

## 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 interproglottidial 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 expansa*, 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.

**Keywords:** *Moniezia* species, Morphology, Sheep, PCR, Phylogenetic study

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

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### الخلاصة

تضمنت الدراسة الحالية فحص ١٠٠ عينة من الأمعاء الدقيقة جمعت عشوائياً 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 اكسينزا في ٩ عينات من الأمعاء وذلك من خلال دراسة الصفات الشكلية والقياسية للديدان خاصة القطع الجسمية الناضجة والتي ظهر فيها كل من المبايض والغدد المحيية 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 أقل 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 المونيزيا وبواقع نتائج تفاعلي ٧٠٠ زوجاً قاعدياً. أظهرت نتائج الدراسة وجود سلالتين من المونيزيا (مونيزيا اكسينزا عراق 1 و مونيزيا اكسينزا عراق ٢) كانت متشابهة مع بعضها البعض ومتباعدة بشكل كبير عن السلالات الأخرى كما أظهرت الدراسة أن نوع مونيزيا اكسينزا المشخص مختلف عن نفس النوع في بلدان أخرى.

### 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 severe in calves and lambs than in adult ruminants (6-8). Mites are regarded as the intermediate host in the *Moniezia* 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. expansa*, *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 and 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).

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

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 cumictatic).

### 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).

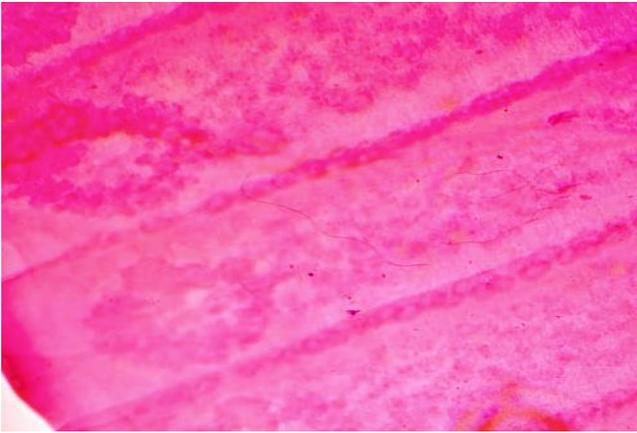


Figure 1: Mature segments of *Moniezia expansa* stained with carmine stain 40X.

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

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.

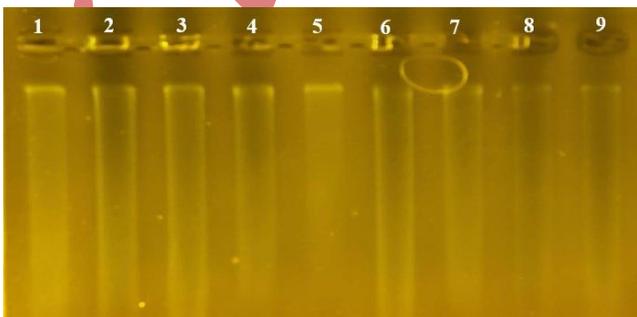
The percentage of infection with *Moniezia* was 10% in sheep aged less than one years and > 2 years, while the percentage of infection in sheep aged 1-2 years was 6.66%, with no significant association between groups of the age of animals (Table 3).

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 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 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 one* 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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#### Article Highlights

- 1- *Moniezia* species are considered as some high prevalence parasites that infect the small intestine of sheep and causes a disease called monieziasis
- 2- Infection with *Moniezia* showed no significant differences appeared between males and females of sheep
- 3- No significant correlation was reported between age of animal and *Moniezia* infection
- 4- *Moniezia expansa* is a dominant species in sheep in Mosul city
- 5- Two strains of *Moniezia expansa*, isolated from Mosul/Iraq (*Moniezia expansa-Iraqi 1* and *Moniezia expansa-Iraqi 2*) were similar to each other had a significant distance to other strains.

**الملاحظات التي يتوجب عليكم تعديلها والتي لا يتم قبول البحث بدون إجرائها (عندما يتم تعديل أية فقرة أو كلمة يجب كتابتها باللون الأحمر):**  
١- إعادة تنسيق المصادر على نسق المجلة العراقية للعلوم البيطرية، ويمكنكم العودة إلى موقع المجلة الإلكتروني من أجل الاطلاع على كيفية كتابة المصادر وتنسيقها، مع تقليل المصادر لتكون بين ٢٠ و ٢٥ مصدراً في أقصى حالة عملاً بتعليمات المجلة. يمكن العودة إلى آخر بحث منشور في المجلة العراقية للعلوم البيطرية والاطلاع على كيفية كتابة المصادر ثم تنسيقها مصادر بحثكم على ضوءها.  
ملاحظة مهمة: يرجى إكمال التصحيحات وخلال يومين من استلامكم البحث لغرض المضي في خطوات قبول النشر اللاحقة