Isolation and Molecular Identification of Enterobius Vermicularis from Patient in Emigrant Campus

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Abstract
Enterobius vermicularis is a pathogenic worm which isolated from 50 patient stool of intense colic, the worm was isolated from stool by microscopic examination and then the worm genomic DNA were extracted and identification by PCR and three new sequences were recorded in NCBI for the first time.

1. Introduction

The worm was first time identified in 1758 by karlLinnaeus, it *Oxyuyris vermicularis*, it is a parasite and the natural host of this worm is human, itis measured approximately 10 mm in length and it lives with their heads embedded in the right colon and adjacent bowel, Enterobiasis infection thatit is known with this worm, it is more commonly in children between the ages 4 to 16 years old, the prevalence of Enterobiasis greatly depends upon living level, education and personal habitat **(1)**. These worm are causing many health and economic issue in different country of the world for bothchildren and adults .It is endemic parasite a worldwide pandemic, it is estimated that pinworms infect more than 410,000,000 peoples throughoutthe world (11% of humans), Enterobiasis may remain causing perianal pruritus stimulated by movement of female, and the albuminous substancethat surrounds the eggs ,other symptoms associated with insomnia, restlessness, irritability, and rarely, impetigo of scratched skin, or enuresis**(2)**. Ribosomal RNA (rRNA) sequences have been used extensively as model genes for phylogenetic inference, although the small gene encoding 5s rRNA in nematodes is extremely conserved, the intergenic regionbetween repeating 5S rRNA coding regions appears to vary greatly in sizeand nucleotide composition (3). The organization of the 5s rRNA gene hasbeen studied in detail in *Cuenorhubditis elegms, Brugia malayi, Ascaris lumbricoides and Entrobius vermicularis* **(4).** The 5S rRNA spacer region has been used for systematic, diagnostic and phylogenetic inferences in nematodes due to its variability in size and sequence **(5).** Combined analyses of the nuclear 5S rRNA facilitated elucidation of the phylogenic relationship between pin from captive chimpanzees and those from human.

2. Materials and methods

DNA extraction from the parasite Enterobius vermicularis:
The analysis kit supplied by (Geneaid) was relied on to extract DNA from samples of the genus Enterobius vermicularis. According to the following steps:

1. The parasite (Enterobius vermicularis) of the pinworm is taken into a
2. ml Eppendorf tube.
3. 200 microliters are added to the Eppendorf tube with Lysozyme enzyme at a concentration of 0.8 mg/200 ml and mixing with Vortex.
4. The tube is incubated at 37 °C for 30 minutes and the tube is turned every 3 minutes during the incubation period.
5. Add 20 µl of Proteinase K and mix Vortex
6. The mixture was incubated at 60°C for 10 minutes.
7. 200 µl of GB buffer is added, mixed using Vortex, and incubated at 70°C.
8. Add 200 microliters of absolute ethanol and mix manually, then transferthe mixture to the GD tube installed in the collection tube and centrifuge with a force of 16000g for 30 seconds and get rid of the filtrate. Centrifuge for 3 minutes to get rid of all remnants of the wash solution.

The GD column is transferred to a new 1.5 ml tube, 100 µl of solute is added, then left for 3 minutes, then centrifuged at 16000 g for 30 seconds,then the DNA is preserved until use.
Preparation of agarose gel and DNA electrophoresis
For DNA migration and detection, an agarose gel is prepared at a concentration of 1%. To obtain this concentration, 0.5 g of agarose powderis dissolved in (50) ml of X1 TBE and 3 microliters of red safe dye is addedusing a heat source with continuous stirring until boiling and left to cool. To a temperature of (60-50) ˚C.
Then the gel solution is poured into the basin of the Tray of the relay deviceafter the special comb is fixed to form the Wells at the edges of the gel, taking into account that the pouring is quiet to avoid the formation of bubbles, and if they are formed, they are removed using the pipette, then leave the gel to solidify.

doi.org/10.31838/hiv23.01.131 Received: 05.09.22, Revised: 02.10.22, Accepted: 04.12.22

The Tray is then placed in an electric relay tank containing an appropriate amount of X1 TBE solution, after which the comb is raised softly.
Migration samples are prepared by mixing (5) µL of the DNA sample with
µL of the loading solution. After that, the relay is operated by passing the electric current with a voltage of (5) volts / cm, and the process takes (2-1.5) hours. After that, the gel is photographed under ultraviolet rays using a gel Documentations device to be able to see the DNA bundles as well as the product of the PCR reaction.

PCR reactions

The DNA concentration in all study samples was adjusted by dilution by TE buffer solution to obtain the required concentration for performing PCR reactions and it was (25) ng/microliter for each sample. The master reaction mixture was prepared for each PCR reaction by mixing the DNA sample and the special primer for each gene with the components of the Master- mix inside a 0.2 ml Eppendorf tube supplied by the English company Promega. The reaction volume was fixed to 20 µl with distilled water, and the mixture was discarded. In the Microfuge device for a period between (5-3) seconds to ensure the mixing of the reaction components, then the reaction tubes were inserted into the Thermocycler for the purpose of conducting the multiplication reaction using the special program for each reaction. Adding the volumetric guide (100 bp) prepared by Biolaps Ladder DNA into one of the holes, adding the Red Safe DNA dye to the gel and then migrating the samples by running the Electrophoresis for a period ranging from (70-60) minutes after which the gel is photographed Using the Gel Documentation device
Molecular diagnosis of the pinworm parasite Enterobius vermicularis based on the 5S rRNA gene region.
The presence of the amplified region was detected as 4 µl (100 nanogram) of template DNA and 1 µl (10 picompl) of each gene-specific primer were added to the contents of the master mix, Table (1)

Table (1): primer used to amplify 5s rRNA	
Primer	Sequence
Forward	5′- CACTTGCTATACCAACAACAC -3′
Revers	5′- GCGCTACTAAACCATAGACG -3′

Then the reaction tubes were inserted into the thermocycler to conduct themultiplication reaction using the special program for the reaction, as shownin table (2).

Table (2): PCR program used to amplify the 5s rRNA				
No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95	5 min.	1
2.	denaturation	95	45 sec.	35
3.	Annealing	55	1.0 min.	
4.	Extension	72	1.0 min.	
5.	Final extension	72	7 min.	1

DNA sequencing analysis

The DNA sequencing technique is the basis for identifying and detecting genetic mutations, SNP variations and the highly conserved region. Usually, the output of the PCR reaction is used to determine the amplifiedsequences of fragments in which genetic variations are required. In recent years, the results presented by DNA technology Sequencing provides highaccuracy in identifying genetic mutations (6).

On the other hand, if the PCR reaction product contains more than one bundle, the required piece is purified and isolated from the gel, but if the reaction product is specific to only one bundle, it can be directly adopted in determining the sequences (7).

Determination of nucleotide sequences for amplified pieces using DNAsequencing

The sequence of nitrogenous bases of the samples under study was determined. The PCR reaction products were sent to the aforementioned samples with the primers of the resulting package. The sequence was readfor the genes based on the 3130 Genetic Analyzer device supplied by the Japanese company Hitachi, (8).
Gene-specific sequences were matched with those documented in the National Center for Biotechnology Information (NCBI) and the results were analyzed using BLAST software.

3. Results and discussion

The isolation and identification of pinworm were done by using microscopic examination in glass slide fig (1).

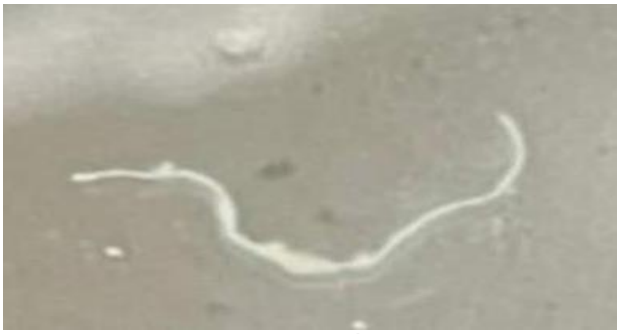

Fig (1): Microscopic picture of pinworm (Enterobius vermicularis)

The pinworm DNA were extracted by using kit ready to use and the

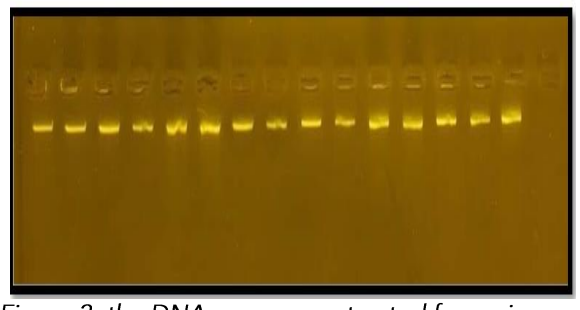

Figure 2: the DNA gen ome extracted from pinworm samples and run 1%agarose gel.

results showed pure and big size of DNA, fig (2). PCR for Amplification 5s rRNA gene:

Enterobius vermicularis Saadia-No DNA, 5S ribosomal RNA intergenic spacer, partial sequence

GenBank: LC655893.1

FASTA Graphics

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REFERENCE   1
  AUTHORS   Yahya,N.A. and Hamad,S.S.
  TITLE     Molecular and epidemic study of Pinworms
  JOURNAL   Unpublished
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  TITLE     Direct Submission
  JOURNAL   Submitted (28-OCT-2021) Contact:Noor Ali Yahya University of
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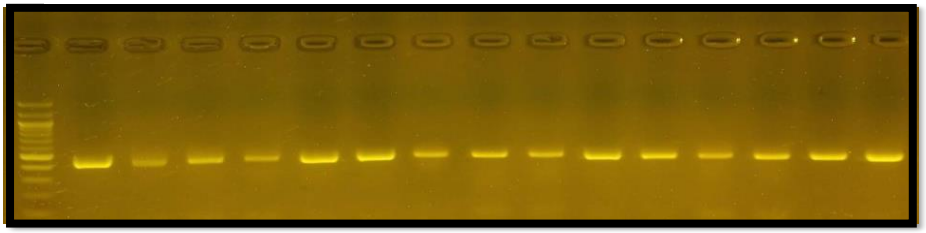

Figure 3: The PCR reaction of 420 bp in size of pinworm samples for the diagnostic region 5srRNA gene migrated into 2% agarose gel.

Enterobius vermicularis Noor-Sa-Di DNA, 5S rRNA NTS region

GenBank: LC657086.1

FASTA Graphics

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ACCESSION   LC657086
VERSION     LC657086.1
KEYWORDS    .
SOURCE      Enterobius vermicularis (human pinworm)
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            Spirurina; Oxyuridomorpha; Oxyuroidea; Oxyuridae; Enterobius.
REFERENCE   1
  AUTHORS   Yahya,N.A., Hamad,S.S. and Muhammad,D.A.
  TITLE     Molecular and epidemic study of Pinworms
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 369)
  AUTHORS   Yahya,N.A., Hamad,S.S. and Muhammad,D.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-NOV-2021) Contact:Noor Ali Yahya Uinversity of
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In the current study, the amplification result of the conserved gene used in pinworm diagnosis was confirmed, whose size was 420 bp in length, and this result agrees with the researcher (9), whose Enterobius vermicularis amplification was performed by PCR at 420 bp. The PCR results for this estimate showed that the amplified DNA had (420bp) of the 5S RNA gene in 15 of the original 50 samples examined, although the percentage of infection in PCR produced less than the percentage of infection

in microscopic diagnosis, but PCR remained a good tool For parasite diagnosis especially those that are established in the human body without symptoms and that failed to diagnose this is microscopically consistent with (10), who demonstrated that the molecular biology approach provides a specific and sensitive diagnostic tool and an opportunity to access the genetic information of the parasite.

This result showed the importance of identifying the gene that plays the central role in diagnosing cryptic entropion, and this result agrees with (11), that the resulting 5S rRNA gene spacer was successfully used as a PCR target for diagnosing E.vermicularis. in Amerindians, (12) and (13), for which the 5S rRNA spacer region has been observed to have been used for systematic, diagnostic and evolutionary inferences in nematodes due to their variability in size and sequence.

The results also indicated the existence of new sequences of pinworms, which may be produced as a result of different environmental conditions, the factor of development and the various variations that work on continuous development to keep pace with the change in the surrounding conditions, and it is one of the tools of adaptation and evolution of organisms.

The conserved region for worm identification were used to identify the pinworm by using oligo sequences of the gene, the result showed amplified DNA in 420 bp of the gene, fig (3).

Sequencing Results:

The result of 15 PCR amplification were sequencing to find out the nucleotides sequencing and the identify the pinworm in species level, the sequencing results showed

matching in 99% of Enterobius vermicularis with three new record of the worm sequencing, fig(4,5,6)

References

Brown, M.D. (2006). Images in clinical medicine: Entrobius vermicularis. NEngl J Med., 334: 12.

Morgenstern, B. 1999. Dialign 2: improvement of the segment-to- segmentapproachto multiple sequence alignment. Bioinformatics 15:211–218.

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), version 4b10. Sinauer Associates, Sunderland, MA.

Liao CW, Chuang TW, Huang YC, Chou CM, Chiang CL, Lee FP, Hsu YT, Lin JW, Briand K, Tu CY, Fan CK. Intestinal parasitic infections: current prevalence and risk factors among schoolchildren in capital area of the republic of Marshall Islands. Acta Trop. 2017; 176:242

Culha, G. and Duran, N.(2006).The relationship between Enterobius vermicularis infection and nocturnal enuresis.Eur.J.Gen.Med.;3(1):16-20.

Erdemir, F. Ozcelik, S.and Saygi, G. (1992). Distrubution of symptoms among the primary School Pupils with or without Enterobiasis. Turkish Parasitology ,16: 53-9.

Hama, A.A. (2007). Intestinal parasites in relation to malnutrition among primary school children in Erbil province with evaluation of some anti-parasitic drugs. M.Sc Thesis, College of Science. Salahadin Univ. Erbil, P76.

an C, Chuang T, Huang Yet al.Enterobius vermicularisinfection: Prev-alence and risk factors among preschool children in kindergarten inthe capital area, Republic of the Marshall Islands.BMC Infect. Dis.2019;19: 536

Remm M, Remm K. Effectiveness of repeated examination to diagnoseenterobiasis in nursery school groups.Korean J. Parasitol.2009;47:235–41.

KIM, D.H., SON, H.M., LEE, S.H., PARK, M.K., KANG, S.A., PARK, S.K., CHOI, J.H., PARK, J.H., YU, H.S. (2015): Negligible egg positive rate of Enterobius vermicularis and no detection of head lice among orphanage children in Busan and Ulsan, Korea (2014). Korean J. Parasitol. 53(4): 497-499. DOI: 10.3347/kjp.2015.53.4.497

M. Moosazadeh, G. Abedi, M. Afshari et al., (2017). "Prevalence of Enterobius vermicularis among children in Iran: A systematic review and meta-analysis," Osong Public Health and Research Perspectives, vol. 8, no. 2, pp. 108–115.

G. Meletis, C. Sdravos, E. Beta et al., (2015). "Moving Enterobius larvae inside their eggs," Clinical Microbiology and Infection, vol. 25, no. 8, pp. 971-972, 2019.

H. M. Li, C. H. Zhou, Z. S. Li et al., "Risk factors for Enterobius vermicularis infection in children in Gaozhou, Guangdong, China," Infectious Diseases of Poverty, vol. 4, p. 28,