Basrah Journal of Veterinary Research,Vol.17, No.3,2018
Proceeding of 6th International Scientific Conference,College of Veterinary Medicine
University of Basrah,Iraq

MOLECULAR CHARACTERIZATION OF *Parabronema skrjabini* IN BASRAH PROVINCE, IRAQ

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Key words: *Parabronema skrjabini*, Nematode, sheep.

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ABSTRACT

Parabronema skrjabini is a spirurid nematode of the family Habronematidae that lives in the abomasum of ruminants such as sheep and goats. The purpose of this study was to investigate the molecular aspects of *Parabronema skrjabini* in Basrah. The worms were collected from slaughtered sheep and goats in Basrah slaughter house between the period from June, 2016 to January, 2017, with total number of abomasum's examined sheep (576) and goat (150). An internal transcribed spacer 2 ribosomal DNA (ITS2-rDNA) fragment of *P. skrjabini* was amplified by polymerase chain reaction (PCR) using a pair of specific primers (Para-Ir-R and Para-Ir-F). ITS2-rDNA sequences by using PCR technique assay which based on a 783-bp long sequence of the 28S rRNA gene, the total genomic DNA has been extracted by extracting kit with some modification. ITS2 homology in different isolates was between (81–86%) compared with the sequence data in GenBank. To our knowledge, this is the first study in Iraq exploring the genetic diversity of *Parabronema* in sheep and goats.

INTRODUCTION

P. skrijabini was originally described from Russian Turkestan and is known to occur in that region in cattle, camel, sheep and goats. The present recorders, there for the first one concerning the occurrence of this genus in north America**(1).**Abomasums in zig-zag manner, their parasite mouth

is provided with pair of lateral psuedolabia. The esophagus has a short narrow anterior and a long wide posterior part. This parasite as, revealed during the postmortem is long, slender and dark red in color found firmly embedded in the mucous according to **(2).** *P. skrijabini* was originally described from Russian Turkestan and is known to occur in that region in cattle, camel, sheep and goats **(1).** The present recorders there for, the first one concerning the occurrence of this genus in North America. This is a parasite of parasites is widespread in various countries in Asia, Africa, it has been recorded in Iran **(3).**

In Iraq *Parabronema skrijabini* is widely distributed in sheep **(4).***P. skrijabini* was recorded in first time in goats**(5).**In camels infected with *Parabronema skrjabini* has been recorder in Iran **(6)** and Iraq **(7).***Parabronemosis is a disease caused by the nematode P. skrijabini parasitized in abomasums of ruminant **(8).**P. skrijabini* are described from horned, *P. skrijabini* is one of the nematodes that inhabits the abomasum of ruminants and has a wide distribution in Africa, Asia and some Mediterranean countries. It has been reported in a number of studies from Mongolia, Kazakhstan, Saudi Arabia, Namibia, Turkey and Iran **(9).**

The ITS2 sequence of *Parabronema* has only been examined in one study in China in which samples were isolated from camels **(10).** Therefore, the present phenotypic study was conducted to accurately identify the *P. skrjabini* infection in sheep and goats in Iran and to assess the level of intraspecies variation in this parasite using the ITS2-rDNA sequences. This work is the first study of ITS2-rDNA sequences of *P. skrijabini* from sheep and goats in Iraq, and its purpose was to investigate the molecular and morphological characteristics of *Parabronema* species collected from slaughtered animals (sheep and goats) in Basrah provinces.

MATERIALS AND METHODS

Samples Collection

A random visit to the Basrah slaughter house in Basrah province (twice a week) from June 2016 till January 2017 to examine abomasum of slaughtered animals (sheep and goats).The abomasum was removed from abdominal cavity, and immediately taken to the laboratory of Parasitology, Department of Microbiology, Veterinary Medicine College in Basrah University, for appropriate examination

Morphological studies.

animals were brought to the laboratory of Veterinary Parasitology at the College of Veterinary Medicine in Basrah University, and examined carefully to detecting parasites, which were isolated from the infected abomasum. After that the worms were washed many times with normal saline (0.9%) according to the method by **(11).** After being recognized, the worms were rinsed in containers with 70% ethyl alcohol which was stored at room temperature for other tests. The worms were mounted in phenol-alcohol on a glass slide and light pressure was applied to cause the worm body to lie flat without being damaged. The males were studied in order to determine body length and females to measure egg dimensions.

DNA Extraction and PCR:

The kit for genomic DNA was used ,the favor prep tissue Genomic DNA Extraction Mini Kit (FAVORGEN / TAIWAN) animal tissue depending to the instructions of manufacturer's and for amplification the ITS2-rDNA gene with (783 bp) long fragment with primers. The forward primer was Para-Ir-F(**5`GTAGGTGAACCTGCGGAAGG3`)**

and and reverse primer was Para-Ir-R (**5`CTGAGCTGAGGTCAACGAAT- 3`**) were In a thermocycler PCR reactions were conducted under conditions The PCR was

performed using the following protocol: 5 min. incubation at 94°C to denature the double stranded DNA, 33 cycles of 45 s at 94°C (denaturing step),45s at 59°C (annealing step), and 45 s at 72°C(extension step). Finally, the PCR was completed with an additional extension step for 5 min at 72°C. Samples without genomic DNA were used as negative controls. The PCR products were analyzed on 1% agarose gel **(12).**

DNA Sequencing

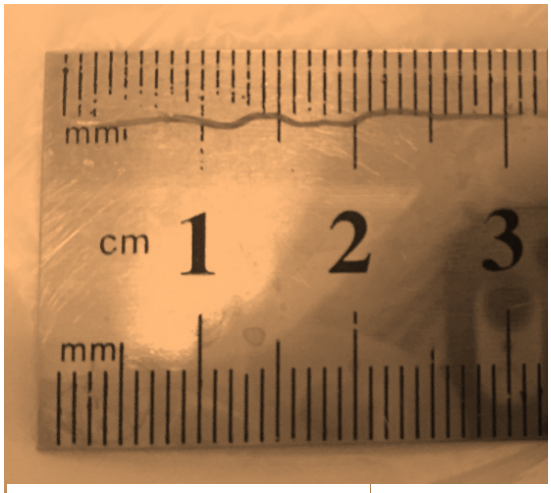
PCR product was purified for sequencing by using EZ-10 spin column DNA cleanup mini preps Kit (Bio Basic Inc., Canada). The PCR products of the *P. skrijabini* and primers were sending to Macro gen company, (USA) for sequencing. The sequence chromatograms were analyzed using the Geneious 5.1.6 software and compared to those deposited in GenBank

(www.ncbi.nlm.nih.gov/) using the 'Basic Local Alignment Search Tool' (BLAST). All sequences for *P. skrjabini* were aligned and compared with one another and with those of the ITS2-rDNA of other spirurids available in GenBank TM. DNA sequences of closely related species were also downloaded and used in the phylogenetic analysis. Multiple sequence alignments were made with the Clustal W. Phylogenetic analyses were performed based on the Neighbor joining and maximum parsimony methods using MEGA5 software.

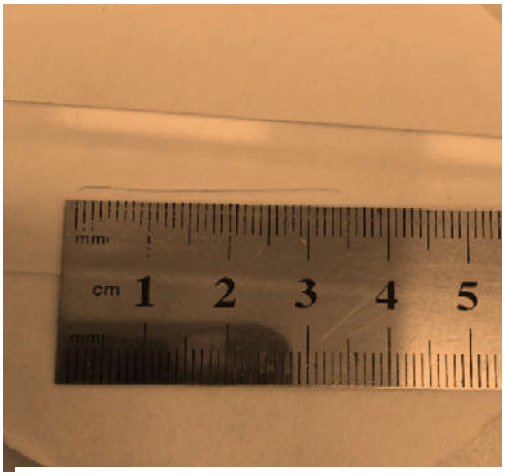
RESULTS

Morphology:

The morphological results showed m**ale** body (25-30) mm long, (photomicrograph1).Female: body 27-34(30.5)mm long(photomicrograph 4),in sheep and goat



Photomicrograph (1): length of *P. skrijabini* male.



Photomicrograph (2): length of *P. skrijabini* female.

Molecular diagnosis has been done, firstly, the DNA of *P. skrijabini* was extracted by used Favor prep Tissue Genomic DNA Extraction Mini Kit(Favorgen Biotech / Taiwan).the detection of genus *Parabronema* was made by using PCR technique. In order to detect the presence of ITS-2 gene, the primer which was used is (Para-Ir-R and Para-Ir-F).whose amplification of the ITS-2 fragment. Extracted DNA samples were measured by Nano-drop to

detection genus *Parabronema* was made by using PCR techniques. The result of PCR assay for the amplification of the ITS-2 rDNA fragment with the designed primer set yielded destined band corresponding to their molecular size of approximately 783bp for *P.skrijabini* independently from the different host species.

Sequencing was done in this study to determine the specific strain in Iraq, PCR result product of 28SrRNA gene of *P. skrijabini* were screened by sequencing of thirteen samples, The samples view satisfactory results depending on NCBI matching. Allresults were compared with *Parabronema* gene sequence and analyzed by using NCBI nucleotide blast in NCBI (http:// NCBI reference sequence) software program chromas and mega version thirteen, The sequences were comparative with an obtainable sequence in GenBank with accession number of (EU375510.1). ITS2-rDNA homology in different isolates was between 81% and 86%. (Table1) presents the comparison of nucleotide sequences in the ITS2-rDNA of *P. skrjabini* from the geographical regions with the referenceITS2 sequence from Gen Bank. Identification with Iranian isolate. The all comparison of nucleotide sequences in the ITS2-rDNA of *P. skrjabini* from the geographical regions with the referenceITS2 sequence from Gen Bank

Table (1): ITS-2 gene Sequences Production Significant Alignments For *P. skrijabini*

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Query  1    CTGCTTTGGCATAAACCATGCAGGATGGGCTAATCCGAATGTACCAACGGTGCCCTCTGT  60
Sbjct  84   CTGTTTTGGCATAAACCATGCATGATGGGCTAATCCGTATGTACCAACGGTGTCCTTTGT  143
Query  61   TGGTGTCTATACTTCATTTGATTTATCGCCCGACCGTCGATAATAATGATGATGAAGTGA  120
Sbjct  144  TGGTGTCTATACTTCATTGGAATTATCGCCCGACCGTCGATAGTGATGATGATGAAGTGA  203
Query  121  TAGCCT--------ATTCTTAATTAGGTAGACTTAATAAGCATTTAGCTAATATGCTGCC  172
Sbjct  204  TAGCCTTATGGAAGATTTTTCACTGGGTAGACTTAATAAGCATTTAGCTAATATGCTGCC  263
Query  173  AACAATTTAACACACACACACACGAACACATTAATATTTTAAAATTTT-GAAATTTTATA  231
Sbjct  264  AACAATTAAACACACACAAACAC-AATA-A-TATTAACTTAAAAATTTAGAAAA---A-A  316
Query  232  TTTAATTTTTTTACTCTTAGCGGTGGATCCCGGGGCTCGTGGAAAGAGGAAAAACGCGGC  291
Sbjct  317  G--AATTGTTTTACTCTTAGCGGTGGATCACTTGGCTCGTGGATCGATGAAGAACGCAGC  374
Query  292  TAAATGCGATAAATATTGCGAATTGCAGACGCATTGAGTTCAAAGATTTCTAACGCACAT  351
Sbjct  375  TAGCTGCGATAAATAGTGCGAATTGCAGACGCATTGAGCACAAAGATTTCGAACGCACAT  434
Query  352  TGCACCATCGCGCTGAGTCCCGATGGTACCTCTGGCTGAGGGTCGATTTTTTGATAAAAT  411
Sbjct  435  TGCACCATCGGGTTGATTCCCGATGGTACGTCTGGCTGAGGGTCGATTTAATGATCATAT  494
Query  412  CCCACTTTTCAATGCAGCAATAGCGGCAACGGCGGAATCGGTATGTTTGAATTTATATCA  471
Sbjct  495  CAAACAACTCAATACAACAGTAGCAA-AA-G----AA---GTATGTTTGAATTTATATCA  545
Query  472  TCGGGTGATA  481
Sbjct  546  TCAGGCGATA  555
```

Score	Expect	Identities	Gaps	Strand
414 bits (224)	**5e-119**	**406/490 (83%)**	**27/490 (5%)**	**Plus/ Plus**
507 bite (274)	**4e -147**	**496 (86%)/427**	**496 (5%)/29**	**plus/Plus**
390 bite (211)	**4e - 112**	**504 (82%)/411**	**504 (5%)/27**	**plus/Plus**
442 bite (239)	**2e - 127**	**458 (85%)/388**	**458 (3%)/17**	**plus/Plus**
370 bite (200)	**6e-106**	**451(82)/372**	**451 (6%)/28**	**plus/Plus**
538 bite (291)	**1e- 156**	**518 (86%)/448**	**518 (6%)/33**	**plus/Plus**
486 bite (263)	**5e- 141**	**495 (85%)/423**	**495 (6%)/31**	**plus/Plus**
374 bite (202)	**4e-107**	**497 (81%)/404**	**497 (6%)/31**	**plus/Plus**
344 bite (186)	**3e-98**	**462 (81%)/374**	**462 (4%)/23**	**plus/Plus**
508 bite (275)	**2e-147**	**497 (86%)/428**	**497 (5%)/29**	**plus/Plus**
514 bite (278)	**2e-149**	**506 (86%)/435**	**506 (5%)/29**	**plus/Plus**
508 bite (275)	**1e-147**	**514 (85%)/439**	**514 (5%)/27**	**plus/Plus**
460 bite (249)	**6e-133**	**495 (84%)/418**	**495 (5%)/29**	**plus/Plus**

Fig.(1):Sequencing of *P. skrijabini* Isolate Genomic ITS-2 ribosomal DNA Gene as Compared with Standard Data from Gene Bank.

The isolated genes were recorded in Gene Bank under the accession number (LC275902 , LC275903 , LC275904 , LC275905 , LC275906 , LC275907 , LC275908 , LC325445.1 , LC325446.1, LC325447.1,LC325448.1, LC325449.1 , LC325450.1)as Iraqi strain

Phylogenetic Analysis

In the current study, the phylogenetic trees were generated depending on the compartment between the sequences in the recent study and the sequences of the other *P.skrijabini* which published in Gene Bank, phylogenetic trees were generated using MEGA software. The phylogenetic relationship among thirteen isolates of *P. skrijabini* obtained during this study and the other strains of *P. skrijabini* (Gene Bank) based on partial nucleotide sequence of ITS-2 rDNA gene view as in Fig. (3) below.

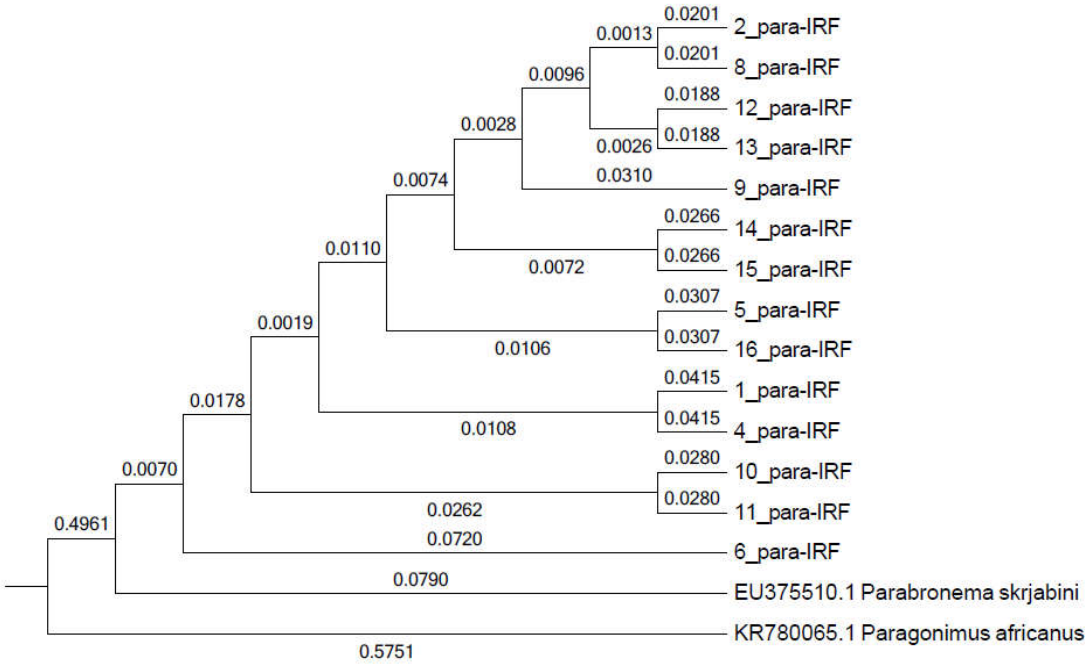

Fig.(2): Phylogenetic Relationships Based on Partial Nucleotide Sequence of the ITS-2 rDNA Genes of *P. skrijabini*

DISCUSSION

In the results, ITS2 sequence identity of isolates from Iraq was between 81% to 86% compared with the sequences in Gen bank, this agree with **(10)**, Molecular studies on the genus *Parabronema* has be chiefly based on rDNA genes obtainable in Gen bank. Studies of ITS1 and ITS2 gene sequences of *P. skrijabini* in camel has shown 30.2–60.1%similarity with other nematodes and **(12)** ITS2 sequence identity of different isolates were between 68% to 77% compared with the sequences in Gen bank. Phylogenetic tree analysis of *P. skrijabini* isolates and compared reference sequence from Gen bank showed that although all of them may have a common ancestor, are grouped on different branches; even the *P. skrijabini* species are distributed on several branches. The sequences in this study were more similar to *Habronema spp.* There was significant difference between the isolates in this study and sequences in Gen bank. The low ITS2-rDNA identity in isolates from Iraq as compared to the reference sequence in GenBank (81–86%) raise questions regarding the species identity of the parasites isolated in Iraq. Very limited number of studies have been published on population genetics of *P.*

skrijabini in ruminants in the world and particularly. This work is the first study of ITS-2 sequences of *P. skrijabini* from sheep and goats in Iraq. The results show genetic diversity which opens fascinating avenues for future studies investigating in depth the phylogenetic relationships of spirurid nematodes and, in particular, those existing among species ranked within the Thelazioidea and Habronematoidea Superfamilie.

التشخيص الجزيئي للـ *Parabronema skrijabini* في مدينة البصرة

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الخلاصة

Parabronema skrijabini تعد من الديدان الخيطية التي تعود العائلة Habronematidaeالتي تعيش في منفحة الحيوانات المجترة للاغنام والمعز. وكان الغرض من هذه الدراسة هو دراسة الجوانب الجزيئية للـ *Parabronema skrijabini* محافظة البصرة / العراق .تم جمع العينات من مجزرة البصرة في الفترة من حزيران (يونيو) ٢٠١٦ إلى كانون الثاني (يناير)٢٠١٧ وخلال هذه الفترة تم جمع (٥٧٦) ، (١٥٠) عينة منفحة من الاغنام والمعز على التوالي .

تم تضخيم الحمض النووي الرايبوسومي (rDNA-ITS2) جزء من (*Parabronema skrijabin*) باستخدام زوج من االبرايمرات محددة ((Para-Ir-R and Para-Ir-F).على أساس طول ٧٨٣bp من الجينات الرنا الرايبوسومي٢٨S،

وقد تم استخراج الحمض النووي الجيني الكلي عن طريق الاستخلاص باستخدام الكت مع بعض التعديل . كانITS2التماثل في عزلات مختلفة بين (٨١-٨٦ ٪) مقارنة مع بيانات التسلسل في بنك الجينات العالمي.

تعد هذه الدراسة هي الاولى في العراق استكشاف التنوع الجيني Parabronemaفي الأغنام والمعز. لوحظ ان انخفاض هوية ITS2-في عزلات مختلفة من العراق بالمقارنة مع التسلسلات المرجعية في بنك الجينات (٨١-٨٦٪).

REFERENCES

1. Sarwar, M. M. (1954). On the Transference of *Parabronema skrijabini* Rassowska, 1924 to Squamanema Thiel, 1925 and its Occurrence in Sheep and Goats in Indo-Pakistan. J. helminthology, 28(3-4), 143-150.

2. Eslami, A. H., & Nabavi, L. (1976). Species of gastro-intestinal nematodes of sheep from Iran. Bulletin de la Société de Pathologie Exotique, 69(1), 92-95.

3. Al-Saeed, A. T. M., & Al-Khalidi, N. W. (1990). Epidemiological studies on abomasal nematodes of sheep in Mosul (Iraq). J. Vet. Parasitol. 4(1), 17-20.

4. Al-Asadi, K. B. F., & Alemarah, G. Y. A. (2014). Detection of Nematode *Parabronema skrijabini* In Goats at Basrah Province. J. Parasitol, 104, 194-199

5. Borji, H., Razmi, G. H., Movassaghi, A. R., Naghibi, A. G., & Maleki, M. O. H. S. E. N. (2010). A study on gastrointestinal helminths of camels in Mashhad abattoir, Iran. Iranian J. Vet. Research, 11(2), 174-179.

6. Al-Fatlawi, M. A. (2017). Identification of the potential vector of some Spirurida (Parabronema skrijabini) in dromedary in Iraq. Kufa J. Vet. Me. Sci., 7(1B).

7. Kaufmaun J. Parasites infection of domastic animals. Diagnostic manual. (1996). Pp: 423.

8. Hasheminasab, S. S. (2015). Molecular characterization of the first internal transcribed spacer of rDNA of *Parabronema skrijabini* for the first time in sheep. Ann Parasitol, 61(4), 241-6.

9. Zhang, X. D., Yang, X. Y., Yang, L. R., LI, L. C., Zuo, H. T., NA, R. H., ... & Wang, J. J. (2009). Cloning and Sequence Analysis of the rDNA-ITS of *Parabronema skrijabini*. Chin. J.Anim. and Vet. Sci.:5

10. Farhan, H. S., Morphological and Molecular Study of *Fasciola gigantica* In Buffaloes and Cows. M.Sc. thesis, Coll. Vet. Med., Univ. Basrah.(2016).

11. Hasheminasab, S. S., Jalousian, F., & Meshgi, B. (2016). Molecular and morphological characterization *of Parabronema skrijabini* of sheep and goats at three different geographical zones in Iran. Ann. Parasitol , 62(1).