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Molecular Diagnosis of *Hymenolepis nana* Parasite in House Rats and Children in Babylon Province, Iraq

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DHAAT and SMKAB did preparation, methodology, writing, investigation and editing. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: *Hymenolepiasis* in humans is typically caused by the dwarf tapeworm *Hymenolepis* nana, or occasionally by the rodent tapeworm *Hymenolepis diminuta*. The current study aimed to detect and *Hymenolepis nana* infecting Children and House rate using molecular techniques.
Methods: Seventy five samples of Children and seventy five of House rats were examined from December 2023 to March 2024 by the molecular techniques.
Results: The results showed that the overall percentage of *Hymenolepis nana* infection was 13.3 (10 out of 75) in (Children) (female 31 and male 44) and in House rats was 16% (12 out of 75). These results also found that the infected males recorded the highest infection rate compared with infected females, where the percentages were (Children) male 10.9% (7/44) and female 9.7%

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(3/31), and (House rats) respectively. In addition, 75 stool samples of humans (children) in different areas (Al- Qasim, Al- Musayyib, Al- Hilla, Al- Kifl, Al- Hamza Al- Gharbi, and Al- Shomali) and study the effects of sex and areas with ages in humans. The infection rates of *Hymenolepis* parasite in humans by using molecular, the study revealed in humans on the infection rates by molecular. In humans *H. nana* was showed an infection rate 13.3% (10/75), the high infection rate was 20% (2/10) in Al Musayyib and 12.5% (1/8) in Al Kifl and it was 5.9% (1/17) in Al- Qasime with no significant difference, 20% (2/10) in Al- Hamza Al Gharbi, and 10% (1/10) in Al Shomali. **Conclusion:** The results were showed the parasite *H. nana* of the infected humans in this study tack three gropes (1-5) years about 20% (5/25), (6-10) years, 9.4% (3/32) and (11-15) years 11.1% (2/18).

Keywords: Hymenolepiasis; children; mt COX1; immunosuppression; cysticercoid.

1. INTRODUCTION

Hymenolepiasis in humans is typically caused by the dwarf tapeworm *Hymenolepis* nana, or occasionally by the rodent tapeworm *Hymenolepis diminuta*. The elaborate life cycles of these tapeworms involve adult stages in the small intestines of humans and rodents and larval stages in insects. The larval forms of H. nana can also enter and mature in the human gut, allowing H. nana to go through its complete life cycle in the human body and multiply through self-infection, thus avoiding the need for an insect host. Research on animals shows that T-lymphocyte–mediated immunity plays a crucial role in protecting against hyperinfection caused by these parasites [1]. Around 93 to 96 hours later, the cysticercoid exits the mucosa and excysts in the small intestine lumen [2].

Arthropods like Tribolium confusum and Tenebrio molitor are the primary intermediate hosts known for transmitting the larvae of H. nana. Fleas like Xenopsylla cheopis, Pulex irritans, and Ctenocephalides spp. have also been linked to spreading this parasite [3].

Hymenolepis nana can be easily passed from one person to another through direct transmission. Even though H. nana lives for just a few weeks, it is continuously replenished by succeeding generations that go through their life cycle within the human intestine. H. nana has the potential to spread widely in children's institutions and cause outbreaks. Immunosuppression, whether by T-cell deprivation or induced steroid treatment, significantly impacts H. nana infection in mice as it promotes the multiplication of abnormal cysticercoids in viscera [4]. Additionally, the presence and spread of Hymenolepis spp. Across 17 different nations, such as Bahrain, Cyprus, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Kingdom of Saudi Arabia (KSA), Syria, Turkey, United Arab Emirates (UAE), and Yemen. The majority of individuals in this area experience low economic status [5]. The region has become a hub for various emerging and re-emerging diseases such as rodent-borne parasitic infections due to factors like cultural diversity, inadequate economic policies, governance issues, population growth, lack of quality education, gender bias, poor infrastructure, and ongoing wars and conflicts [6].

In underprivileged areas, humans experience the highest infection rates as a result of potential direct fecal-oral and human-to-human transmission. Numerous studies have been conducted globally to assess and establish the prevalence and associated risk factors of gastrointestinal parasites in house mice, laboratory animals, particularly mice and rats, and humans [7]. Identifying the morphological features of causative species and diagnosing Hymenolepiasis often involves using eggs found in the host's feces [8]. However, PCR-based molecular techniques not only increase detection rates of parasites, but also provide the accurate species differentiation and their genetic characterizations also the polymerase chain reaction (PCR) has provided procedure in identification of parasites [9].

The ITS1 and ITS2 regions of nuclear ribosomal RNA gene can assist in solving taxonomic problems and differentiating between closely related genera and species. Additionally, mitochondrial genome sequences have been shown to be valuable and dependable markers for population genetics and systematic research. Molecular biology involves methods like PCR and RFLP that are quick and easy ways to identify parasites (Navone, 2007).

Mitochondrial genome sequences have demonstrated their utility and dependability as genetic markers for population genetics and

systematic studies [10,11]. The mt COX1 marker has been effectively utilized to determine Cyclophyllidea phylogenetic relationships at family and genus levels [10]. This research was conducted to determine the frequency of Hymenolepis nana parasites in both house rats and children in Babylon governorate/Iraq in order to assess the potential risks to children.

2. MATERIALS AND METHODS

2.1 Samples Collection

The present study was done in department of parasitology of veterinary medicine in the AL-Qasim green university, the study was persistent from December 2023 till April 2024 a surveillance study was done at Babylon.

Seventy-five individuals of various age ranges, from one to fifteen years old, were involved in the research (31 female and 44 male). Each participant provided one stool sample, which was collected directly. Samples of stool were examined to detect parasitic forms (such as scolex, segments, and eggs), and details about the individuals' gender and location were documented. The sample of feces needs to be gathered in a sterile and empty container with a secured lid [12]. Microscopic examination is the initial method used to identify an egg under a microscope. Seventy-five samples were obtained from feces of various elderly rats. Before starting the experimental trial, it was necessary to make sure that the rats were not carrying any parasites by examining their feces with traditional methods. The rat is euthanized and then the intestines are examined during a post-mortem. Fecal samples are collected from the intestines using a swab placed in a cup, and then brought to the laboratory. To check for the presence or absence of the parasite. The stool samples were gathered and examined with a microscope to demonstrate the presence of eggs.

Dissecting and gathering parasites: Rats caught by the tail are euthanized in a humane manner with anesthetic (9:1, ketamine, and xylazine) per 100 gm of body weight. The method of concentrating formalin ethyl acetate was used to identify eggs in stool samples fixed with formalin. Cestodes were directly removed from the intestine and then transferred to different plastic containers. The next step was to take the samples to the Parasitology Laboratory at the School of Veterinary Medicine for analysis [13]. For the purpose to prepare and stain the permanent slides, they were first dehydrated in various alcohol grades, cleaned in xylene, and then mounted in Canada balsam. Following their morphological classification under a microscope were used.

2.2 Molecular Results

The current study noted that infection in Al Musayyib and Al Hamza Al Gharbi was highly asignificant than other area then followed by Al Hilla center, but significantly lowered in Al Qasim to reach percent about 5%.

The present study according to sex with PCR technique showed that male infection rate was 15.9% which was highly a significant than female 9.7.

2.2.1 Human (PCR)

Hymenolepis nana isolate sub-unit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, complete sequence; and 5.8S ribosomal RNA gene, partial sequence. The PCR primers for house rats and Children was designed in this study by using NCBI dbSNP database.

Table 1. Infection rate of *Hymenolepis nana* in humans according to areas of study in (PCR)

Areas	No. of the exam. Samples	Positive Samples	
		No.	% of total
Al Musayyib	10	2	20
Al Hilla	20	3	15
Al Kifl	8	1	12.5
Al Hamza Al Gharbi	10	2	20
Al Qasim	17	1	5.9
Al Shomali	10	1	10
Total	**75**	**10**	**9.3**
X^2	**1.735011**		
P value	**0.884460 NS**		

NS: No Significant differences at (P≤0.05)

Table 2. Infection of Hymenolepis nana in humans according to sex in (PCR)

Gender	No. of Samples Examined	Positive Samples	
		No. of positive	Percentage of total (%)
Male	44	7	15.9
Female	31	3	9.7
Total	75	10	13.3
X^2	0.611183		
P value	0.434343NS		

NS: No Significant differences at (P≤0.05).

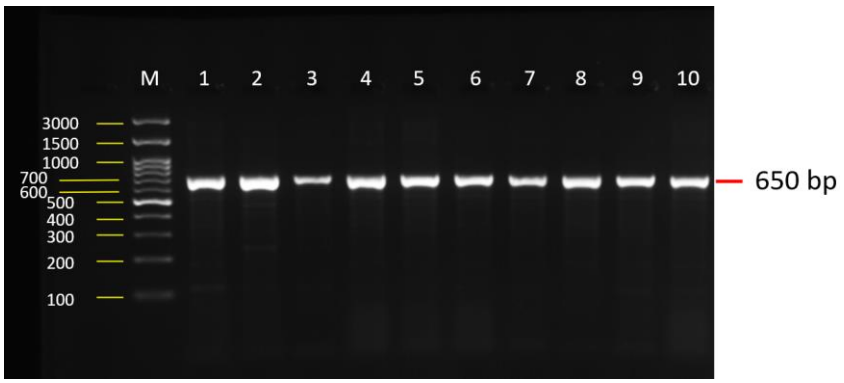

Fig. 1. Agarose gel electrophoresis image (agarose 1.5 %) shows the amplicons of *Rodentolepis nana* (1-10) represent positive samples isolated from human infection within a specific genetic region (internal transcribed spacer 1). M is molecular marker from (Genedirex, Korea)

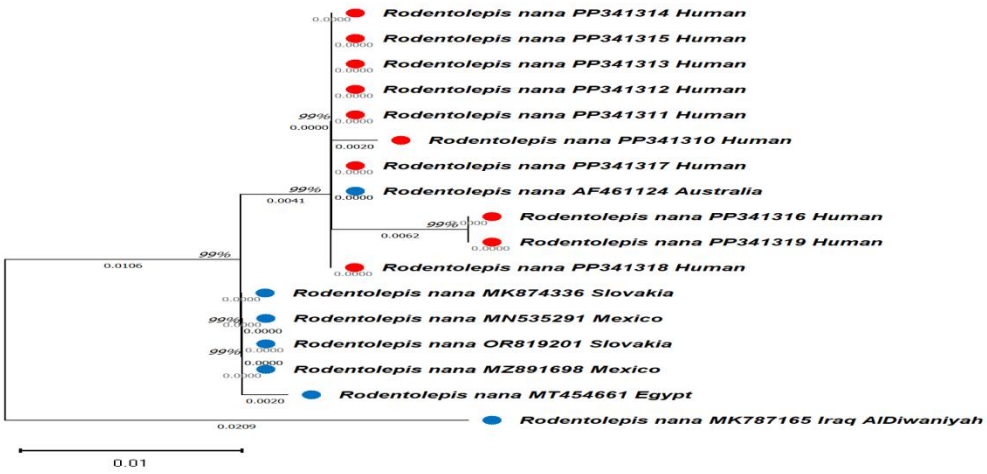

Fig. 2. Evolutionary analysis by maximum likelihood method of *Rodentolepis nana* in human infection

2.3 Phylogenic Tree

The Maximum Likelihood method was utilized to deduce the evolutionary history. The tree's branches are drawn to scale, measured in substitutions per site. The percentage of locations on the tree where there is a minimum of 1 clear base in any one sequence in every branch of the family is indicated alongside every inner node. This examination included 17 nucleotide sequences. The final dataset contained a combined total of 493 positions. MEGA11 was used for conducting evolutionary analyses.

2.4 The NCBI-BLAST Homology Sequence

The NCBI-BLAST Homology Sequence identity (%) between local isolates from rat infection that were deposited in gene bank with obtained accession numbers (PP341320, PP341321, PP341322, PP341323, PP341324, PP341325, PP341326, PP341327, PP341328, and PP341329) and compared with other NCBI-BLAST deposited global isolates.

2.4.1 Moleculer result of house rats

The present study in tab (3) noted that Infection in rate of Hymenolepis nana in House rats according to sex was there is no asignificant difference in male and female rats.

The evolutionary history was inferred by using the Maximum Likelihood method. The tree is accurately depicted, with branch lengths measured in substitutions per site (below the branches). Next to each internal node in the tree, the percentage of sites containing at least one clear base in at least one sequence for every descendant clade is displayed. This examination comprised of 17 nucleotide sequences. The final dataset contained a grand total of 493 positions. MEGA11 was used to perform evolutionary analyses.

2.5 Analysis by Comparison of Human and Rat Infection

The Maximum Likelihood method and the Tamura-Nei model were utilized to deduce the evolutionary history. The tree is accurately depicted, with branch lengths representing substitutions per site (below branches). This examination included 27 nucleotide sequences. The final dataset contained a grand total of 493 positions. MEGA11 was used for conducting evolutionary analyses.

The result of this study is agreement with some previous study was done by Franssen et al. [14] who showed that the results of brown rats in the Netherlands, recorded 10.2% for H. diminuta and 4.1% for H. nana. Also, Yang et al. [15] recorded that the H. diminuta (14.9%) a higher infection rate than H. nana (6.1%) using PCR in China. However, the results are in disagreement with Cheng et al. [16]. recorded higher infection rate of H. nana 72.97% than H. diminuta 71.04% in China; and Tresnani et al. [17] who showed that from PCR results 35 DNA samples suspected for Hymenolepis worms, only three samples were positive for Hymenolepis spp. 2 samples for H. nana and 1 sample for H. diminuta from rats in Indonesia.

The results of current study shown the genomic DNA that extracted from 100 mice samples; include 19(19%) worms 10(52.63%) worms of H. nana and 9 (47.36%) worms of H. diminuta in house and laboratory mice. The results agree with Okamoto et al. [18] who examined partial sequences from the COX1 gene and were infection rate of H. nana 18.2% comparative H. diminuta was 16.6%. Also Mohammadzadeh et al. [19] who reported the genomic diversity of 16 H. nana with the origin of Shiraz and Tehran

Table 3. The NCBI-BLAST Homology Sequence identity (%) between local isolates of *Rodentolepis nana* from human infection that were deposited in gene bank with obtained accession numbers (PP341310, PP341311, PP341312, PP341313, PP341314, PP341315, PP341316, PP341317, PP341318, and PP341319) and compared with other NCBI-BLAST deposited global isolates

Sample #	Accession #	The Homology Sequence identity (%) of NCBI-BLAST			
		Identification	Accession number of Gene Bank	Region	The Identity (%)
1	PP341310	*Rodentolepis nana*	AF461124	Australia	99.59
2	PP341311	*Rodentolepis nana*	MN535291	Mexico	99.59
3	PP341312	*Rodentolepis nana*	OR819201	Slovakia	99.59
4	PP341313	*Rodentolepis nana*	MZ891698	Mexico	99.59
5	PP341314	*Rodentolepis nana*	MK874336	Slovakia	99.19
6	PP341315	*Rodentolepis nana*	MT454661	Egypt	99.19
7	PP341316	*Rodentolepis nana*	MK787165	Iraq_AlDiwaniyah	95.49
8	PP341317	*Rodentolepis nana*	AF461124	Australia	99.80
9	PP341318	*Rodentolepis nana*	MN535291	Mexico	99.59
10	**PP341319**	*Rodentolepis nana*	OR819201	Slovakia	98.78

Table 4. Infection in rate of Hymenolepis nana in House rats according to sex in (PCR)

Gender	No. of Samples Examined	Positive Samples	
		No. of Positive	Percentage of Total (%)
Male	47	8	17
Female	24	4	16.7
Total	71	12	16.9
X^2	0.001422		
P value	0.969915 NS		

NS: No Significant differences at (P≤0.05)

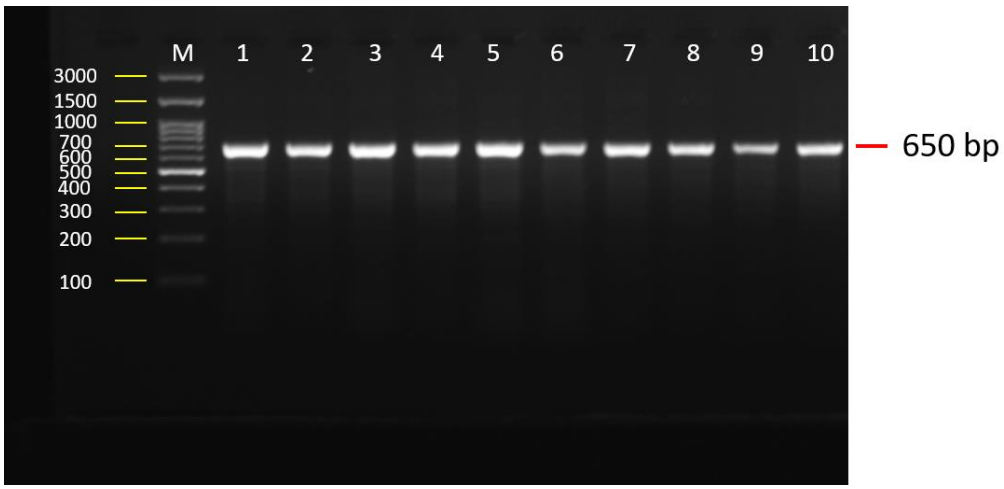

Fig. 3. Agarose gel electrophoresis image (agarose 1.5 %; at 5 volt/ cm for 1 hour) shows the amplicons of *Rodentolepis nana* (1-10) represent positive samples isolated from rat infection within a specific genetic region (internal transcribed spacer 1). M is molecular marker from (Genedirex, Korea)

Table 5. The NCBI-BLAST Homology Sequence identity (%) between local isolates from rat infection that were deposited in gene bank with obtained accession numbers (PP341320, PP341321, PP341322, PP341323, PP341324, PP341325, PP341326, PP341327, PP341328, and PP341329) and compared with other NCBI-BLAST deposited global isolates

Sample #	Accession #	The Homology Sequence identity (%) of NCBI-BLAST			
2	PP341321	*Hymenolepis nana*	MN535291	Mexico	99.59
3	PP341322	*Hymenolepis nana*	OR819201	Slovakia	99.39
4	PP341323	*Hymenolepis nana*	MZ891698	Mexico	99.59
5	PP341324	*Hymenolepis nana*	MK874336	Slovakia	99.19
6	PP341325	*Hymenolepis nana*	MT454661	Egypt	98.98
7	**PP341326**	***Hymenolepis nana***	MK787165	Iraq AlDiwaniyah	96.11
8	**PP341327**	***Hymenolepis nana***	AF461124	Australia	99.80
9	**PP341328**	***Hymenolepis nana***	MN535291	Mexico	99.59
10	**PP341329**	***Hymenolepis nana***	OR819201	Slovakia	99.59

were studied among the worms of mice and rats by randomly amplified polymorphic DNA (RAPD-81 PCR), and Jarońová et al. (2019) who found the parasite of H. nana 17.1 % and H. diminuta 15.9% by using PCR for COX1 gene. Study was described the occurrence of H. nana and H. diminuta human in Baghdad Province. Results of human cases of Hymenolepiasis caused by H. nana 8/10(80%) and H. diminuta 2/10(20%) have been reported in the investigated areas. Our findings support Kandil et al. (2010) who focused on the cytochrome C oxidase gene, particularly codons in subunit 1 (COX1), of H. diminuta and H. nana Egyptian isolates. They analyzed samples from adult eggs and worms, as well as hosts (human and rat), by amplifying,

sequencing, and aligning them. Panti-May et al. [20] also discussed molecular characterization and phylogenetic analysis using the COX1 gene and ribosomal ITS1 region, confirming the identity of cestodes from Yucatan/Mexico [21-23]. The phylogeny showed genetic differences within H. nana (0-5%), H. microstoma (0-0.4%), and H. diminuta (0-6.5%), indicating the presence of diverse species infecting humans and rodents [24,25]. Future studies may explore why the male ratio is greater than female that suggested in this study [26,27].

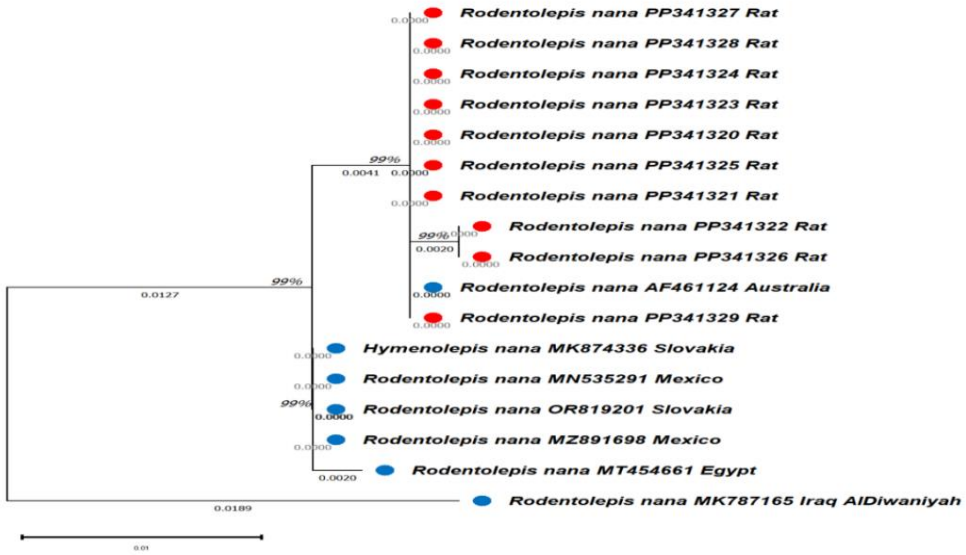

Fig. 4. Evolutionary analysis by maximum likelihood method of the identified sequences of Rodentolepis nana from rat isolates

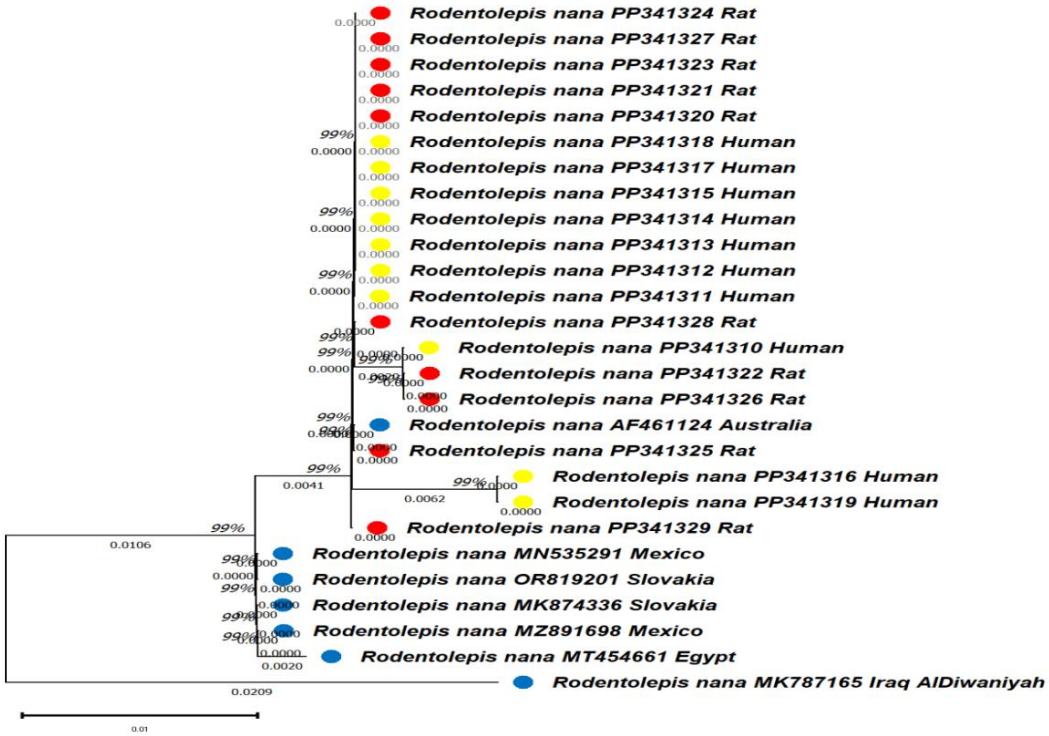

Fig. 5. Evolutionary analysis by maximum likelihood method of the identified sequences in human and rat isolates

3. CONCLUSION

The study identified Hymenolepis nana infections in both children and house rats using molecular techniques. The overall infection rate was 13.3% in children and 16% in house rats. Male children exhibited a higher infection rate (10.9%) compared to females (9.7%). Among the house rats, males also had a higher infection rate. Geographical variations in infection rates were observed in children, with the highest rate (20%) in Al Musayyib and Al Hamza Al Gharbi. The infection was most prevalent in children aged 1-5 years (20%), followed by those aged 11-15 years (11.1%), and least in those aged 6-10 years (9.4%). These findings highlight the need for targeted interventions to control H. nana infections in both children and house rats.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Depending on scientific committee instructions in Al-Qasim Green University at number of 411/2023.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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