MOLECULAR IDENTIFICATION AND PHYLOGENETIC-TREE ANALYSIS OF MONIEZIA SPECIES FROM SHEEP IN AL-DIWANIYAH CITY

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ABSTRACT

The present study was performed to detect the molecular and the phylogenetic identification of species that belonging to the genus of Moniezia Blanchard, 1891 which affected intestines of sheep in Al-Diwaniyah city, Iraq; fifty intestine samples were sought for the infestation of Moniezia spp. from the city slaughterhouse from 1 October to 30 November 2017, this tapeworm was found to infest the intestines of 13 sheep.

For morphological identify the genus of this tapeworm, eggs from one gravid proglottid of the thirteen worms were examined, polymerase chain reaction (PCR) and the PCR-product-based sequencing were applied on 4 Moniezia tapeworms targeting a specific region of the 18S rRNA gene.

The sequencing has shown 2 species of Moniezia, SP1 and SP2, these two species revealed close matching on the phylogenetic tree to an according to the current study findings, Moniezia spp. affect on sheep in the city of Al-Diwaniyah, Iraq, these findings give interesting information about the evolution history of this worm in the studied city.

Keywords: Cestoda, Moniezia, PCR, Phylogeny, Sheep.

INTRODUCTION

The genus of Moniezia are considered as high prevalent worms that infest sheep intestines, the disease conditions by these worms lead to risky-economic crises around the world (Soulsby, 1982; Maziyad and El-Nemr, 2002). The characteristic scolex, neck, and strobili are the highly recognized parts of the worms. Cyclophyllidea and Anoplocephalidae are the order and the family of this genus respectively, each proglottid has repeated sexual parts for better differentiation of these worms; mites are considered the main intermediate hosts for Moniezia species that provide a source of infestation via feeding on grass (Denegri et al., 1998).

Monieziasis is the term of illness that is caused by species of Moniezia, for this genus as a tapeworm has limited species such as M. expansa (Rudolphi, 1810), M. benedeni (Moniez 1879) and M. monarda (Ohtori et al., 2015). M. expansa affects sheep (high incidence), cattle, goats, swine, and very rarely human (El-Shazly et al., 2004; Gómez-Puerta, 2008). Young animals appear to be the main targets for the infestation by M. expansa (Wymann, 2008);
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several proglottids that have sensory-organ-based anterior-scolexes, neck, and the strobilus are the main parts of this species. According to Brusca and Brusca, (1990), the sensory parts are present along the body of the worm and used for tactile stimulation; metrics of the parasite could be expanded as 8-10 meters in length and 1.5 centimeters in width (Chilton et al., 2007).

To study the evolution history of Moniezia species in the city of Diwaniyah, Iraq, the present study was initiated to evaluate the identity matching or mismatching of the city species with global species that belonging to this genus.

MATERIALS AND METHODS

Intestines from 50 sheep (22 male, 28 female; 20 with age <6 months, 18 with age 6 to <12 months and 12 with age > 12 months) were examined for the infestation of Moniezia spp. from the city slaughterhouse.

To identify the genus of this tapeworm morphologically, eggs from one gravid proglottid of the thirteen worms were examined (Rahif, 1998); sequencing of the polymerase chain reaction (PCR) products were applied on four Moniezia tapeworms targeting a specific region of the 18S rRNA gene (743bp). The protocol of gSYAN DNA Extraction Kit (Gene aid, USA) was followed to extract the genomic DNA from the mature proglottids of the worms. Accu Power TMPCR Pre Mix (Bioneer, Korea) was performed to prepare the master mix using the manufacturer’s instructions. The primers (AY752651.1), F: TGCTACCGCAGATGGTG and R: ACACAGTTGGCTGCACTT were used in this study (Wickström et al., 2005).

The thermocycler reaction-based conditions were 1 cycle of initial denaturation at 95°C for 5min, 30 cycles of (denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 72°C for 1min), and 1 cycle of final extension at 72°C for 5min. We had optimized these conditions previously to fulfill the amplification requirements for this study.

Electrophoresis was used to separate the PCR products on a 1.5% agarose gel at 100 volts and 80 amp for 1hour; a UV-light-based imager was used to identify these products in the gel.

Sequencing was applied on the positive-PCR products (Macrogen Company, Korea) employing AB DNA sequencing system. NCBI Websites and MEGA 6.0 software were utilized to analyze the evolutionary history of the species included in this study. The phylogenetic tree was generated via the use of the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (Saitou and Nei, 1987; Tamura et al., 2013).

RESULTS AND DISCUSSION

Fifty intestines were examined for the infestation of Moniezia spp. in the city slaughterhouse; this tapeworm was found to infest the intestines of 13 sheep. Genital pore, cirrus sac, vitelline gland, testes, and inter-proglottid gland were noticed on the mature segments of the tapeworm (Pl.1).

Polymerase chain reaction (PCR) showed the product amplification at 743bp of the 18S rRNA gene (Pl.2); the PCR-product-based sequencing was applied on 4 *Moniezia* tapeworms targeting a specific region of the 18S rRNA gene.

Plate (2): Agarose-gel-based electrophoresis. (SP 1 and 2 are positive for *Moniezia* spp. VC 1 and 2 are negative controls, M is the ladder (2000-100bp)).

The sequencing has shown 2 species of *Moniezia*. SP1 (MH298620.1) and SP2 (MH298621.1), these species revealed close matching on the phylogenetic tree to an isolate from China (GU817405.1) (Diag.1).
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A phylogenetic tree analysis was performed using NCBI-based nucleotide nucleotide website. The comparison was performed using NCBI-based nucleotide nucleotide website.

Diagram (1): Phylogenetic tree analysis relied on 18S ribosomal RNA gene sequence. The sequencing has shown 2 species of *Moniezia* spp., SP1 (MH298620.1) and SP2 (MH298621.1). These two species revealed close matching on the phylogenetic tree in an isolate from China (GU817405.1). The comparison was performed using NCBI-based nucleotide nucleotide website.
According to the present study, the *Moniezia* spp. were found to be wide-prevalent and caused the infestation in sheep intestine, the morbidity of *Moniezia* infestation in the current study was 26%, which indicates a risky situation in which the disease caused by these tapeworms may lead to economic crises in Al-Diwaniyah city (Diop et al., 2015).

In 2012, the species of this genus were detected in the intestines of camels, and that was according to a study performed by Anisimova (2012), this study was estimated the rate of infestation to be as 32.35% and 15.38% in Al-Diwaniyah and Al-Najaf cities respectively. The present study gives information that agrees partially with Fadl et al. (2011) who showed that the infestation of this tapeworm was 0.9% in sheep of Baghdad sampled regions; the infestation prevalence of these tapeworms may go high during spring and summertime, especially when having high numbers of mites.

Identifying the morphology of the five *Moniezia* tapeworms were performed using a modified Carmen stain in which genital pore, cirrus sac, vitelline gland, testes, and interproglottid gland were noticed on the mature segments of the tapeworms, and these results agree with Melhorn (2001).

The PCR results showed the amplification of the specific region of the 18S rRNA gene (743bp) in these tapeworms, and this agrees with (Nguyen et al., 2012) who used the same technique; the results of the sequencing identified these tapeworms in the intestine of the tested sheep in the city, and the phylogenetic tree provided information that our species were matched up with a Chinese strain; this matching may indicate a certain relation between our strain and the Chinese one which could be as a result to have come from the same ancestor. According to the current study findings, *Moniezia* spp. affect sheep in the city of Al-Diwaniyah, Iraq; these findings give interesting information about the evolution history of this worm in the studied city.

**LITERATURE CITED**


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التحديج الجزيئي وتحليل شجرة النشوء لأنواع الجنس Moniezia

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الخلاصة

أجريت الدراسة للكشف عن التحديد النشوئي والجزيئي لديدان الأنواع العائدة للجنس Moniezia Blanchard, 1891 التي تؤثر على امعاء الاغنام في مدينة الديوانية، العراق.

تم استخراج 50 معى اغنم للبحث عن الاصابة بأنواع هذا الجنس في مجزرة المدينة، إذ وجدت هذه الديدان الشريطية في امعاء 13 فردًا من الاغنم لغرض التشخيص المظهري للجنس هذه الديدان. صبغت خمسة قطع جسمية ناضجة بصبغة الكارمن باستعمال الكارمن لدراسة النشأة للعظام، النشأة، وتشخيص الفرد.

عند تطبيق تفاعل البلمرة المتسلسل ودراسة تعابق القواعد النتروجينية لأربعة 16S rRNA من جنس Moniezia، نشأ عنها نوعان من هذا الجنس، SP1 و SP2. كما أظهر النوعان تطابقًا متقاربًا في شجرة النشأة من عزلة من الصين، استنادًا نتائج الدراسة الحالية، فأن النوعان Moniezia spp. من الأنواع في مدينة الديوانية، العراق. تعطى هذه النتائج معلومات مفيدة لالتقاء من تاريخ التطور لهذه الدودة في منطقة الدراسة.