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Morphological identification and phylogenetic analysis of Moniezia species isolated from sheep in Wasit province/ Iraq

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***Abstract*---**The current study was conducted during the period from December 2020 to August 2021 for detect the infection of Moniezia parasite in sheep using the traditional and molecular diagnostic methods as well as for confirmation of Moniezia species in sheep by the phylogenetic analysis. The samples fresh adult worms Moniezia were collected from intestines of sheep the locations of samples were obtained from abattoirs in Wasit province and also regarding months, sexes and ages affecting on infection prevalence rate. Results of microscopically, the samples fresh adult worms Moniezia spp. were collected from intestines of 125 sheep and stained with semichon's acid carmine and examined by light microscope, the results were revealed on 29 (23.2%) positive. Concerning to Moniezia spp. have been identified and classified out of 29 from sheep showed that M. expansa and M. benedeni 26 (89.65%) and 3(10.34%) respectively, with a significant difference (P≤0.01). Regarding to risk factors (sex, age and months), significant (P≤0.01) increase in sheep Monieziasis was reported in age groups <1year (37.5%) more than 1-2 year (14.28%) and > 2year (7.40%). No significant difference was observed in infection rates between male and female. The study revealed that the infection rate was April (57.14%) more than other months.Underwent twenty one PCR positive samples from sheep for DNA sequencing method was performed for Moniezia species typing of some positive local Moniezia spp. and multiple sequence alignment analysis was used in present study to detect of internal transcribed spacer1 and 5.8S ribosomal RNA gene in local M. expansa and M. benedeni sheep isolates and NCBI-Genbank M. expansa and M. benedeni global related isolates. Moreover M. expansa in sheep, worm tissues analysis of genetic relationship between the local M. expansa isolate (ON454618, ON454619, ON454620, ON454621, ON454622,

ON454623, ON454624, ON454625, ON454626, ON454627, ON454628, ON454629, ON454630, ON454631, ON454632, ON454633, ON454634 and ON454635) were showed more closed related to NCBI-BLAST Moniezia expansa Japan, China and India isolates at (100%, 99% and 83%), respectively, and total genetic changes (1.4%). Whereas M. benedeni worm tissues analysis of genetic relationship between the local M. benedeni isolates (ON528685, ON528686 and ON528687) were showed more closed related to NCBI-BLAST M. benedeni Japan isolates at (99%) and a total genetic changes (0.0015%).

***Keywords*---**M. expansa, M. benedeni, sheep, risk factor, phylogenetic analysis, Iraqs.

Introduction

Moniezia species are the most common intestinal cestodes in ruminants, with a worldwide distribution (Diop *et al.*, 2015). Intestinal parasites are responsible for the increased the mortality and decreased animal production as they could cause many pathological change which may lead to severe illness or death of the host (Soulsby, 1982; Al-Rubaie *et al.*, 2019). 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 infection via feeding on grass (Denegri *et al.*, 1998).

Moneizia spp. is one of the most widespread tapeworm infections in sheep and goats recognized to occur in the small intestine, *Moniezia* is a common tape worm with a broad spectrum of definitive hosts, including cattle, sheep, goats and other wild ruminants, the most important species is *M. expansa* which more frequently parasitizes sheep and goats compare to other ruminants (Al-Qureishy, 2008). The whole life cycle required two hosts, ruminants as final hosts, and oribatid mites as intermediate hosts, eggs are passed out from the intestine of the ruminant host along the gravid proglottids in the feces into the soil, the eggs are eaten by soil mites (Aboma *et al.*, 2015). Although most of the *Moniezia* infection are asymptomatic in ruminants but heavy parasitic load may cause poor hair coat, constipation, diarrhoea, dysentery and sometimes anemia, mentioned clinical signs are mainly seen in the young animal which are especially on a poor diet (Constable *et al.*, 2017). However, the determination of *Moniezia* species by morphological observations is difficult, many controversial opinions exist on the validity of any species or individual features (Lamka *et al.*, 2007). Therefore, Polymerase Chain Reaction (PCR) for differentiation of these two species has been developed (Ali *et al.*, 2018). This study determined the prevalence of *Moniezia* species in sheep in Wasit province, Iraq by molecular diagnosis and determined the genetic identity of these *Moniezia* species by phylogenetic analysis.

Materials and Methods

Samples collection

The study was conducted during the period December 2020 to August 2021, fresh adult worms *Moniezia* were collected from intestines of 125 sheep the locations of samples were obtained from abattoirs in Wasit province ,Iraq. The sheep sampling were included both sex (67) male and (58) female. Worms were collected in a petri dish and rinsed several times with physiological normal saline then fixed with 70% alcohol and stained with semichon's acid carmine for morphological features were described according to (Garcia and Ash, 1979). And keep in 70% ethyl alcohol at –20°C for DNA extraction (using in PCR technique). The laboratory processes were done in laboratory of the Parasitological laboratory - College Veterinary Medicine -University of Baghdad.

Molecular study

Molecular study was performed for detection *Moniezia* spp. based on *internal transcribed spacer1 and 5.8S ribosomal RNA gene* (ITS1 and 5.8S rRNA gene) that isolated sheep (Table1). This method was carried out according to method described by (Ohtori *et al.*, 2015). Genomic DNA from worm tissue samples were extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions.

DNA extraction

Extraction of DNA from 29adult worms collected, Genomic DNA of *Moniezia* spp. isolate was extracted by using gSYAN DNA mini kit extraction kit (Tissue protocol) Geneaid. USA, and done according to company instructions, primer were used in this study were obtained from Scientific Reserecher. Co. Ltd, Iraq, *internal transcribed spacer1*(ITS1), *5.8S ribosomal RNA gene*(5.8S rRNA gene). These primer were prepared according to the information of the company (Table 1)

Table 1
The primers used to detect the *Moniezia* spp. based on ITS1 and 5.8S rRNA gene

Primer Name	Primer sequence (5′ to 3′)		Product size (bp)	References
ITS1, 5.8S rRNA gene	F	GCAAGGCATAAGACGTTTGG	400	Ohtori *et al.*(2015)
	R	TGATCCACCGCACACAGT		

Genomic DNA estimation

The extracted genomic DNA from worm tissue sample was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity and concentration of DNA through reading the absorbance in at (260 /280 nm).

PCR Thermocycler Conditions

PCR thermocycler conditions by using convential PCR thermocycler system table (2).

Table 2
PCR cycling condition

PCR step	Temperature	Time	repeat
Initial Denaturation	94ºC	5min.	1
Denaturation	94 ºC	30sec.	35cycle
Annealing	53 ºC	30sec	
Extension	72 ºC	1min.	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	—

The PCR products of was analyzed by agarose gel electrophoresis, 1.5% Agarose gel was prepared in using 1X TBE and dissolving in microwave for 1-3 minutes, after that, left to cool 50°C, then 3µl of Ethidium bromide stain was added into agarose gel solution, agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer, and therefore 10µl of PCR product were added in to each comb well and 3µl of (100bp Ladder) in first well, Then electric current was performed at 100 volt and 80 A. M. for 1hour and PCR products were visualized by using UV Transilluminator.

DNA sequencing method

DNA sequencing method was performed for *Moniezia* species typing of some positive local *Moniezia* spp. 21 PCR positive samples from sheep isolates as the PCR product of *ITS1, 5.8S rRNA gene* were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system. The DNA sequencing analysis (Phylogenetic tree analysis) was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis based ClustalW alignment analysis and the evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA (Unweighted Pair Group Method with Arithmetic) method. The *Moniezia* species typing analysis was done by phylogenetic tree analysis between local *Moniezia* spp. isolates and NCBI-Blast related *Moniezia* species and finally identified *Moniezia* spp. isolates were submitted into of NCBI-GenBank to get Genbank accession number.

Statistical analysis

The Statistical Analysis System- SAS (2012), program was used to detect the effect of difference factors in study parameters. Chi-square (X^2) test was used to significant ($P \leq 0.01$) in this study.

Results

Total results of microscopic

A totally 125 samples isolated from small intestines sheep carcasses slaughtered at abattoir of Wasit province, and then fixed with 70% alcohol and stained with semichon's acid carmine the results were revealed on, 29(23.2%) were given positive results when examined by Microscopically (Table 3).Based on the morphological identification, the body of *Moniezia* spp. consists of three parts, the scolex, the neck and the strobila. The neck section was a very small part that occurs after the scolex section, and the strobila segments could be divided into immature, mature and gravid proglottids, the scolex carries four large suckers, there were no hooks and without spines. These two species could be distinguished from one another by the shape of the interproglottidal glands (IG), *M. expansa* has a row of rosette-like glands extending the width of mature segments whereas the interproglottid glands of *M. benedeni* has arranged in a short continuous row close to the midline of each segment according to (Wang *et al.*, 2010; Liu *et al.*, 2019). (Figure 1; 2; 3; 4; 5; 6).

Table 3
Total infection rate of *Moniezia* spp. in sheep by Microscopically

Diagnostic test	No. of examined	No. of infected	(%)
Microscopy	125	29	23.2

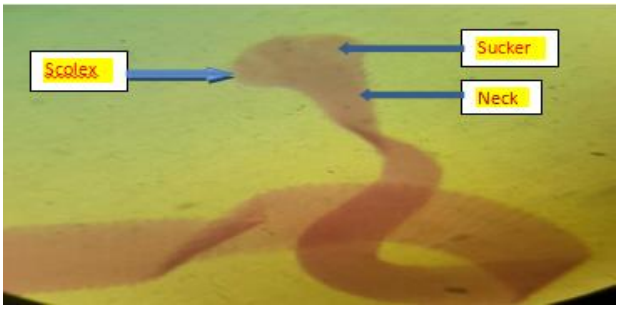

Figure 1. Scolex (contain sukers) and neck of *Moniezia* spp. stained with semichon's acid carmine (4×)

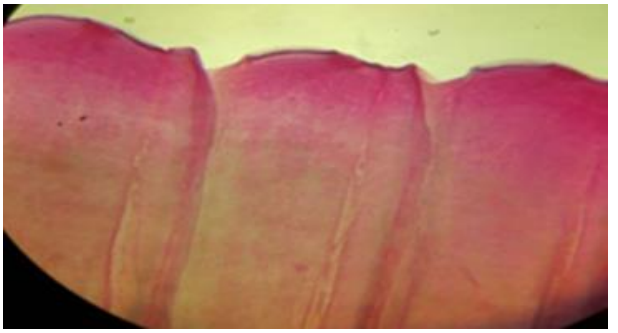

Figure 2. Immature proglottids of *Moniezia* spp. stained with semichon's acid carmine (4×).

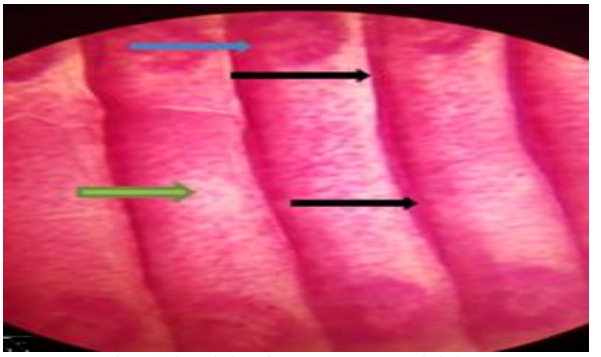

Figure 3. Mature proglottids (black, green and blue arrows indicate IGs, numerous testes and vitelline gland, respectively) of *Moniezia expansa* stained with semichon's acid carmine (4×)

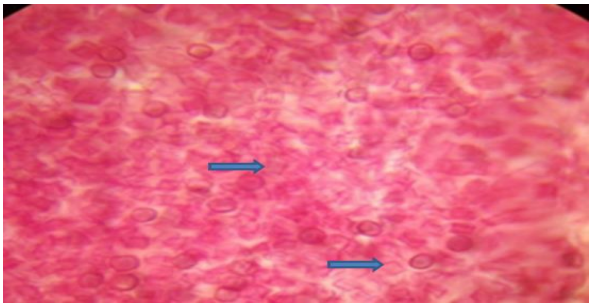

Figure 4. Gravid proglottids (blue arrows indicate eggs). of *Moniezia expansa* stained with semichon's acid carmine (4×)

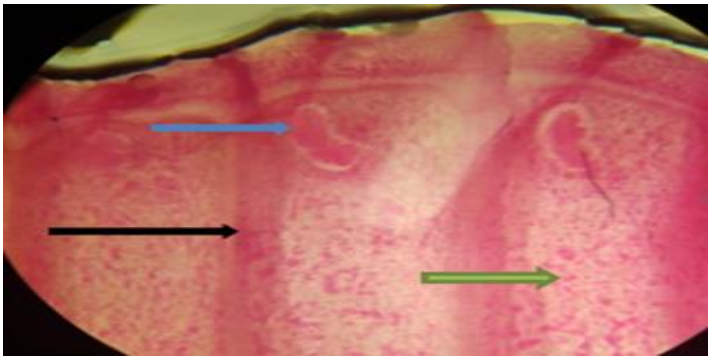

Figure 5. Mature proglottids (black, green and blue arrows indicate IGs, numerous testes, vitelline gland and ovary, respectively) of *Moniezia bendeni* stained with semichon's acid carmine (4×)

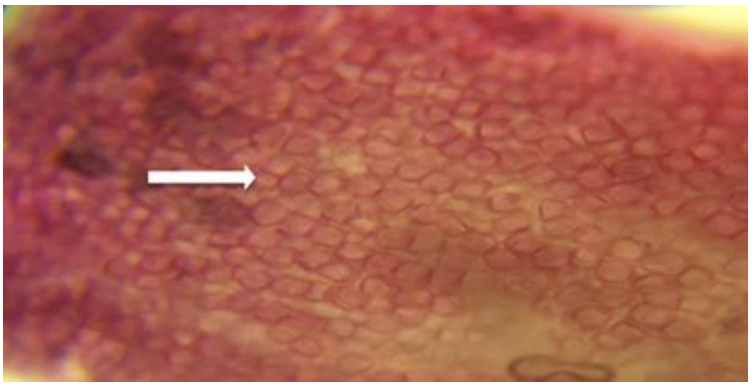

Figure 6. Gravid proglottids (white arrows indicate eggs) of *Moniezia bendeni* stained with semichon's acid carmine (4×)

Identification and classification of *Moniezia* spp. in sheep

All 29 number of infected were results of microscopically for *Moniezia* spp. that identified and classified to *Moniezia expansa* and *Moniezia bendeni*, 26/29 (89.65%) and 3/29 (10.34%), respectively. (Table 4)

Table 4
Moniezia spp. have been identified and classified from sheep

No. of infected	*Moniazia* spp.	Positives	(%)
29	*M. expansa*	26	89.65
	M. bendeni	3	10.34
Chi-Square (x^2)	--	--	18.241` **
** (P≤0.01).			

Infection rate of *Moniezia* species according to sex

Taking 125 samples from isolated from small intestines sheep carcasses slaughtered at abattoir of Wasit province,16/67 (23.88%) male were given positive results when examined by Microscopically, while included female 13/58 (22.41%). (Table 5)

Table 5
Infection rate of *Moniezia* species according to sex

Sex	No. of examined	No. of infected	Percentage (%)
Male	67	16	23.88
Female	58	13	22.41
Total	125	29	23.2
Chi-Square (x^2)	--	--	0.307 NS
NS: Non-Significant.			

Infection rate of *Moniezia* species according to age

Sheep were placed into three groups according on their age: three months to less one year (3 months <1year), one to two years (1-2 years), and more than two years (> 2 years). The largest of positive cases (37.5%) were discovered within 3 months <1year (Table 6).

Table 6
Infection rate with *Moniezia* species according to age

Ages	Examined No.	Positive No.	(%)
<1year	56	21	37.5
1-2 year	42	6	14.28
> 2 year	27	2	7.40
Total	125	29	23.2
Chi-Square (x^2)	--	--	9.163 **
** (P≤0.01).			

Infection rate of *Moniezia* species in sheep according to study months

According to the months of the study the high rate was observed that April, May and March; 8/14 (57.14%), 6/14 (42.85%) and 5/14 (35.71%) respectively; while the lowest rate 1/14 (7.14%) was recorded at January and February. (Table 7).

Table 7
Infection rate of *Moniezia* species in sheep according to study month

Months	Examined No.	Positive No.	(%)
December 2020	13	1	7.69
January 2021	14	1	7.14
February	14	1	7.14
March	14	5	35.71
April	14	8	57.14
May	14	6	42.85
Jun	14	3	21.42
July	14	2	14.28
August	14	2	14.28
Total	125	29	23.2
Chi-Square (x^2)	--	--	19.528 **
** (P≤0.01).			

Molecular study results
Polymerase chain reaction (PCR) results

A total of 29 genomic material (DNA) of adult worms *Moniezia* spp. from sheep were subjected for PCR assay. The result showed that all samples were positives for *Moniezia* spp. with 400 bp. for sheep (Figure 7)

Figure 7. 1.5% agarose gel electrophoresis image that showed the PCR product analysis of *internal transcribed spacer1, 5.8S ribosomal RNA gene* in *Moniezia* spp. Sheep isolates. Where M: marker (2000-100bp) and the lane (1-15) showed some positive *Moniezia* spp. sheep at (400bp) PCR product

Submission of local Iraq isolate in NCBI

21 PCR samples positive sequencing by forward and reverse primers. The sequences were employed in the NCBI gene bank database of *Moniezia expansa*: (ON454618, ON454619, ON454620, ON454621, ON454622, ON454623, ON454624, ON454625, ON454626, ON454627, ON454628, ON454629, ON454630, ON454631, ON454632, ON454633, ON454634 and ON454635). While *Moniezia benedeni*: (ON528685, ON528686 and ON528687).

Moniezia expansa
Phylogenic Analysis

The phylogenetic tree genetic relationship analysis was showed that the local *Moniezia expansa* isolates (ON454618, ON454619, ON454620, ON454621, ON454622, ON454623, ON454624, ON454625, ON454626, ON454627, ON454628, ON454629, ON454630, ON454631, ON454632, ON454633, ON454634 and ON454635) were showed more closed related to NCBI-BLAST *Moniezia expansa* Japan, China and India isolates total genetic changes (1.4%) as showed in figure (Figure 8). (Table 8).

Table 8
NCBI-BLAST Homology Sequence identity percentage between local *M. expansa* sheep isolates and NCBI-BLAST Japan, China and India isolate submitted *M. expansa* isolate

Accession		Country	Host	Source	identity
1.	D: AB367793.1	Japan: Iwate	sheep	*M. expansa*	100%
2.	D: KX377890.1	China	goat	*M. expansa*	99%
3.	D: MZ374056.1	India	Cervus hanglu hanglu	*M. expansa*	83%

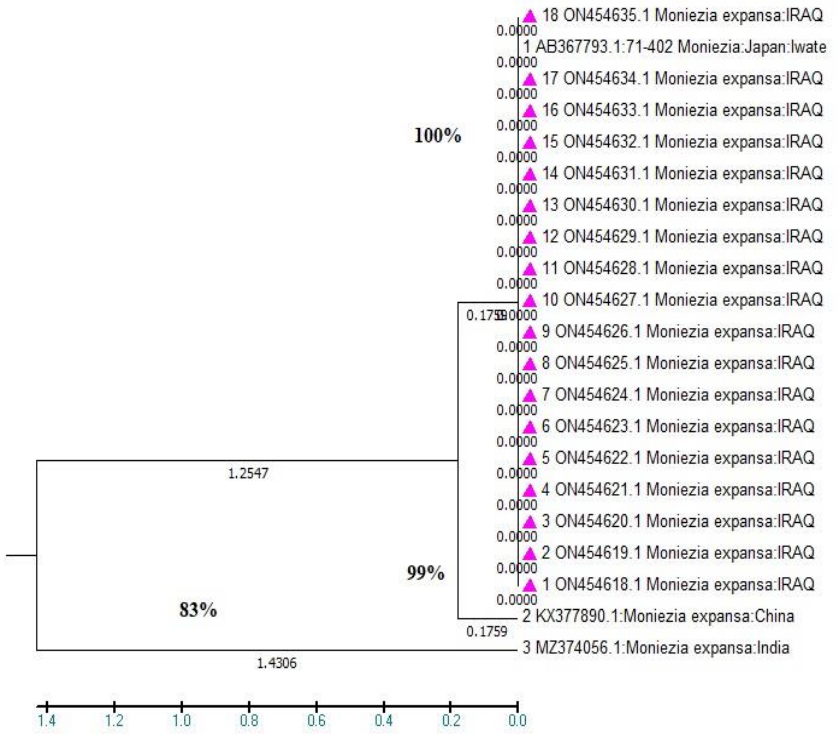

Figure 8. Phylogenetic tree analysis based *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence in local *Moniezia expansa* sheep isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version)

Moniezia benedeni

The phylogenetic tree genetic relationship analysis was showed that the local *Moniezia benedeni* isolates (ON528685, ON528686 and ON528687) were showed more closed related to NCBI-BLAST *Moniezia benedeni* Japan isolates total genetic changes (0.0015%) as showed in figure (Figure 9) (Table 9).

Table 10
NCBI-BLAST Homology sequence identity percentage between local *M. benedeni* sheep isolates and NCBI-BLAST Japan submitted *Moniezia benedeni* isolate

Accession		Country	Host	Source	identity
1.	ID: AB367792.1	Japan: Iwate	cattle	*M. benedeni*	99%

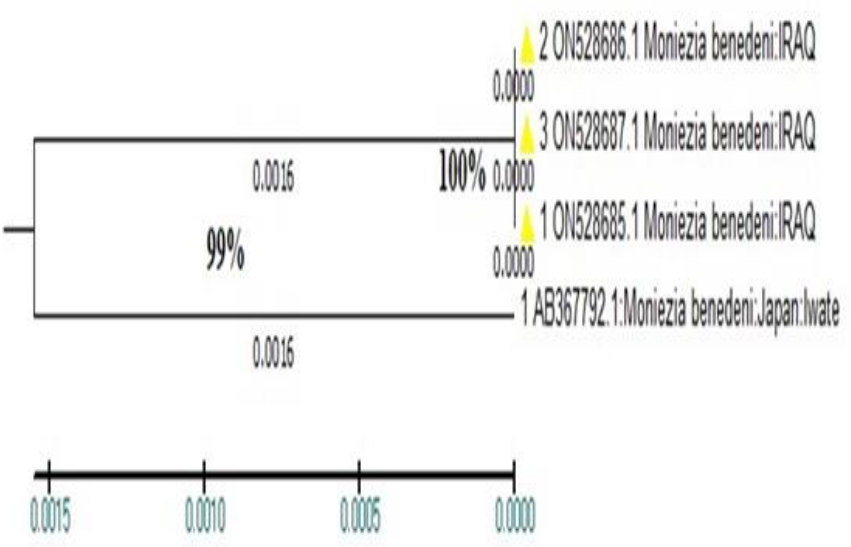

Figure 9. Phylogenetic tree analysis based *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence partial sequence in local *Moniezia benedeni* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version)

Discussion

The *Moniezia* spp. were common parasites which affect ruminants, including sheep, cattle and other species (Prchal *et al.*, 2015). *Moniezia expansa* was considered as the most important cestode parasites infected sheep (Hassanain *et al.*, 2014), whereas *M. benedeni* was more common in cattle. However, both species had been reported in sheep and cattle (Nguyen *et al.*, 2012).

Microscopic examination, Samples isolated from small intestines of sheep

The results of microscopic examination for 125 samples isolated from small intestines of sheep carcasses and then fixed with 70% alcohol and stained with semichon's acid carmine revealed 29 (23.2%)) were given positive results to *Moniezia* spp. The rate of infection in this study was nearly similar to certain studies did in Iraq by Ali *et al.* (2018) who recorded 26% in sheep of Al-Qadisiyah Province. While Anisimova and Al-Fatlawi (2012) were recorded 32.35% and 15.38% in camels of Al-Diwaniyah and Al-Najaf cities, respectively. This results were in agreement with that detected previously by El-Dakhly *et al.* (2012), 25.3% *Moniezia* spp. In Kenya 21% by Kanyari *et al.*, (2009), and Eranska *et al.* (2005) 19.2% in Slovak Republic. But higher than the rate of infection in studies of Fadl *et al.* (2011) who recorded 0.9% in Baghdad, while Al-Qureishy, (2008) in Saudi Arabia and (Moazeni and Nili, 2004) in Iran, they recorded less than 4% of *Moniezia* infection in sheep.

The variation in above results may be attributed to differences in sampling times and variations in geographic locations and shown that in the wet warm season

the rate of *Moniezia* infection (Bashtar *et al.*, 2011 and Nguyen *et al.*, 2012). The intermediate host of the *Moniezia* spp. is pasture mites, exposure of the ruminants to soil and grass will increase the chances of infection and the factors responsible for variations in the prevalence of different parasitic diseases might be the different climate and immune status of the individual animal (Zainalabidin *et al.*, 2019; Win *et al.*, 2020) . According to *Moniezia* spp. have been identified and classified from sheep, the positive findings of microscopy were revealed on highly Significant differences in their values ($P \leq 0.01$), the *M. expansa* and *M. benedeni* in sheep were 89.65% and 10.34%, respectively.

These results were in agreement with the results that have been reported *M. expansa* 88% and *M. bendeni* 12% for Vietnam (Nguyen *et al.* 2012), and Bashtar *et al.* (2011) in Egypt mentioned that *M. expansa* and *M. bendeni* were 74%, 4.8%, respectively. While these results disagree with (Memmendov, 2009) the *M. expansa* 69.3% and *M. bendeni* 30.7%, in Turkey, El-Dakhly *et al.* (2012), reported 34.6% for *M. expansa* and 22.7% for *M. benedeni* in Beni-Suef, Egypt. According to sex, analysis of the data on the basis of sex revealed to male of higher than female, non-significant (NS) difference in the overall prevalence of *Moniezia* spp. in sheep male (23.88 %) and female (22.41%). This was agreement with Win *et al.*, (2020) male 10.7% and female 10.5%, and (Bhat *et al.* 2012: Minnat, 2014) recorded non-significant difference prevalence of sex. There is no significant difference due to the exposure of males and females to the same atmosphere or conditions surrounding them.

The current study referred to the relationship between age of animal and the infection with *Moniezia*sis and significant differences ($P \leq 0.01$), while the high rate 37.5% of infection recorded among sheep with age group that less one year (<1year) and decrease toward the old ages. This result agreement with Squire *et al.* (2019), mentions to *Moniezia* infections were higher in the young animals (under 12 months) than adults animals (above 12 months). Young ages are more susceptible to infection because immunity is incomplete compared to older ages, as well as at older ages, immunity is higher as a result of repeated infections. During studying the effect of different months on the rate of infection the study found that there is significant differences ($P \leq 0.01$), and it showed that the positive results were existed among all months of study period. However, the highest and lowered positive results to *Moniezia* spp. in sheep were detected in April, May and March (Spring), 57.14%, 42.85% and 35.71%, respectively. While the lowest rate 7.14% was recorded at January and February (winter). Anywise the infection prevalence of these tapeworms may go high during spring and summer time, especially when having high numbers of mites (Ali *et al.*, 2018), and other reports observed higher infection rates during spring (Arvinder, 1995; Faraj *et al.*, 2007) and during summer (Arvinder *et al.*, 1993). These results disagree with Tramboo *et al.* (2015), who recorded highest in winter 11.67% followed by spring 7.67%, summer 7.33%, and autumn 5%, in Kashmir Valley.

The variation in months results, due to the distribution of oribatid mites (intermediate host) were affected by a variety of factors, temperature, soil moisture, sun radiation, and food availability were all important environmental elements that influence oribatid mite dispersal and also environmental conditions, particularly temperature and humidity, affect the distribution of

species and the presence of parasites. These mites decrease when the temperature increases and rainfall (Baihaqi *et al.*, 2019).

Multiple sequence alignment and phylogenetic tree analysis

DNA sequencing method was performed for *Moniezia* spp. typing of some positive local *Moniezia* spp. 21 PCR positive samples. The present study, phylogenetic tree genetic relationship analysis was showed that the local *Moniezia* spp. isolated from small intestines of sheep and cattle carcasses (worm tissue) based on *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence and homology sequence identity from local *Moniezia* spp. isolates with the cosmopolitan isolates.

Moniezia expansa

The phylogenetic tree genetic relationship analysis was showed that the local *Moniezia expansa* isolated from small intestines of sheep carcasses based on *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence and homology sequence identity from local *Moniezia expansa* isolates with the global isolates. The homology sequence identity between *Moniezia expansa* sheep isolates of the Eighteen (ON454618, ON454619, ON454620, ON454621, ON454622, ON454623, ON454624, ON454625, ON454626, ON454627, ON454628, ON454629, ON454630, ON454631, ON454632, ON454633, ON454634 and ON454635) and NCBI BLAST related *Moniezia expansa* Japan, China and India isolates were showed genetic homology sequence identity 100%, 99% and 83%, respectively and total genetic changes (1.4%). The Japanese *M. expansa* (AB367793.1) isolate originated from sheep (Ohtori *et al.*, 2015). While the Chinese *M. expansa* (KX377890.1) isolated originate from goat (Lin and Yang., NCBI Genbank, 2018 direct submission) and the Indian *M. expansa* (MZ374056.1) isolated originate from Cervus hanglu hanglu (Khurshid *et al.*, 2021).

Based on the foregoing, we suggested that our strains might have descended from the ancestor of the Japan and China isolates, and that there are certain factors might play a role in distribution of this ancestor around the world such as intermediate host, moving of the animals, importation and exportation processes. Important that genetic markers be established for the accurate identification of *Moniezia* spp. and to provide the basis for taxonomic and population based studies, particularly given that *M. expansa* and *M. benedeni* are capable of parasitizing the same hosts (Ba *et al.*, 1993), and also the type species, *Moniezia expansa*, is a widely distributed cestode of ungulates in Europe, Asia, Africa, America and Australia (Chilton *et al.*, 2007).

Moniezia bendeni

The phylogenetic tree genetic relationship analysis was showed that the local *M. bendeni* isolated from small intestines of sheep carcasses based on *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence and homology sequence identity from local *Moniezia bendeni* isolates with the global isolates. The homology sequence identity between *M. bendeni* sheep isolates of the three

(ON528685, ON528686 and ON528687) and NCBI BLAST related *M. benedeni* Japan isolate were showed genetic homology sequence identity (99%) with the Japan and total genetic changes (0.0015%). The Japanese *M. bendeni* (AB367792.1) isolate originated from cattle (Ohtori *et al.*, 2015).

(Ohtori *et al.*, 2015), who used the same technique, the results of the sequencing identified these *Moniezia* species isolated from intestine of the ruminants in the city, and the phylogenetic tree provided information that our species were matched up with a Japanese strain, this matching may indicate a certain relation between our strain and the Japanese one which could be as a result to have come from the same ancestor. According to the current study findings, *M. benedeni* affect sheep, these findings give interesting information about the evolution history of this worm existed in Iraq.

Conclusion

Molecular analysis of *Moniezia* spp. with its sequencing used for the first time in Wasit-Iraq Province to confirm the detection of *Moniezia expansa* and *Moniezia benedeni* from carcasses (worm tissue). The local *M. expansa* sheep isolates from carcasses (worm tissue) are close related to isolates of Japan and China. While the local *M. bendeni* sheep isolates from carcasses (worm tissue) are identical to the isolates of Japan only.

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