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Morphometric and molecular characterization of *Moniezia* species in sheep in Mosul city, Iraq

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Abstract

The current study examined 100 small intestines collected randomly from sheep slaughtered in the abattoir and butcher's shops from different Mosul city / Iraq areas of both sexes (55 females, 45 males) and different ages. *Moniezia expansa* was diagnosed in 9 samples of intestines by studying the morphometric characteristics of these tapeworms, especially the mature segments, in which both the ovaries and vitelline glands appeared in the ring shape on either side of the body segments and the rosette-like shape of the interproglotidial glands. No significant difference was noticed between males and females of sheep in our study, and the infection rate was 10% in sheep less than a year old and older than two years, with no significant difference between the age groups. The results of the molecular analysis by using conventional polymerase chain reaction technique confirmed the diagnosis of these worms, which belong to the genus *Moniezia*, with a product reaction of 700 base pairs**.** The sequencing result shows two strains of *Moniezia expanza*, which isolated from Iraq *(Moniezia expansa-Iraqi one and Moniezia expansa-Iraqi 2)* were similar to each other had a significant distance to other strains. The study also showed that *Moniezia expansa* is different from the same species in other countries.

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Introduction

Sheep is considered the most crucial preferable livestock for human consumption in an Arab country (1-5). The genus *Moniezia* belongs to the Family Anopocephalidae. Order Cyclophyllidea is considered a high prevalence parasite that infects the small intestine of sheep and causes a disease called moniliasis. Pathogenicity and GIT disorder is less sever ein calves and lambs than in adult ruminants (6-8). Mites are regarded as the intermediate host in the *Momiezia* life cycle. When ruminants ingest them, their larvae (Cysticercoid) are actively attached to the small intestine and become adults (9). Many species of *Moniezia* were recognized in both domestic and wild ruminants, especially *M. expans*e, *M. manardi*, and *M. benedeni* (10). Depending on the morphological of interproglottidial glands, *Moniezia* spp could be differentiated into those with glands arranged in a rosette shape (*M. expansa*) as a short row (*M. benedeni*) (6,10,11) or even lack glands (12). Molecular diagnosis of helminthes has been developed, and the PCR is used to differentiate the species of *Moniezia*. Therefore, the present study was designed to determine the infection of *Moniezia* spp. in the intestine of slaughtered sheep in different areas of Mosul city and confirm the diagnosis of *Moniezia* species by using conventional PCR and studying the phylogenetic tree.

Materials and methods

Collection of samples

Small intestines of 100 slaughter sheep (55 females, 45 males) were examined for the infection with *Moniezia* species. These intestines were collected randomly from slaughterhouse sand butchers shops in different areas of Mosul city during the period from November 2020 to June 2021. The worms were placed in a slightly hot physiological salt solution, and the morphology of these worms was

identified using a light microscope. Some portions of tapeworms (mature segments) were fixed in 70% ethanol and stained with carmine stain and mounted in Canada balsam (5), and another portion of tapeworms were kept at -20°C for molecular study.

DNA extraction

The genomic DNA of these tapeworms was extracted using a DNA extraction kit (Geneaid) following the manufacturer's instructions. The DNA Pellet was rehydrated by adding 100µl of rehydration solution and kept at -20°C until further assay.

Polymerase chain reaction (PCR)

PCR was done to confirm the diagnosis of *Moniezia* spp. by using the primers: Forward: 5′-TGCTACCCGCATGATGTTGT-3′. Reverse: 5′-ACACAGTTGGCTGCACTCTT-3′ (13). The PCR reaction mixtures were prepared in 20µl containing 10µl of Master mix (Promega 2X) with 1µl of each primer, 4µl of DNA template, and 4µl of PCR grade water. The PCR was done using a thermocycler (Optimum 96 G Germany), and PCR cycles were performed as shown in (Table 1) (13).

The amplified products were separated using electrophoresis in 2% agarose gel pertained with a 4µl red safe. A 4µl of each PCR product was loaded into the well of agarose gel. The electrophoresis was carried out at 60 V for 45 min using a power supply containing 1X TBE buffer. A 100 bp DNA marker (Biolaps), 4 µl, was used as a standard molecular marker. The gel was examined under UV light (Gel Do cumictatioc).

Table 1: Cycling conditions of PCR for amplification of *Moniezia*

Step	°C	Time (min)	Cycle
Initial denaturation	95	5	1
Denaturation	95	1	
Annealing	53	1	35
Extension	72	1	
Final extension	72	5	1

Determination the nucleotide

Sequences of nitrogenous bases of *Moniezia* were done by the Genetic Analyzer 3130 (Hitachi, Japan) and matched with NCBI according to the BLAST program.

Statistical analysis

The results were analyzed statistically using chi-square, with a significance level of $P \leq 0.05$.

Results

One hundred intestines of sheep were examined for the infection with *Moniezia* species. The result found that nine sheep infect with these tapeworms with a percentage was 9%. These worms appeared very long, reached up to 6 meters in length and 1.5 cm in width. The body has hundreds and up to thousands of segments. When these segments were stained with carmine stain and examined by a light microscope, it was found that these segments contained two sets of genital organs with marginal pores. The ovaries and vitelline glands have a ring shape on either side. The testes are distributed through the central proglottid. The inter-proglottid glands appear as a row of rosette-like on the middle portion of the posterior border of each segment (Figure 1).

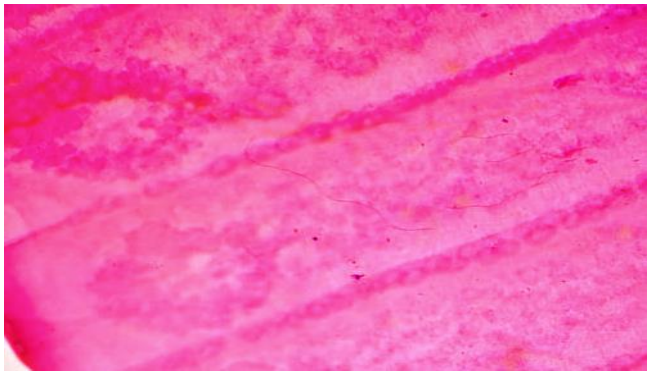

Figure 1: Mature segments of *Moniezia expansa* stained with carmine stain 40X.

A high percentage of infection with *Moniezia* species appeared in sheep females with 9.09% with no significant differences between both sexes (Table 2).

The percentage of infection with *Moniezia* was 10% in sheep aged less than one years and > 2 years, while the percentage of infection in sheep aged 1-2 years was 6.66%, with no significant association between groups of the age of animals (Table 3).

Figure 2 shows the bands of DNA extracted from 9 tapeworms of *Moniezia* in a concentration of 25 ng/µl. The concentration of extracted DNA was 50- 100 ng with a purity of 1.7.

The polymerase chain reaction results showed the possibility of diagnosing *Moniezia* in the DNA samples extracted and used in this reaction. PCR showed the product of amplification 700 bp (Figure 3).

Table 2: The relationship between the infection with *Moniezia* species and sex of animals

Sex	No. examined	No. positive	% Infection
Female	55	5a	9.09
Male	45	4a	8.88
Total	100	9	9

The same letters referred to no significant differences between females and males of sheep with infection with *Moniezia* spp.

Table 3: The relationship between the infection with *Moniezia* spp. and age of animals

Age	No. examined	No. positive	%Infection
> 1 year	30	3a	10
1-2 years	30	2a	6.66
>2 years	40	4a	10
Total	100	9	9

The same letters referred to no significant differences between the infection with *Moniezia* spp. and the age of animals.

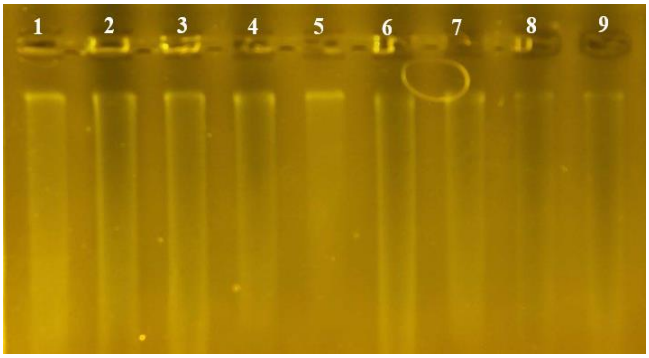

Figure 2: DNA bands extracted from *Moniezia* species migrated into 2% agarose gel with loading dye.

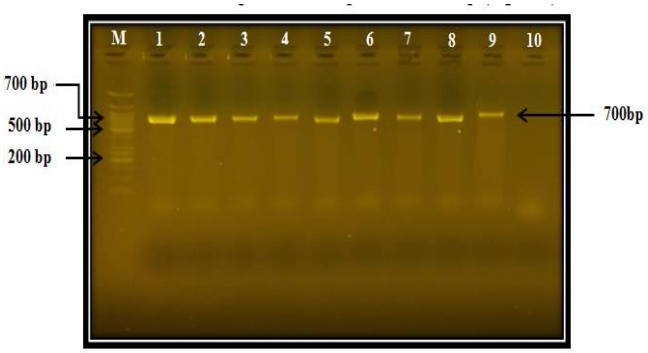

Figure 3: Gel agarose electrophoresis of amplified *Moniezia* DNA using specific primers, M:100 bp DNA size marker, lanes 1 to 9 positive samples (700 bp).

The results of the sequencing showed that the two strains of *Moniezia expansa*, which isolated from Mosul city, were similar to each other and had a significant distance to other strains recorded in different countries when matched with the World Gene Bank, it had the taxonomic symbol ID: AB367793.1 length 1376 Number of matches:1 Range1:84 to586 GenBank and ID: AB367793.1 length 1376 Number 1 Range 1:85 to 684 GenBank (Figures 4 and 5).

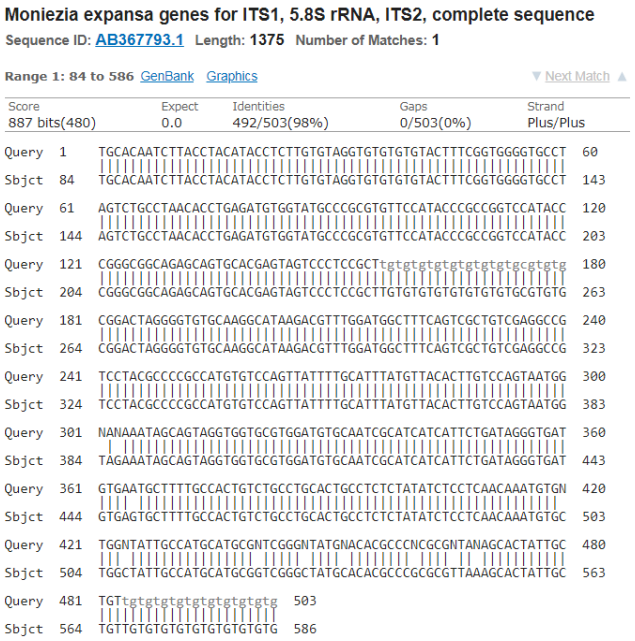

Figure 4: Taxonomic symbol of strain 1 of *Moniezia expansa*.

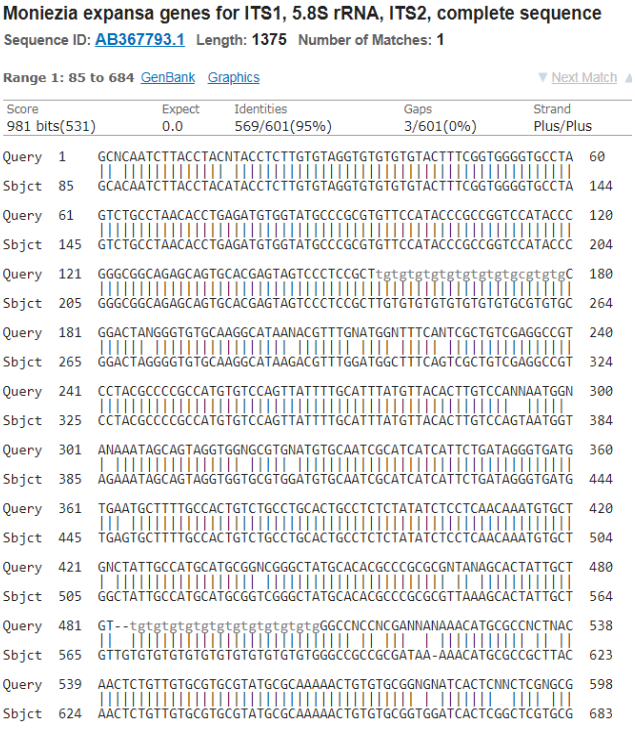

Figure 5: Taxonomic symbol of strain 2 of *Moniezia expansa*.

The results also showed the species of *M. expansa* in Mosul /Iraq differs from some species in other countries while the species of *M. expansa* from India close to species *M. sichanesis* the same thing with *M. benedini* and *M. expansa* from Japan (Figure 6).

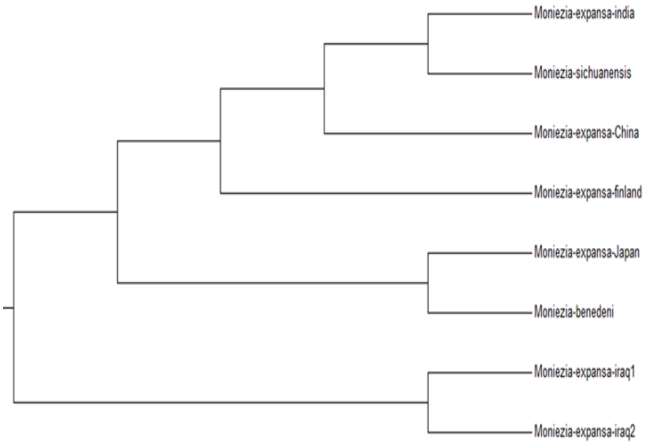

Figure 6: Phylogenetic-tree analysis according to the 18srRNA gene sequencing from *Moniezia expansa*.

Discussion

Moniezia species are common tapeworms in sheep and cattle worldwide, but their detailed morphology and molecular information are securing, except in some limited countries. In the current study, the tapeworms which were diagnosed as *Moniezia expansa* according to the characteristic features, especially the interprologtidial glands which appear as a row of rosette-like shape, this result was the agreement with Diop *et al.* (6), Ali *et al.* (12), Diipeolu *et al.* (14) and Tam *et al.* (15) who referred that *Moniezia expansa* was the predominant species occurs in both small ruminants, sheep and goats.

In our investigation, infection with *Moniezia* showed high prevalence in sheep females, with no significant differences appearing between males and females. This finding was in agreement with Nurlign and Admasu (16). At the same time, this study was disagreement with Molla and Bandyopadhyay (17), and the high infection of parasites in females than males of sheep may be related to various depending factors including lambing, lactation contributing peculiar stress factors responsible for female malnutrition and weakness (18), on contrary sex of animals played no role on the epidemiology and occurrence of *Moniezia* spp (19).

The prevalence of infection with *Moniezia expansa* was 10% in both sheep aged less than one year and sheep whose age was > 2 years, but no significant correlation was reported between animal and *Moniezia* infection.

Our findings agreed with Nurlign and Admasu (16), who reported that GIT parasites affect all ages of animals. In contrast, the present finding disagrees with other studies, which indicated that younger animals (<1year) were more susceptible to *Moniezia* spp infection than older ones >1 year of age due to some degrees of immunity in ruminants to *Moniezia expansa* in older ages (1,17,20).

The PCR analysis showed the amplification of the specific gene 18 srRNA genes 700 bp to *Moniezia* spp. These results are in the same line with Ali *et al.* (12), Wicksrum (13), and Nguyen (21), who referred to the usefulness of PCR technique in the elucidation of *M. expansa* as a dominant species in sheep and goats while *M. benedeni* is one that dominant in cattle. The phylogenetic tree sequence in our results determines the distance and proximity between *Moniezia* strains. The crucial role of the environmental factors in emerging or changing the strains to new ones is in case nucleotides availability in the sequence and their adaptation to environment niche.

Regarding the sequencing *of M. expansa* in Diwaniyah governorate, phylogenic tree matches certain closeness with Chinese strains (12). These molecular findings, coupled with detailed morphological study, could clarify the taxonomic status of *Moniezia* species in various geographical areas (15).

Conclusion

The predominant *Moniezia species* in sheep reared in Mosul city is *Moniezia expansa*. Two strains of *Moniezia expansa* isolated from Iraq *(Moniezia expansa-Iraqi 1 and Moniezia expansa-Iraqi 2)* were similar and had a significant distance to other strains.

Acknowledgments

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Conflict of interest

The authors confirm no conflicts of interest in the publication of this paper.

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دراسة وصفية وجزيئية لأنواع المونيزيا في الأغنام في مدينة الموصل، العراق

إيمان غانم سليمان و نادية سلطان الحيالي و أحلام فتحي الطائي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تضمنت الدراسة الحالية فحص ١٠٠ عينة من الأمعاء الدقيقة جمعت عشوائيا من الأغنام المجزورة في المجزرة ومن محلات القصابين في مناطق مختلفة من مدينة الموصل / العراق من كلا الجنسين (٥٥ إناث و٤٥ ذكور) ومن أعمار مختلفة، تم تشخيص ديدان المونيزيا اكسبنزا في ٩ عينات من الأمعاء وذلك من خلال دراسة الصفات الشكلية والقياسية للديدان خاصة القطع الجسمية الناضجة والتي ظهر فيها كل من المبايض والغدد المحية بالشكل الحلقي على جوانب القطع الجسمية والشكل المسبحي للغدد ما بين القطع الجسمية. أظهرت نتائج الدراسة عدم وجود فرق معنوي في نسبة الخمج بين ذكور وإناث الأغنام كما وبلغت نسبة الخمج ١٠% في الأغنام التي عمرها اقل من سنة والتي عمرها أكبر من ٢ سنة مع عدم وجود فرق معنوي بين الفئات العمرية. أشارت نتائج الفحص الجزيئي وباستخدام تقنية تفاعل البلمرة المتسلسل التقليدي تأكيد تشخيص هذه الديدان والتي تعود لجنس المونبزا وبواقع ناتج تفاعلي ٧٠٠ زوجا قاعديا. أظهرت نتائج الدراسة وجود سلالتين من المونيزيا (مونيزا اكسبنزا عراق1 و مونيزا اكسبنزا عراق٢) كانت متشابهة مع بعضها البعض ومتباعدة بشكل كبير عن السلالات الأخرى كما أظهرت الدراسة أن نوع مونيزا اكسبنزا المشخص مختلف عن نفس النوع في بلدان أخرى.