Comparative Study morphological and Molecular for infected Rodent with two cestoda Hymenolepis nana & H. diminuta In Al-Diwaniyah City, South Of Iraq

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Abstract. The study aimed at comparing infection of Hymenolepis nana with that of H. diminuta during the study period from November 2016 to August 2017. A total of 220 rodents were identified including Mus musculus(89), Rattus norvegicus(37), R. rattus(48) & Swiss albino(46). Under anesthesia by chloroform, specimen were dissected, seeking parasitic helminths within digesting bowels. It was found that Mus musculus, Rattus rattus, Rattus norvegicus and Swiss Albino were infected by H. nana (4.4%, 4.1%, 8.1%, 13%) respectively, comparison with Mus musculus, Rattus rattus and Rattus norvegicus were infected by H. diminuta (31.4%, 39.5%, 13.5%) respectively. Diagnosis is confirmed by using PCR technique for H. nana using ITS1 gene, and for H. diminuta using ITS2 gene. In addition to diagnosis is confirmed by using Gen Sequencer technique, throughout the analyzing of phylogenetic tree leading to learn the extent of convergence between the local specimens and the global ones, the diagnosis of these helminthes by using this technique is considered the first in Iraq.

Key words: Rodents, Hymenolepis nana, H. diminuta, Molecular, ITS1, ITS2.

Introduction:
Rodents play a big role in conveying a number of viral, riktsia, bacterial and fungal diseases, as well as parasitic ones to the human and his tame and wild animals (Al-Musawi, 1982)[1]. Rodents have sanitary and economic important. Sanitarily, they are regarded as a dangerous conveyer of diseases as they convey and spread a number of diseases either directly though contaminating food with urine and feces. They form intermediate hosts, reservoir, carrier or vector of parasites, or indirectly through arthropoda – blood suckers that intrudes externally in rodents such as: Mites, Lice, Fleas and Ticks(Meehan, 1984)[2].

In Baghdad, through a study made by Al-Zahidi (2001)[3] comprising 97 specimen of Mus musculus, other 36 of Rattus rattus and other 29 of Rattus norvegicus, revealed that Mus musculus was infected with H. diminuta at 1% while Rattus rattus was infected with H. nana at 13.9% and Rattus norvegicus was infected with H. diminuta at 20.7 and H. nana at 20.7 as well. In the study made by Al-Murshidi (2001)[4] in Al-Hilla investigating internal parasites infecting Mus musculus and Rattus rattus finding...
that *Rattus rattus* was infected with *H. nana* at 8.1% and *Rattus rattus* was infected with *H. diminuta* at 13.5%, while *Mus musculus* was infected with the same parasite at 6.9% . Al-Zubeidi study (2007)[5] found that *Mus musculus* was infected with *H. nana* at 8.4% and *H. diminuta* at 15.3%. Hasson (2010)[6] had accomplished through a study he made investigating parasites. The study included anatomizing 186 rodents (62 specimen of *Rattus rattus*, 89 of *Rattus norvegicus* and 35 of *Mus musculus* . It found that the *Mus musculus* was infected with *H. nana* at 3.5% and with *H. diminuta* at 4.4% while the *Rattus norvegicus* was infected with *H. nana* at 7.3% and with *H. diminuta* at 12.5%. In study of Okamoto et al., (1997)[7] was made on the Cestoda that belonged to hymenolepidid family of *Rattus rattus* and hamster. The ratio Neoclitides diphrene among *H. diminuta* was 16.6%, *H. microstoma* was 18.2%, for the CO1 gene in *H. diminuta* was 21.3% and the ITS1 gene in *H. nana* was 22.9%. Awwad et al., (2001)[8] in his study made in Egypt an investigation for the *H. diminuta* and *H. nana* in rats that was molecularly diagnosed by using the PCR/RFLPs technique. In Iran, Mohammadzadeh et al., (2007)[9] molecularly diagnosed *H. nana* from mice and rats by using the RAPD-PCR technique. In Spain, particularly in Caari Islands, Foronda et al., (2011)[10] made his study on *Mus musculus* , *Rattus norvegicus* and *Rattus rattus* using the PCR technique to diagnose the *H. diminuta*, the result was infection at 9% using CO1 gene. In East Asia countries, particularly in Indonisia, Tresnani et al., (2016)[11] made his investigation for *H. diminuta* and *H. nana* totally and molecularly using the PCR technique with the help of ITS1 gene of *Rattus rattus*. In China, Yang et al., (2017)[12] made his molecular diagnostic study on the *Rattus norvegicus* , the study found an infection of *H. nana* at 6.1% and *H. diminuta* at 14.9% for the ITS2 gene.

**Materials & Methods**

Metal hunting devices with dimension of 23cm length, and 11cm width & height have been used cucumber, carrots, bread and fat have given as baits. Chloroform has been used for anesthesia of rodents before making examinations to check up internal parasites. Rodents have been anatomiized by making a cut along the abdominal zone starting from the anus to abdomen the head using scissor so that abdomen can be clearly observed. After anatomizing a rodent, abdomen are cut from their two connection points, anus and pharynx, then divided into four parts: Esophagus, stomach, small intestine and large intestine. These parts are put in petri dishes containing normal saline, then opened with a little scissor, and left for 5-10 minutes to let cestoda get down the bottom. Clear liquid then is poured out leaving cestoda at the bottom. Enlarging lens is use to examine the left. The parts having the cestoda are put in a water bath at a 37°C for 15-30 minutes, in order to isolate the scolex from the intestines of the infected rodent. Worms are cleaned with normal saline to remove undesirable material with a dropper. They are then pressed within two pieces of glass isolated by Vaseline so as to get best extension of the worms without damaging their bodies. They are fixed and kept in the ethanol 70% and then conveyed to the acitocarmine dye for no more than five minutes. They are conveyed to the gradually concentrated of ethanol (70%, 80%, 90%, 100%) for one hour for each concentration. They are then conveyed the ethanol 100% and xylenes (1:1) for half an hour. Finally, they are conveyed, for three minutes, to the xylene. The sample id relayed on a glass slides for examining and diagnosing (Garcia and Ash, 1979)[13].

**Polymerase chain reaction(PCR)**

We use two types of primers for (PCR): ITS1 to diagnosing *H. nana* ;according to primers that used from researcher trsnsani et al., (2016)[11], and ITS2 to diagnosing *H. diminuta nana* ;according to primers that used from researcher Rammath et al., (2014)[14].

| Primer | Sequence | Amplicon |
|--------|----------|----------|
| **ITS1** | 5’ GCAGAAAGGATACCTACACGTTC 3’ | 646 bp |
| F      | 5’ GCTCGACTCTTCATCGATCCACG 3’ |          |
| R      | 5’ GGTACCGGGTGGATACGCTCGTG 3’ | 791 bp  |
| **ITS2** | 5’ GGGATCTCTGAAGTTATTCTTTCTCCGC 3’ |          |
(Genomic DNA extraction kit) from DSBIO company, extracting accomplished according to company protocol.

The PCR products were visualized using 0.8 % agarose gel through electrophoresis at 80 V for 1 h. The agarose gel was stained with Ethidium bromide (Promega).

**Results**

An *H. nana* been extracted off intestines of rodents, it's found that the highest infection is in the Swiss Albino at 13%, *Rattus norvegicus* at 8.1%, *Mus musculus* at 4.5% and *Rattus rattus* at 4.1%.

**Table 5:** Infection percentage of pinworms isolated from rodents:

| Rodent species | Number examined | Parascript species         | Total % infected | Females % infected | Males % infected |
|----------------|-----------------|-----------------------------|------------------|--------------------|------------------|
| Mus musculus   | 89              | *Hymenolepis nana*          | 4                | 2.2                | 2.2              |
| Rattus norvegicus | 37          | *Hymenolepis diminuta*      | 31.5             | 14.6               | 16.8             |
This diagnosis has been ascertained using polymer chain Reaction (PCR). The electrical relaying by using the agarose gel at 0.8% has shown results of examining the PCR of *ITS1* gene of the cestoda in the molecular size 646 bp. *H. diminuta* has been extracted off intestines of rodents too, finding that the highest infection was in *Rattus rattus* at 37.5%, in *Mus musculus* at 31.5%.

The diagnosis is ascertained by using the examination of the Polymer Chain Reaction (PCR). The electrical relaying has revealed, by using agarose gel, the results of examining the PCR gene *ITS2* which is diagnosed by the molecular size 791 bp of the cestoda.
Phylogenetic tree analysis of *H. nana* & *H. diminuta*: Consequences of (PCR) examination has shown, by using (Mega 6) program, for the analysis of the phylogenetic tree of the two types *H. nana* & *H. diminuta* a clear convergence in the nitrogen bases of these sample with those globally registered which have the serial No. : MF067416.1, MF143799.1, AF461124.1, LM405059.1 and KJ917784.1. Consequences have shown congruency of the nitrogen bases of *H. nana* (local sample No.1) with the one globally registered at 96%, and with the other one globally registered at 96%, and with the that globally registered at 95% as well. Congruency of the nitrogen bases sequences of the *H. nana*, local sample No.2 with the one globally registered at a rate of 97% has been noticed, as well as congruency with the sample globally registered at a rate of 97% and that one globally registered at rate of 96%. Congruency of nitrogen bases sequences of the *H. diminuta* of the local sample No.3 with the one globally registered at a congruency rate of 91% has been noticed. Rate of congruency of the sample globally registered represents 91%. Congruency of the nitrogen bases sequences of the *H. diminuta* local sample No.4 with the one globally registered at a congruency rate of 92% is made sure. This congruency represents a rate of 92% with the one globally registered. Consequences has shown a genetic dimension at a rate of 3% between the *H. diminuta*, which is locally registered, with those globally registered.
Discussion:
The length of *H. diminuta* is 20-60mm with a width of 4mm in comparison with the length of *H. nana* that comes to 20-40mm with a width of 1mm (Zeibig, 2013)[15]. The *H. nana* scolex is formed of four suckers and is armed with retractable rostellum bearing a single row of 20–30 hooks, in comparison with *H. diminuta* whose rostellum has no hooks (Robert & Janovy, 2009)[16]. The shape of egg of the *H. diminuta* is spherical contains an embryo which possesses three pairs of small hooks in comparison with the egg of *H. nana* that has an oval shape and the embryo having polar scars ending with eight polar filaments each (Baker, 2007)[17].

Rodents infection with *H. nana* is respectively: 4.4%, 8.1%, 4.1% and 13% in the Mus musculus, Rattus rattus, Rattus norvegicus and Swiss Albino. The diagnosis is ascertained by using the (PCR) technique. Those consequences came less that what Al-Zahidi (2001)[3] registered in the Rattus norvegicus and Rattus rattus. They were : 13.9% and 20.7% respectively. Al-Zubeidi study (2007)[5] foud that it was 8.4% in the Mus musculus. It was approaching the study made by Al-Murshidi (2001)[4] on the Rattus rattus which 8.1% and less than that registered in the Mus musculus which was 8.2% approaching the study made by Hasson (2010)[6] in the Mus musculus and Rattus norvegicus at 3.5% and 7.3% respectively. That infection variation might be due to the variation of places from where samples were collected, and to the availability of insects that had an important role in increasing their being epidemic as rodents were infected through insects that consequently transferred to human through food and water being contaminated with the feces of infected rodents containing eggs. Respectively, the infection in Mus musculus, Rattus norvegicus and Rattus rattus was 31.4%, 13.5% and 37.5%. That diagnosis was ascertained by using the (PCR) technique, while in Al-Zahidi study (2001)[3] the infection in the Mus musculus was at 1% lesser that registered in the current study and by 20.7% in the Rattus norvegicus higher than that registered by Al-Zubeidi (2007)[5] in the Mus musculus by 15.3%, and Hasson study (2010)[6] in the Mus musculus and Rattus rattus by 7.3% and 4.4% respectively approaching the one registered in the Rattus norvegicus at 12.5 and lesser that registered by Karim & Al-Salihi (2014)[18] in the Rattus norvegicus by 75%. That difference might be due to the difference in the type of places where samples were collected from. The infection by this cestoda is regarded as one of the common parasitic infections.

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 *H. nana* in the current study is approaching that one having the serial No. KJ917784.1 registered in Tehran-Iran by Mowalavi et al., (2014), and the one having the serial No. LM405059.1 registered by Aslett & De the one having Serial No. AF 461124.1 registered by Macnish et al., (2003)[19]. Besides, it has been noticed that the sample of the *H. diminuta* in the current study is asymptotic to that having the Serial No. MF 143799.1 registered by Younis et al., (2017a)[20] in Egypt, and the one having serial No. MF067416.1 registered by Younis et al., (2017b)[21] in Egypt as well. Nevertheless, findings have shown genetic dimension at a stated rate amid the *H. diminuta* that is locally registered.
and the globally registered types. This genetically dimension refers to the difference in the successions of the nitrogen bases between the local sample and the one globally registered. 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 18s rRNA gene of the *H. nana* and 5.8s rRNA gene of the sample of *H. diminuta* 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.

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