MOLECULAR DIAGNOSIS OF NEMATODE WORMS *PARABRONEMA SKRJABINI IN CAMELS* (*Camelus dromedaries*) IN IRAQ

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ABSTRACT
The current study was designed for analysis of the pattern of the nucleotide sequence of the tissue DNA isolates based on the ITS2,*28SrDNA gene* by the traditional polymerase chain reaction. All amplicons were well suited for the prepared primer with size 873 bp . and identical ratio ranged 87.2- 98% of the same species, as included a high similarity of the isolates taken camels in Iran and Iraq related to *Parabronema skrjabini* . Phylogenic tree inferred the degree of relatedness between 28SrDNA sequences deposited in the international nucleotide bank sequence database (NCBI). The sequence of *Parabronema skrjabini* was recorded in the Genbank under accession number MT742154.1.

Keyword: *Parabronema skrjabini,* Nematoda, phylogenic tree, camels, Iraq

مجلة العلوم الزراعية العراقية -2022 :53(3):584-588 فاضل وآخرون

التشخيص الجزيئي لديدان المثقوبة *Parabronema skrjabini* في الجمال(*Camelus dromedarius*) في العراق

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المستخلص
صممت الدراسة الحالية لتحليل نمط تسلسل النوكليوتيدات لعزلات الحمض النووي بناءً على جين ITS2، 28SrDNA عن طريق تفاعل البلمرة المتسلسل التقليدي. كانت جميع الأمبليكون مناسبة تمامًا للبرايم المحضر بحجم 873. وتراوحت نسبة التماثل 87.2– 98٪ من نفس النوع، حيث اشتملت على تشابه كبير بين العزلات المأخوذة من الإبل في إيران والعراق المرتبطة *Parabronema skrjabini*. استنتجت شجرة النشوء والتطور درجة الارتباط بين تسلسلات *28SrDNA gene* المودعة في قاعدة بيانات الدولي (NCBI). تسلسل *Parabronema skrjabini* المسجل في Genbank تحت رقم الانضمام MT742154.1.

الكلمات المفتاحية: ببرابرونيما شكرجباني, الديدان ,الشجرة الوراثية,الجمال, العراق

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INTRODUCTION

Gastro-intestinal (GI) nematode and trematode infections of ruminant livestock cause major problems in the developing world. These parasites are difficult to manage because in some cases they develop resistance to all available commercial dewormers and resistance to dewormers is now see worldwide (2,16). Economic losses incurred by these parasites include reduced animal performance and weight gain, condemnation of whole carcass or affected organs at slaughter, cost of treatments, and mortality in severe cases (1). Parabronemosis is a disease caused by the roundworms *Parabronema skrjabini* parasitized in abomasum's of the ruminant, it is a disease that severely threatens camel health, causing huge economic losses to industries involved in camel husbandry. these worms are distributed in different areas around the world such as the Mediterranean, Asia, Africa (12,13). Parabronemosis causes lack of absorption, weakness, low weight, and histopathological changes in the abomasa mucosa, followed by reduced productivity and working efficacy (4,10). The confirmation of Parabronemosis depends on the general morphology and specific molecular characteristics of the parasites (7,14,17)

MATERIALS AND METHODES

Samples collection: The abomasa of the slaughtered camels were collected between August 2018 and May 2019 from the slaughterhouse of AL-Najaf AL-Ashraf, Iraq. Abomasum samples were cut longitudinally and examined the mucosal surface directly for any attached worms; the identified adult worms were collected carefully to prevent its damage, washed with physiological saline solution than kept in 70% Ethanol, transferred to the laboratory in the faculty of Veterinary Medicine, University of Baghdad.

Parasitology Test: A total of 20 adult *Parabronema* worms isolates from camels (50 male and 50 female) were examined morphologically. After washing by saline and kept in 70% ethanol at –20°C for their molecular examination (DNA extraction). Adults parasites identification was done regarding to (9) .

DNA Extraction and PCR. : DNA extraction from worms was performed using a DNA extraction kit**(**WizPrep™ gDNA Mini Kit (Cell/Tissue) Korea, wizbio co. regarding to the manufacturer's notes.

Primers

The PCR primer preformed in this analysis for the amplified ITS2,28S rDNA gene of *P. skrjabini* by thermocycler PCR system(Table1).

Table 1. Oligonucleotide primers used to amplify and sequence parasite *ITS2,28S rDNA genes*

Gene	Primer sequence (5' to 3')	size (bp)	References
ITS2 fragment	Fwd_seq:5'- GTAGGTGAACCTGCGGAAGGATCATT -3'	873bp	(17)
28S*rDNA* gene	Rev_seg: 5'- AGCGGAGGAAAAGAAACTAA-3'		

PCR master mix was implemented by (WizPrep™ gDNA Mini Kit), according to the manufacturer's instructions as in (Table2).

Table2. Protocol of PCR reaction mixture volume

PCR Master mix	Vol.
DNA template 5-5ng/µl	5µl
Primer- forward (10pmol)	1µl
Primer - reveres (10pmol)	1µl
Nuclease free water	13µl
final volume	20µl

After then, This PCR mix constituents transferred into thermocycler (Thermal cycler BioRad. USA).

PCR Conditions

PCR thermocycler conditions were implemented by utilizing a conventional PCR thermocycler system as in (Table3).

Sequencing and phylogenetic analysis: PCR products purified using (INTRON) kit and analyzer (Macrogen) using terminator cycle sequencing and BLAST analysis (http://blast.ncbi.nlm.nih.gov), edited with (Mega 6) then analyses by (Neighbour Joining Method).

Table3. The optimum condition of detection *Parabronema*

PCR step	Temperature	duration	reiterate
Primary denaturatio	95ºC	3min	1
Denaturation	95 ºC	18sec.	
Annealing	55ºC	40sec	35 run
Extension	72 ºC	40 min	
last extension	72 ºC	5min	1
hold	4 ºC	Forever	-

RESULTS AND DISCUSSION

Microscopic investigation results showed characteristics features of adult Male about 102-117 µm in wide. Buccal cavity 93-112 µm deep. The tail is coiled ventrally, with lateral alae at the posterior extremity. Spicules are distinctly unequal. Adult Female worm**,** Worm is 192 µm in width. The tail is pointed or blunt and distinctively curved on the dorsal side. These features matched with (9, 3) (Figure 1).

Figure 1.A, Anterior and B, posterior end of *Parabronema skrijabini* (male)

Our present study showed that the PCR amplification was positive on all isolated nematodes for the ITS2,28S *rDNA* gene. The selected amplified fragment size was 873bp. As showed in (figure 2). Molecular method is a more sensitive diagnostic method for differentiation of nematode worms in animals, allows identification of genetic links between different isolates and the valuation of parasite control by identifying the source from infection (5,6, 8,11,15).

Phylogenic Analysis

The current study was for the study of *Parabronema skrijabini* fresh isolated from camels in Iraq. Regarding the obtainable information from National Center for Biotechnology Information Search database GenBank. One sample selected on random base and send to NICEM co. (South Korea) for sequencing. The results have been registered in NCBI with accession number (MT742154.1) to *Parabronema skrijabini*. For phylogenic analysis. Comparative analysis *28S rDNA* gene sequences Iraq of *Parabronema skrijabini* with global breeds of *Parabronema skrijabini* was similarities of 87%-98%. (accession no. MT742154.1), Iraq of *Parabronema skrijabini* was similarities of with (accession no. MK7620918.1), Iran, (accession no. LC325449.1), Iraq, accession no. LC325450.1), Iraq, accession no. LC275904.1), Iraq and (accession no. LC275908.1), Iraq. (Figure 3).

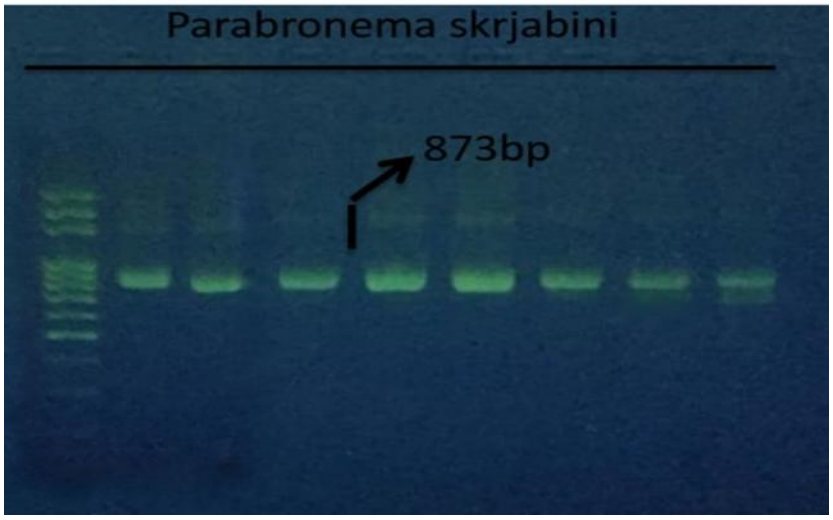

Figure 2. Gel electrophoresis of PCR product of ITS2,28S*rDNA* gene (873bp), for *Parabronema skrijabini* using 2% agarose gel at 5volt /cm for 2 hours.

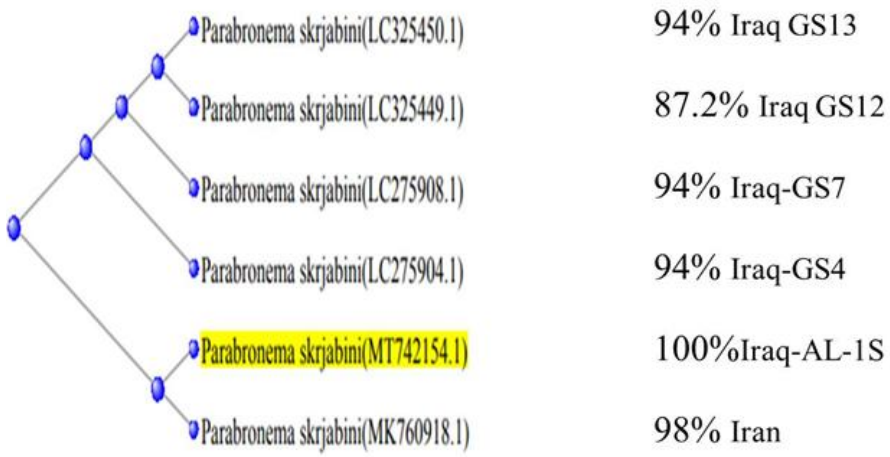

Figure 3. *Parabronema skrijabini* Phylogenetic analysis

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