How to Cite:
Sray, A. H. K., & Faraj, A. A. (2022). Prevalence and molecular study of moniezia species isolate from cattle in Wasit Province, Iraq. *International Journal of Health Sciences*, *6*(S5), 936–953.
https://doi.org/10.53730/ijhs.v6nS5.8796

Prevalence and molecular study of *moniezia* species isolate from cattle in Wasit Province, Iraq

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Abstract*---Moniezia*** spp. parasitize the intestines of ruminants causing Monieziasis, *M. expansa* and *M. benedeni* (Cyclophyllidea: Anoplocephalidae) are large species of tapeworm that occurs in sheep and cattle and inhabits the small intestine.The current study was conducted during the period from December 2020 to August 2021 for detect the infection of *Moniezia* parasite in cattle using the traditional and molecular diagnostic methods as well as for confirmation of *Moniezia* spp. in cattle by the phylogenetic analysis. The samples fresh adult worms *Moniezia* were collected from intestines of cattle 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 cattle and stained with semichon's acid carmine and examined by light microscope, the results were revealed on 6 (4.8%) positive. Concerning to *Moniezia* spp. have been identified and classified out of 6 from cattle showed that *M. expansa* and *M. benedeni* 3 (50%) and 3(50%)respectively, without a significant difference. Regarding to risk factors (age, months and sex), significant (P≤0.05) increase in cattle Monieziasis was reported in age groups <12 months (12.19%) compared to 12-24 months (2.08%) and >24 months (0%); in study months April and March (14.28%) compared to months others, and the sex without significant the males (5.71%) compared to females (3.63%). Underwent Six PCR positive samples from cattlefor 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* cattle isolates and NCBI-Genbank *M. expansa* and *M. benedeni* global related isolates.Moreover*M. expansa* in cattle, worm tissues analysis of genetic relationship between the local *M. expansa* isolate the local *M. expansa* isolate (ON528682, ON528683 and ON528684) were showed more closed related to NCBI-BLAST *Moniezia expansa* Japan, China and India isolateswere showed genetic homology sequence identity 99%, 99% and 83%, respectively and total genetic changes (1.5%).Whereas *M. Benedeni* worm tissues analysis of genetic relationship between the local *M. benedeni* isolates (ON454642, ON454643, ON454644)were showed more closed related to NCBI-BLAST *M.benedeni* Japan isolates at (100%) and a total genetic changes (0.00%).

Keywords*---M. expansa, M. benedeni*, cattle, Risk factor, Phylogenetic analysis, Iraq.

Introduction

Moniezia is a parasite that is commonly found in the small intestines of cattle, sheep, goats, and deer (Haukisalmi *et al.*, 2018). 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). Among the intestinal parasites that cause economy loss for animal husbandry, it is an important problem in domestic ruminants (Nguyen *et al.*, 2005). 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).

Monieziasis 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. monardi* (Ohtori *et al.*, 2015). *M. expansa* affects sheep (high incidence), cattle, goats, swine, and very rarely human (Gómez-Puerta, 2008). Young animals appear to be the main targets for the infection by *M. expansa* (Wymann, 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, diarrhea, 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 (Nguyen *et al.*, 2012). This study was carried out to investigate *Moniezia* species in cattle at Wasit province, Iraq with indicating importance of

genetic variation among geographic populations to establishment a reliable identification and conventional taxonomy.

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 cattlethe locations of samples were obtained from abattoirs in Wasit province, Iraq. The cattle sampling was included both sex (70) male and (55) 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 featureswere 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 studywas performed for detection *Moniezia* spp. based on *internal transcribed spacer1, 5.8S ribosomal RNA gene* (*ITS1, 5.8S rRNA gene*) that isolated cattle (Table 1). 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 6 adult 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 Researcher. 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 and 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)**.**

Table (2)
PCR thermocycler conditions by using convential PCR thermocycler system

PCR step	Temperature	Time	repeat
Initial Denaturation	94°C	5min.	1
Denaturation	94 °C	30sec.	35cycle
Annealing	53 °C	30sec	
Extension	72 °C	1min.	
Final extension	72 °C	5min.	1
Hold	4 °C	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, andtherefore 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. 6PCR positive samples fromcattleisolates asthe 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 Clustal W 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 the significant ($P \leq 0.05$)in this study.

Results

Total results of microscopic

A totally 125 samples isolated from small intestines cattle 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, 6(4.8%) 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 (El-Dakhly *et al.*, 2012; Tam *et al.*, 2019) (Figure 1; 2; 3; 4; 5; 6).

Table (3)
Total infection rate of *Moniezia* spp. in cattle by microscopically

Diagnostic test	No. of examined	No. of infected	(%)
Microscopy	125	6	4.8

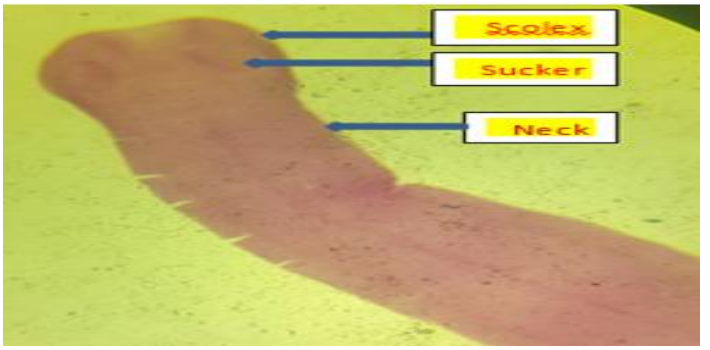

Figure 1: Scolex (contain sukers) and neck of *Moniezia* spp. stained with semichon's acid carmine (4×)

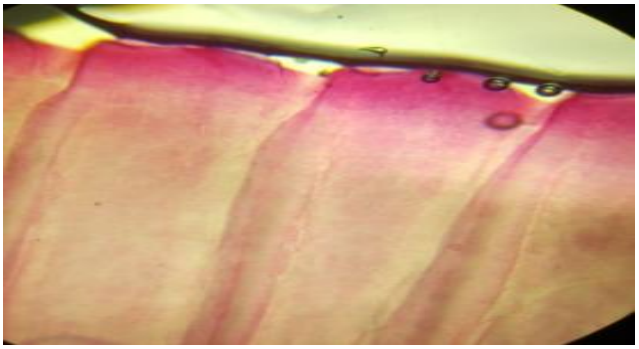

Figure 2: Immature proglottids of *Moniezia* spp.stained with semichon's acid carmine (4×)

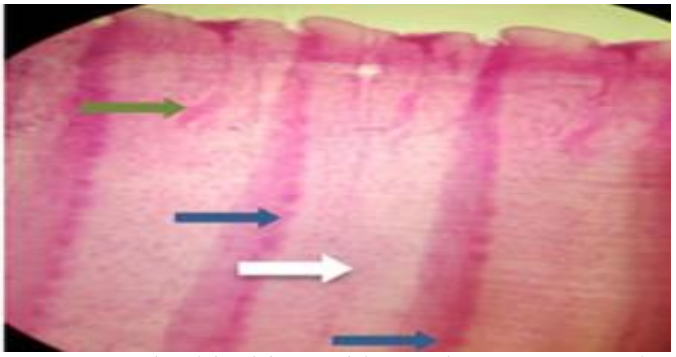

Figure 3: Mature proglottids (blue, white and green arrows indicate IGs, numerous testes, vitelline gland and overy, respectively) of *Moniezia expansa* stained with semichon's acid carmine (4×)

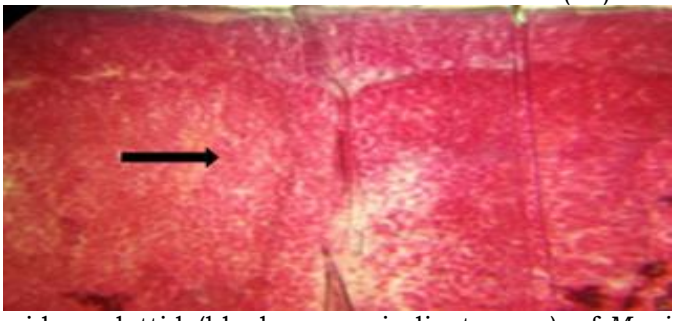

Figure 4: Gravid proglottids(black arrows indicate eggs). of *Moniezia expansa* stained with semichon's acid carmine (4×)

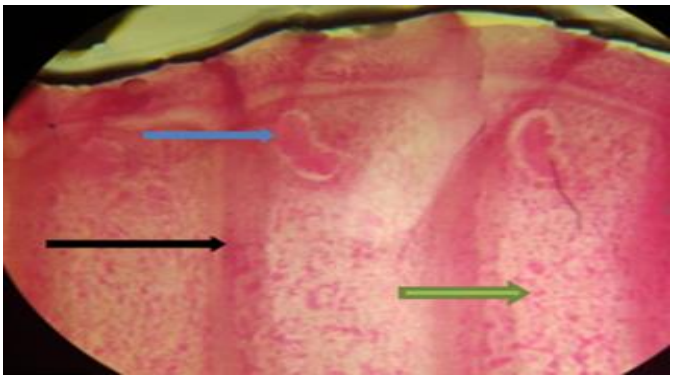

Figure 5: Mature proglottids (black, green and blue arrows indicate IGs, numerous testes, vitelline gland and ovary, respectively) of *Moniezia bendeni* stained with semichon's acid carmine (4×)

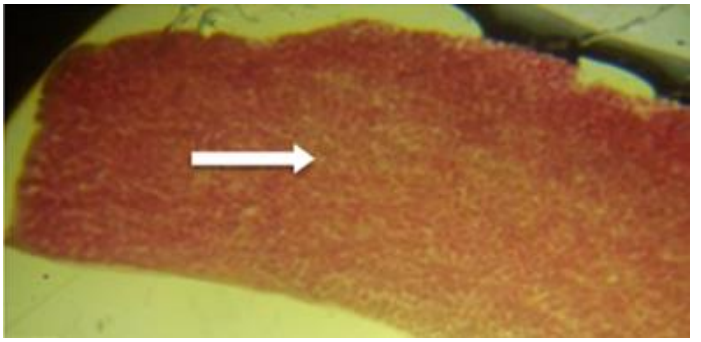

Figure 6: Gravid proglottids (white arrows indicate eggs). of *Moniezia bendeni* stained with semichon's acid carmine (4×)

Identification and classification of *Moniezia* spp. in cattle

All six infected *Moniezia* spp. were discovered microscopically and categorized as *Moniezia bendeni* and *Moniezia expansa*, 3/6(50%) and 3/6(50%), respectively, without significant differences (Table 4)

Table (4)
Moniezia spp. have been identified and categorized from cattle

No. of infected	*Moniazia* spp.	Positives	(%)
6	*M. bendeni*	3	50
	M. expansa	3	50
Chi-Square (x^2)	--	--	1.00 NS
NS: Non-Significant.			

Infection rate of *Moniezia* spp. in cattle according to sex

Out of 125 samples from small intestines of male and female carcasses slaughtered at a slaughterhouse in Wasit province were evaluated microscopically,4/70(5.71%) males showed positive results, while females given 2 /55 (3.63 %) without significant differences.(Table 5)

Table (5)
Infection rate of *Moniezia* spp. in cattle by microscopically

Category	No. of examined	No. of infected	(%)
Male	70	4	5.71
Female	55	2	3.63
Total	125	6	4.80
Chi-Square (x^2)	--	--	0.892 NS
NS: Non-Significant.			

Infection rate of *Moniezia* spp. in cattle according to age

Cattle were divided into three age groups based on their age, less one year (<12months), one to two years (12-24 months), and more than two years (>24 months). Within less one year, the majority of positive cases (12.19%) were detected**,** with significant differences at (P≤0.05) (Table 6).

Table (6)
Infection rate of *Moniezia* spp. in cattle according to age

Ages	Examined No.	Positive No.	(%)
<12 months (<1year)	41	5	12.19
12-24 months (1-2year)	48	1	2.08
>24 months (> 2year)	36	0	0.00
Total	125	6	4.80
Chi-Square (x^2)	--	--	4.837 *
* (P≤0.05).			

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

According to the month of the study the high rate was observed that March and April; 2/14 (14.28%), while the lowest rate zero was recorded at January,February, Jun, July and August with significant differences at (P≤0.05).(Table 7).

Table (7)
Infection rate of *Moniezia* species in cattle according to study months

Months	Examined No.	Positive No.	(%)
December / 2020	13	1	7.69
January / 2021	14	0	0
February	14	0	0
March	14	2	14.28
April	14	2	14.28
May	14	1	7.14
Jun	14	0	0
July	14	0	0
August	14	0	0
Total	125	6	4.8
Chi-Square (x^2)	--	--	5.027 *
* (P≤0.05).			

Molecular study results
Polymerase chain reaction (PCR) results

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

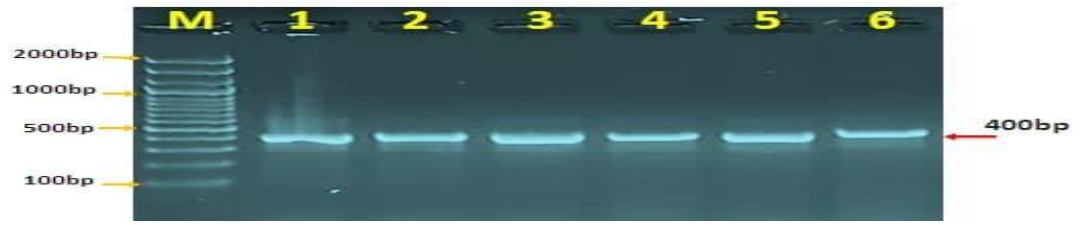

Figure 7: 1.5% agarose gel electrophoresis image that showed the PCR product analysis of *internal transcribed spacer1, 5.8S ribosomal RNA gene* in *Moniezia* spp. Cattle isolates, where M: marker (2000-100bp) and the lane (1-6) showed some positive *Moniezia* spp., cattle isolates at (400bp) PCR product.

Submission of local Iraq isolate in NCBI

SixPCR samples positive sequencing by forward and reverse primers. The sequences were employed in the NCBI gene bank database of *Moniezia expansa*: (ON528682, ON528683 and ON528684). While, *Moniezia benedeni*: (ON454642, ON454643, ON454644).

Moniezia expansa
Phylogenic Analysis

The phylogenetic tree genetic relationship analysis was showed that the local *Moniezia expansa* isolates (ON528682, ON528683 and ON528684) were showed more closed related to NCBI-BLAST Moniezia *expansa* Japan, China and India isolates total genetic changes (1.5%) as showedin (Table 8)(Figure 8).

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

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

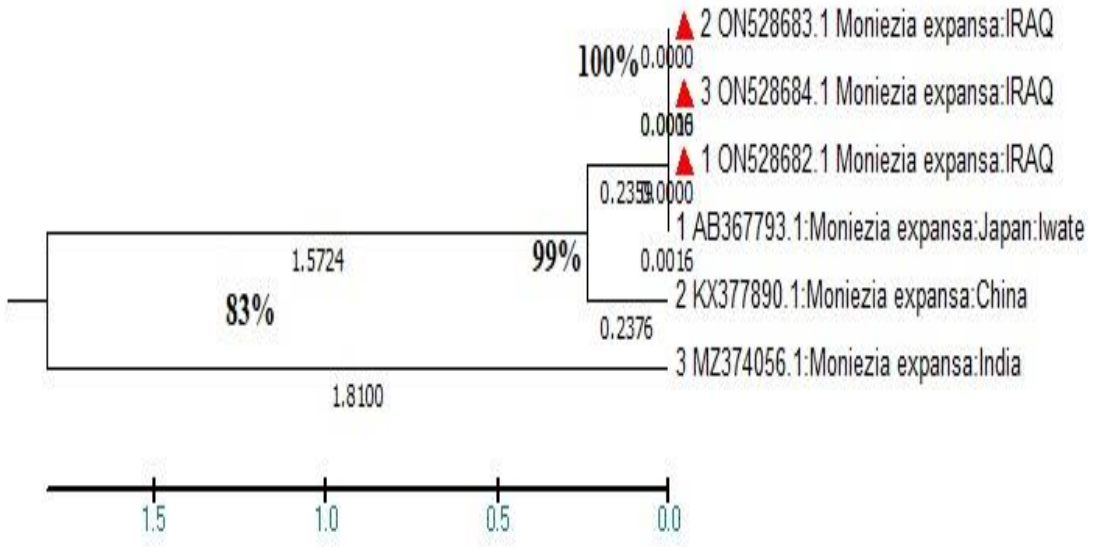

Figure 8: Phylogenetic tree analysis based *internal transcribed spacer1, 5.8S ribosomal RNA gene* partial sequence in local *Moniezia expansa* cattle isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA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

Moniezia benedeni

The phylogenetic tree genetic relationship analysis was showed that the local *Moniezia benedeni* isolates (ON454642, ON454643 and ON454644) were showed more closed related to NCBI-BLAST *Moniezia benedeni* Japan isolates total genetic changes (0.00%) as showedin (Table 9)(Figure 9).

Table (10)
NCBI-BLAST Homology Sequence identity percentage between local *M. benedeni* isolates and NCBI-BLAST Japan submitted *M. benedeni* isolate

	Accession	Country	Host	Source	identity
1.	ID: AB367792.1	Japan: Iwate	cattle	*M. benedeni*	100%

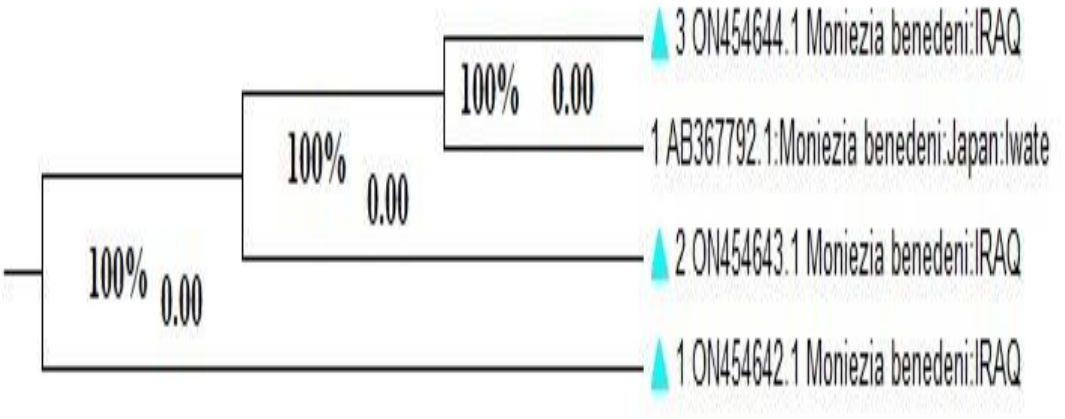

Figure 9: Phylogenetic tree analysis based *internal transcribed spacer1, 5.8S ribosomal RNA gene* in local *Moniezia benedeni* cattle isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA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

Discussion

Moniezia species are the most common intestinal cestodes in ruminants, with a worldwide distribution (Jyoti *et al.*, 2014), *Moniezia expansa* was considered as the most important cestode parasites infected sheep (Poec, 1990), 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 cattle

A total of 125 samples extracted from small intestines cattle corpses slaughtered at a slaughterhouse in Wasit province, and then fixed was 70% alcohol and stained with semichon's acid carmine, the results revealed that 6 (4.8 %) of the samples were positive when inspected microscopically for *Moniezia* spp. This result is agreement with Ntonifor *et al.*, (2013), who recorded the infection in

North West Region of Cameroon was 3.6% and similar with the report from Brazil (Borges *at al.*, 2001) 4,76%, Marskole *et al.*, (2016) 7.90% in Jabalpur, India and *Moniezia* spp. 2.3% by Squire *et al.* (2013) in Southern Ghana. While, high than the rate of infection in studies, Gupta *et al.*, (2012) 0.28%, and Gunathilaka *et al.*, (2018) 0.68% in Gampaha District, Sri Lanka.But much more lower compared with the reported from Hassan and El-Bahi, 1992 the infection with Monieziasis among animals reached to 28% in some areas of Egypt, and reported from France (Achi *et al.*, 2003) 31%. On the other hand, the observed of high infection (29-61%) have been reported in Czech Republic (Chroust, 1997).The variation in the prevalence might be due to the opportunity of exposure to the intermediate host and the free-living soil mites on pasture (Al-gharban and Dhahir, 2015; Marskole *et al.*, 2016). And also the infection rates reported due to differential management practices (Mandonnet *et al.*, 2003), natural resistance (Pal and Quyyum, 1992), drug treatment (Ali *et al.*, 1997).

During studying discovered microscopically and categorized as *Moniezia bendeni* and *Moniezia expansa*, 50%, with non-significant (NS) difference. It seems that infection with the same parasites of sheep was due to grazing on common pastures (Anisimova and Al-Fatlawi, 2012). Akande (2020) in Nigeria recorded, *M. benedini* (24%) and *M. expansa* (16%) with no significant difference, and Laha*et al.* (2017) recorded to *M. expansa* (0.64%) and *M. benedeni* (0.35%) in cows and buffaloes in different places of Manipur. In Indonesia the beef cattle infected *M. expansia* only (9%) recorded by Susana *et al.* (2019). Whereas Nguyen (2012) explained the species *Moniezia benedeni* and *Moniezia expansia* was 88% and 12%, respectively in central Vietnam.

Concerning of sex, from 125 samples from small intestines of male and female carcasses slaughtered at a slaughterhouse in Wasit province were evaluated microscopically, 4/70(5.71%) males showed positive results, while females given 2/55 (3.63 %). The present study showed no significant difference, between sex groups. This finding agrees with the report by Marskole *et al.*, (2016), andKabada *et al.* (2020) explained revealed that there was no significant association between parasite infection and sex of the animal. Because males and females are exposed to the same environment or conditions, there is no significant difference.

Regarding to age, cattle were divided into three age groups based on their age, less one year (<12 months), one to two years (12-24 months), and more than two years (>24 months). Within less one year, the majority of positive cases (12.19%) were detected with significant difference (P≤0.05).The present study showed reported the significant association of *Moniezia* infection and age, this study similar nearly with Squire *et al.* (2013) in Southern Ghana, recordedto(0-12 months) 10.4%, (13-24 months) and(>24 months) were zero, and León *et al.* (2019) recorded to (<12 months) 5.8%, (12-24 months) and (>24 months) were zero percentage, in the Colombian Northeastern Mountain. Whereas, Singh *et al.* (2008) in Faizabad, reported to (0-6 month) 5.35%, (7month - 1year) 12.5%, (>1 year) 0.72%. *Moniezia* spp. infection is quite common in young calves during their first year of life and relatively less common in older animals (Deepak *et al.*, 2020). The present study disagree with Rashid *et al.* (2015) recorded to (<12 months) zero, (12-24 months) 4.60% and (>24 months) 1.08%. However, occurrence of this parasite in the areas is associated with the ingestion of oribatid mites infected

with larval stages of *Moniezia* spp. (Ntonifor *et al.*, 2013). That besides age, climate and educational level of farmers, type of farm management is also one of the risk factors for gastrointestinal parasite infection according to Kantzoura *et al.* (2012). Young animals are susceptible to many parasites and become more resistant to primary infection with some parasites as they reach maturity (Taylor *et al.*, 2016). Due to immunity is insufficient at young ages compared to later ages, as well as immunity is higher at older ages as a result of repeated infections, young ages are more susceptible to infection.

According to the months of the study the high rate was observed that March and April; 2/14 (14.28%), while the lowest rate zero was recorded at January, February, Jun, July and August, with significant difference (P≤0.05). The present study similar nearly with Irie *et al.* (2013) in Japan, recorded in March 18.8% and in April 16.1% in cattle, and Renwal *et al.* (2017) recorded that rainy 6.53%, Winter and Summer zero in India, and other reports observed higher infection rates during spring (Arvinder, 1995; Faraj *et al.*, 2007).While Sodha *et al.* (2021) in Rajasthan India, recorded that 1%, 2.84%, 7.94% in Winter, Summer and rainy, respectively. The study disagrees with Nath *et al.* (2016) in Madhya Pradesh, India, summer 0.24%, Monsoon 0.79%, winter 0.48%. Seasonal dynamics of gastrointestinal helminth infections revealed high prevalence during rainy season followed by summer and winter seasons which reports of Choudhary *et al.* (2018).The differences in prevalence might be due to climatic variations (Krishna and Souza, 2016). Besides, Oribatid mites (intermediate hosts) have to be also present, cysticercoids or larval stages of *Moniezia* spp. develop in them and in this way, the life cycle is completed and the infection is maintained, also wet soils and vegetation permit a better living for these intermediate hosts, in contrast with dry lands where their survival is more difficult (León *et al.*, 2019).

Roczee*t al.* (2016) explained to Oribatid mites (Oribatida) are prevalent throughout the world and constitute one of the largest mites orders. They play an important role in the ecosystem as decomposers of plant detritus. Meanwhile, some of them may be of epidemiological and medical significance because they act as intermediate hosts in the life-cycle of tapeworms of the Anoplocephalidae and Mesocestoididae families. At present, there are about 10,000 spp. of the described oribatid mites. Andcontrol is often achieved by prophylactic use of anthelmintic treatments and deworming and management practices lead to low prevalence of gastrointestinal parasites (Gunathilaka *et al.*, 2018).

Multiple sequence alignment and phylogenetic tree analysis

DNA sequencing method was performed for *Moniezia* spp. typing of some positive local *Moniezia* spp. six PCR positive samples. The present study, phylogenetic tree genetic relationship analysis was showed that the local *Moniezia* spp. isolated from small intestines of 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 cattle carcassesbased 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* cattle isolates of the three (ON528682, ON528683 and ON528684) and NCBI BLAST related *Moniezia expansa* Japan, China and India isolates were showed genetic homology sequence identity 99%, 99% and 83%, respectively and total genetic changes (1.5%). 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) andthe Indian *M. expansa* (MZ374056.1)isolated originate from Cervushangluhanglu (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).

Moniezi abendeni

The phylogenetic tree genetic relationship analysis was showed that the local *M. bendeni* isolated from small intestines of cattle 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. benedeni* cattle isolates of the three (ON454642, ON454643 and ON454644) and NCBI BLAST related *Moniezia benedeni* Japan isolate were showed genetic homology sequence identity (100%) and a total genetic changes (0.00%). The Japanese *M. benedeni* (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 cattle, 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* cattle isolates from carcasses (worm tissue) are close related to isolates of Japan and China. While the local *M. bendeni* cattle isolates from carcasses (worm tissue) are identical to the isolates of Japan only.

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