hymenolepis* nana at 535bp and *Hymenolepis diminuta* at 361bp duplex PCR product size (Figure: 7).

Multiple sequence alignment analysis of cytochrome c oxidase subunit 1 (cox1) gene in local Rat *Hymenolepis diminuta* isolates and NCBI-Genbank Hymenolepis diminuta country related isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool.Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in cytochrome c oxidase subunit 1 (cox1) gene between isolates. (Figure: 8).

Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat *Hymenolepis diminuta* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis diminuta* isolates (MW454844, MW454845, MW454848 and MW454851) were showed closed related to NCBI-BLAST *Hymenolepis diminuta* Iran isolates at total genetic changes (0.4-0.010%). Also Multiple sequence alignment analysis of cytochrome c oxidase subunit 1 (cox1) gene in local Rat *Hymenolepis nana* isolates and NCBI-Genbank Hymenolepis nana country related isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool. Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in cytochrome c oxidase subunit 1 (cox1) gene between isolates (Figure: 10).

Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat Hymenolepis nana isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean

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(UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis nana* isolates (MW454842.1) were showed closed related to NCBI-BLAST Hymenolepis nana Egypt isolate. The *Hymenolepis nana* the local *Hymenolepis nana* isolates (MW454843.1, MW454846.1, MW454847.1, and MW454850.1) showed closed related to NCBI-BLAST *Hymenolepis nana* Japan isolate. Whereas, the local *Hymenolepis nana* isolates (MW454849.1) showed new variant isolate at total genetic changes (0.025-0.0050%) (Figure; 11).

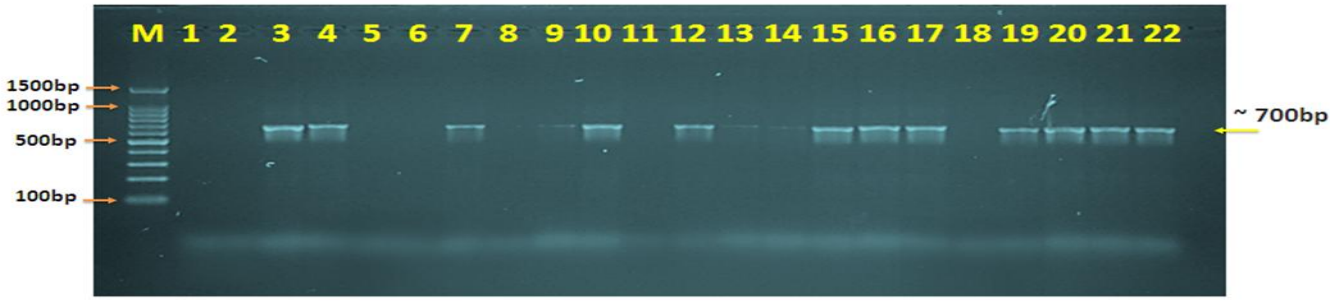

Figure (6): Agarose gel electrophoresis image that showed the PCR product analysis of mitochondrial cytochrome c oxidase subunit 1 (cox1) gene in *Hymenolepis* sp. from Rats feces samples. Where, the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-22) were showed some positive PCR amplification of (cox1) gene in *Hymenolepis* sp. at 700bp PCR product size.

Figure (7): Agarose gel electrophoresis image that showed the Duplex PCR product analysis of 18S rRNA-ITS1 gene in *Hymenolepis nana* and *Hymenolepis diminuta* from Rat stool samples. Where, the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-20) were showed some positive PCR Hymenolepis nana at 535bp and Hymenolepis diminuta at 361bp duplex PCR product size.

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MW454844.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
MK614218.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
MW454851.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
MW454848.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
MW454845.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
MN536015.1    TTGAGTTTAATTCCTGATGCTTTTGGGTTTTATGGGCTCTTGTTTGCCATGTTTTCTATT
KY079338.1    TTGAGTTTAATACCTGACGCTTTTGGATTTTATGGACTTTTATTTGCCATGTTTTCTATT
AF096244.2    TTGAGTTTAATACCTGACGCTTTTGGATTTTATGGACTTTTATTCGCCATGTTTTCTATT
              *********** ***** ******** ******** ** ** ** ***************

MW454844.1    GTTTGTTTAGGTAGAAGTGTTTGAGGGCATCATATGTTTACTGTTGGTTTAGATGTAAAG
MK614218.1    GTTTGTTTAGGTAGAAGTGTTTGAGGTCATCATATGTTTACTGTTGGTTTAGATGTAAAG
MW454851.1    GTTTGTTTAGGTAGAAGTGTTTGAGGGCATCATATGTTTACTGTTGGTTTAGATGTAAAG
MW454848.1    GTTTGTTTAGGTAGAAGTGTTTGAGGGCATCATATGTTTACTGTTGGTTTAGATGTAAAG
MW454845.1    GTTTGTTTAGGTAGAAGTGTTTGAGGGCATCATATGTTTACTGTTGGTTTAGATGTAAAG
MN536015.1    GTTTGTTTAGGTAGAAGTGTTTGAGGGCATCATATGTTTACTGTTGGTTTAGATGTAAAG
KY079338.1    GTTTGTTTAGGAAGAAGTGTTTGGGGCCATCATATGTTTACTGTTGGTTTAGATGTGAAG
AF096244.2    GTTTGTTTAGGAAGAAGTGTTTGGGGCCATCATATGTTTACTGTTGGTTTAGATGTGAAG
              *********** *********** ** ****************************** ***

MW454844.1    ACGGCAGTGTTCTTTAGATCTGTAACTATGATTATAGGGGTACCTACAGGAATTAAGGTG
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MK614218.1    ACGGCAGTGTTTTTTAGATCTGTAACTATGATTATAGGGGTACCTACAGGAATTAAGGTG
MW454851.1    ACGGCAGTGTTCTTTAGATCTGTAACTATGATTATAGGGGTACCTACAGGAATTAAGGTG
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MW454845.1    ACGGCAGTGTTCTTTAGATCTGTAACTATGATTATAGGGGTACCTACAGGAATTAAGGTG
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MW454845.1    TTTACTTGGTTATACATGCTTTTAAACTCTAAAGTTAATAAGGGGGATCCTATTGTTTGA
MN536015.1    TTTACTTGGTTATACATGCTTTTAAACTCTAAAGTTAATAAGGGGGATCCTGTTGTTTGA
KY079338.1    TTTACTTGGTTATATATGCTTTTAAACTCTAAAGTTAATAAGGGTGATCCTGTTGTTTGA
AF096244.2    TTTACTTGGTTATATATGCTTTTAAACTCTAAAGTTAATAAGGGTGATCCTGTTGTTTGA
              ************** **************************** ****** ********

MW454844.1    TGAATAGTGTCTTTTATCGTGTTATTTAGATTTGGAGGAGTTACAGGAATTATTTTATCT
MK614218.1    TGAATAGTGTCTTTTATTGTGTTATTTAGATTTGGAGGAGTTACAGGGATTATTTTATCT
MW454851.1    TGAATAGTGTCTTTTATCGTGTTATTTAGATTTGGAGGAGTTACAGGAATTATTTTATCT
MW454848.1    TGAATAGTGTCTTTTATCGTGTTATTTAGATTTGGAGGAGTTACAGGAATTATTTTATCT
MW454845.1    TGAATAGTGTCTTTTATCGTGTTATTTAGATTTGGAGGAGTTACAGGAATTATTTTATCT
MN536015.1    TGAATAGTGTCTTTTATCGTGTTATTTAGATTTGGAGGAGTTACAGGAATTATTTTATCT
KY079338.1    TGGATAGTGTCTTTTATTGTGTTATTTAGGTTTGGGGGAGTCACAGGAATTATTTTATCT
AF096244.2    TGGATAGTGTCTTTTATTGTGTTATTTAGGTTTGGGGGAGTCACAGGAATTATTTTATCT
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Figure (8): Multiple sequence alignment analysis of cytochrome c oxidase subunit 1 (cox1) gene in local Rat *Hymenolepis diminuta* isolates and NCBI-Genbank Hymenolepis diminuta country related isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool.Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in cytochrome c oxidase subunit 1 (cox1) gene between isolates.

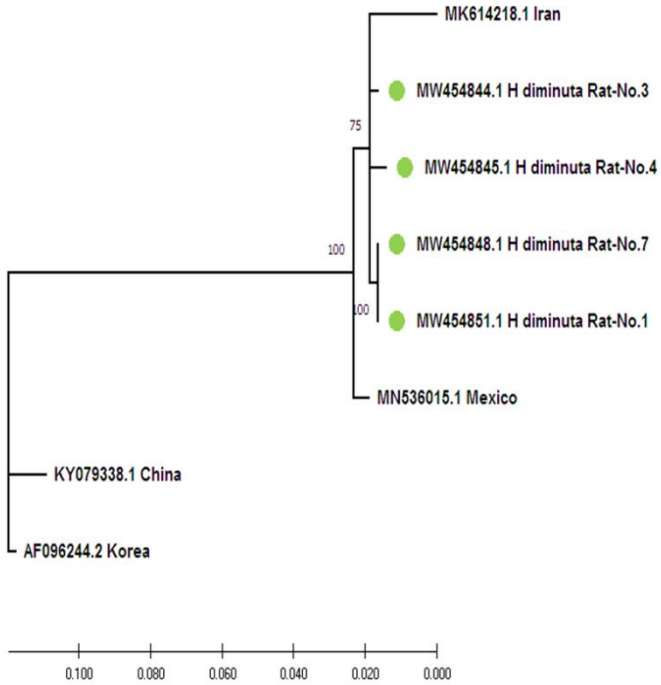

Figure (9): Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat *Hymenolepis diminuta* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis diminuta*

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isolates (MW454844, MW454845, MW454848 and MW454851) were showed closed related to NCBI-BLAST *Hymenolepis diminuta* Iran isolates at total genetic changes (0.4-0.010%).

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AP017666.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
MW454850.1    GGTATTATAGGTCATATAAATTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
MW454846.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
MW454843.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
MW454847.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
KY079336.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
MW454849.1    GGTATTATAGGTCATATATGTATAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
HM447234.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTAATTCCTGATGCTTTTGGGTTTTAT
GU433104.1    GGTATTATAGGTCATATATGTTTAAGATTGAGTTTGATTCCTGATGCTTTTGGGTTTTAT
MW454842.1    GGTATTATAGGTCATAAATGTTTAAGATTGAGTTTGATTCCTGATGCTTTTGGGTTTTAT
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AP017666.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454850.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454846.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454843.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454847.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
KY079336.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454849.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
HM447234.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTCGTTAGGTTGTAGTGTGTGGGCTCATCAT
GU433104.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
MW454842.1    GGTTTATTATTTGCTATGTTTTCTATAGTGTGCTTAGGTTGTAGTGTGTGGGCTCATCAT
              ******************************** ****************************
AP017666.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454850.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454846.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454843.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454847.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
KY079336.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454849.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
HM447234.1    ATGTTTACTGTTGGTTTGGATGTTAAGACGGCTGTATTTTTTAGGTCTGTGACTATGATT
GU433104.1    ATGTTTACTGTTGGTTTGGATGTTAAGACAGCTGTATTTTTTAGGTCTGTGACTATGATT
MW454842.1    ATGTTTACTGTTGGTTTGGATGTTAAGACAGCTGTATTTTTTAGGTCTGTGACTATGATT
              ***************************** ******************************
AP017666.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
MW454850.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
MW454846.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
MW454843.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
MW454847.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
KY079336.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
MW454849.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAACTCTATG
HM447234.1    ATAGGAATACCTACGGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAATTCTATG
GU433104.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAATTCTATG
MW454842.1    ATAGGAATACCTACTGGTATTAAGGTATTTACGTGGTTATATATGCTTTTAAATTCTATG
              ************** ****************************************** *******
AP017666.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
MW454850.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
MW454846.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
MW454843.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
MW454847.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
KY079336.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTCAGATTT
MW454849.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
HM447234.1    GCTAAAAAGAGTGATCCGGTGATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
GU433104.1    GCTAAAAAGAGTGATCCGGTAATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
MW454842.1    GCTAAAAAGAGTGATCCGGTAATATGGTGAATAGTATCATTTATTGTGTTGTTTAGATTT
              ******************** ********************************** *******
AP017666.1    GGTGGTGTGACTGGTATTATTTTGTCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454850.1    GGTGGTGTGACTGGTATTATTTTGTCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454846.1    GGTGGTGTGACTGGTATTATTTTGTCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454843.1    GGTGGTGTGACTGGTATTATTTTGTCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454847.1    GGTGGTGTGACTGGTATTATTTTGTCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
KY079336.1    GGTGGTGTGACCGGTATTATTTTATCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454849.1    GGTGGTGTGACTGGTATTATTTTATCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
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HM447234.1    GGTGGTGTGACTGGTATTATTTTATCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
GU433104.1    GGTGGTGTGACTGGTATTATTTTATCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
MW454842.1    GGTGGTGTGACTGGTATTATTTTATCAGCTTGTGTTTTGGATAAAGTTTTACATGATACT
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Figure (10): Multiple sequence alignment analysis of cytochrome c oxidase subunit 1 (cox1) gene in local Rat *Hymenolepis nana* isolates and NCBI-Genbank Hymenolepis nana country related isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool. Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in cytochrome c oxidase subunit 1 (cox1) gene between isolates.

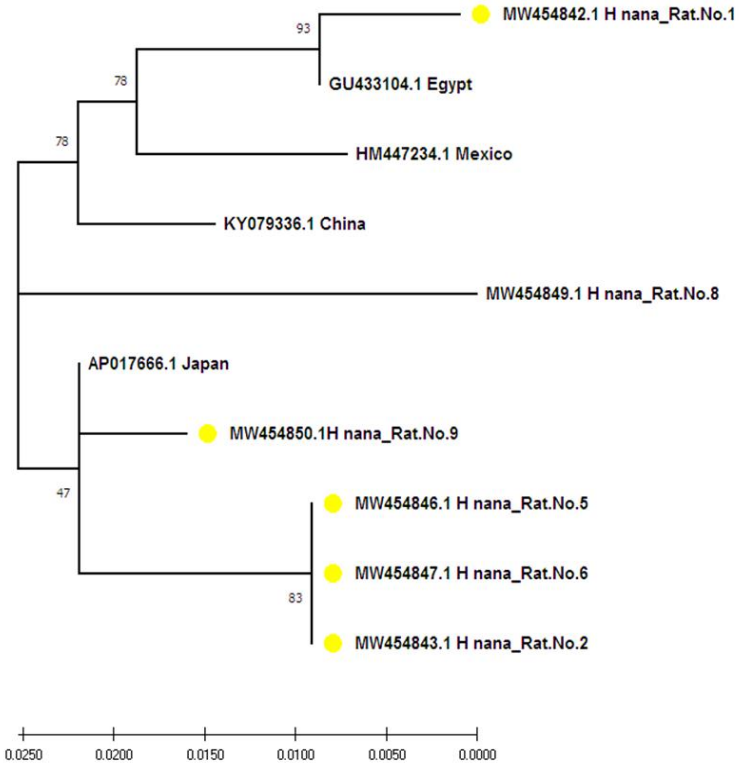

Figure (11): Phylogenetic tree analysis based cytochrome c oxidase subunit 1 (cox1) gene partial sequence in local Rat Hymenolepis nana isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *Hymenolepis nana* isolates (No.1) were showed closed related to NCBI-BLAST Hymenolepis nana Egypt isolate. The Hymenolepis nana the local Hymenolepis nana isolates (No.1, No.5, No.6, and No.9) showed closed related to NCBI-BLAST Hymenolepis nana Japan isolate. Whereas, the local *Hymenolepis nana* isolates (No.8) showed new variant isolate at total genetic changes (0.025-0.0050%).

Discussion

The results showed that the range measurement of length and width agreement with other measurement reported by some researchers Rawat *et al*. (2020) who mention that eggs specific to *H. diminuta.* it was yellowish-brown in colour and round to oval shaped and measured about 70-80

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(length)×60-70 µm(width) and having hexacanth embryo with three pairs of hooks and the absence of polar filaments. Also Panti-Maya *et al*. (2020) who recorded that the egg of *H. nana* was 44–54 (length)×38–44(width)µm with polar filaments. While disagreement with Ash *et al*. (1994) and Pérez-Chacón *et al*. (2017) recorded that the measurement of *H. nana* eggs was 30–47µm in diameter, and contain an oncosphere with six hooks surrounded by an inner membrane with polar thickenings from which four to eight polar filaments arise and extend to the space between the oncosphere and the outer shell whereas *H. diminuta* their eggs are yellowish and larger. They measure 60–80 µm in diameter and the oncosphere is separated from the outer membrane by a clear space without polar filaments.

The results of the present study of prevalence rats are in the ratio is close to with Fitte *et al*., (2017) who stated of total Hymenolepiasis prevalence at 20.4%. While the results are in disagreement with other scientific studies by Guddissa *et al*. (2011) who reported that the prevalence rat was 33.93 % in Ethiopian and Karim and Al-Salihi (2014) who recorded 3/4 (75%) rats with infected rats revealed 5- 8 adult worm that were attached on the intestinal wall in Iraq.

The present study showed relatively higher prevalence of *H. diminuta* (18%) followed by *H. nana* (7%). This result is in agreement with those observed by Guddissa *et al*. (2011) where the author reported the high prevalence of *H. diminuta* (26.79 %) followed by *H. nana* (7.1 %) in rats at Ethiopian. Further, Singh *et al*., (2020) who recorded that higher prevalence of *H. diminuta* (35%) followed by *H. nana* (23.3%). On the other hand, this result is in disagreement with Tijjania *et al*. (2020) who referred that the *H. nana* (19.1%) was higher than *H. diminuta* (16.8%) Also Tung *et al*. (2013) who found that *H. nana* was 21.8% whereas *H. diminuta* was 6.3% in the Taiwan.

The results of the present study revealed the differences in percentage of positive samples according to the sex. This result is in agreement with those observed by Ahmed *et al*.(2014) who stated the prevalence in male were much higher than female 43.8% and 29.3% respectively. Panti-May *et al*. (2017) who referred that the prevalence rates of male were higher than female 15.2% and 12.8%, respectively. On the other hand, this result is in disagreement with Mazhari et al. (2019) who referred that the prevalence rates of male were higher than female 58.3% and 41.7% respectively.

The highest percentage in male in comparison with female to be referable to travel of male from one population to the other getting more chance to contact infection from infected intermediate hosts (Ahmad *et al*., 2014).Our results are agreed with Ahmed *et al*. (2014) who found that the highest (48.0%) month-wise prevalence of *H. diminuta* during August whereas the lowest (28.0%) during January and the highest seasonal prevalence peak (45.4%) was during summer followed by spring (35.1%), autumn (31.9%) and the lowest (29.3%) in rats / mice captured during winter.

Isolates of *H. nana* and *H. diminuta* infecting rodents are morphologically identical; the only way they can be reliably distinguished is by comparing the parasite in each host using molecular techniques (Cheng *et al*., 2016). Genetic diversity of *H. nana* and *H. diminuta* has been studied using some genetic makers, such as cytochrome c oxidase subunit 1 (cox1) and the first and second internal transcribed spacer (18S rRNA-ITS1) regions of nuclear ribosomal DNA (rDNA) (Okamoto *et al*., 1997 and Macnish *et al*., 2002). In present study, genomic DNA was extracted from 100 rat specimens, from different geographical locations in Baghdad/Iraq. It was used three primers *Hymenolepis* sp Mitochondrial cox1 gene, *H. nana* 18S rRNA-ITS1 gene and *H. diminuta* 18S rRNA-ITS1 gene but the lengths of the amplified fragment were different for *H. nana* and *H. diminuta*, PCR amplification of (cox1) gene in *Hymenolepis* sp. at 700bp, PCR H. nana at 535bp

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and H. diminuta at 361bp duplex PCR product size. Using this technique aims at asserting the diagnosis of the sample already registered in the current study then being compared with the global sample in order to get knowledge on the nitrogen bases of cox1 gene and 18S rRNA-ITS1 gene in *H. nana* and *H. diminuta* from Rat stool samples; as this technique is regarded as one of the most precise methods of diagnosis, and that can be useful in the genetic and biological studies in comparison with the previous local or global studies (Shubber *et al*., 2019)**.**

By the analysis of the phylogenetic tree; convergence of nitrogen bases for the two types of cestoda (*H.nana* & *H.diminuta*) with those samples globally registered has been noticed: the samples of the *H. diminuta* in the current study is asymptotic to that having the Serial No. MK614218.1 registered in Iran by (Makki *et al*., 2017), the Serial No. MN536015.1 registered in Mexico by (Panti-Maya *et al*., 2020), the Serial No. KY079338.1 registered in China by (Yang *et al*., 2017) and the Serial No. AF096244.2 registered in Korea by (LEE *et al*., 2007).

Besides, it has been noticed that the sample of *H. nana* in the current study are approaching that one having the serial No. GU433103.1 registered in Egypt by (Kandil *et al*., 2010), the serial No. AP017666.1 registered in Japan by (Cheng *et al*., 2015), the serial No. KY079336.1 registered in China by (Yang *et al*., 2017) and the serial No. HM447234.1 registered in Mexico by (Panti-May *et al*., 2020).

In conclusion, two species of *Hymenolepis* were identified in Baghdad/Iraq *H. nana* and *H. diminuta* from Rats. The morphological and phylogenetic analysis allowed us the correct identification of the *Hymenolepis* spp. The occurrence of these *Hymenolepis* cestodes in Baghdad to monitor rodent populations and potential intermediate hosts to identify sources of Hymenolepiasis of poor communities and avoid transmission of *Hymenolepis* spp. to human

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