How to Cite:
Aziz, M. A.-K., & Alshaebani, K. (2022). Molecular characterization of Sarcocystis species Isolated from sheep in Al-Qadisiyah Governorate – Iraq. *International Journal of Health Sciences*, *6*(S6), 9491–9511. https://doi.org/10.53730/ijhs.v6nS6.12473

Molecular characterization of Sarcocystis species isolated from sheep in Al-Qadisiyah Governorate – Iraq

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Abstract---Sarcocystis is a disease that affects both humans and animals alike(Dubey, Calero-Bernal, et al., 2015). the parasite needs to complete its life cycle two hosts(El-Dakhly et al., 2011). a definitive host represented by carnivores(Prakas & Butkauskas, 2012).and an intermediate host herbivores(Motamedi et al., 2020). belonging to the phylum Apicomplexa(Rubiola et al., 2021). Sporozoasida Class(Shnawa & Swar, 2021). During the current study period, a collection of 415 sheep heads to check for infection Sarcocystosis. The diagnostic test is carried out in two ways Examination was carried out first with the naked eye ,and secondly with a light microscope. The first method recorded an infection rate of (7.46%) , While it was recorded by the microscopic method, the percentage of Sarcocystosis cases was 95.18%. Male sheep recorded a percentage of infection with macroscopic Sarcocystis that amounted to 2.73%. In comparison, the percentage of infection in females was 11.71%, with significant differences under the level of significance($p<0.05$). between male and female infections. The molecular study also proved that sheep were infected with the parasite Sarcocystis type S.tenella. Sarcocystis is a zoonotic infection caused (Dubey, 2015)by different species of the genus Sarcocystis(Mousa et al., 2021). parasite that are spread geographically all over the world. The current study verified the presence of Sarcocystis parasite in sheep slaughtered in a slaughterhouse Al-Diwaniyah Governorate - Iraq.

Keywords---Sarcocystis in sheep, Iraq, isolated.

Introduction

Sarcocystis parasite, one of the obligatory parasites(Mohamed et al., 2020a) Sarcocystis is a parasite of zoonotic origin (Mohamed et al., 2020b).that is widespread throughout the world (Shnawa & Swar, 2021), infecting humans, livestock, birds and wild animals. (Shahabi et al., 2022). belonging to the phylum Apicomplexa (Shahabi et al., 2022). Sarcocystis is a Greek name that consists of two syllables (Shnawa & Swar, 2021), the first means meat, Sarkos, and the second syllable, Kystis, means bladder (Al-Saadi et al., 2020). the disease was first discovered by the scientist Miescher in 1843 AD(Fayer, 2004) in the form of milky white (Elmishmishy, 2017). threads in the skeletal muscles of house mice in Switzerland (Castro-Forero et al., 2020). The parasite needs two hosts to complete its life cycle(Abdel-Ghaffar et al., 2009) . Asexual reproduction occurs with cyst formation in the extra-intestinal muscle tissue of the sheep intermediate host, While sexual reproduction takes place in the small intestine, the definitive host is carnivores such as humans. (Prakas et al., 2021). It also infects livestock, poultry and humans (Dubey, Calero-Bernal, et al., 2015). It lives within the striated muscles and nervous system (Farhang-Pajuh et al., 2014). in the intermediate hosts that represent the herbivores (Ayazian Mavi et al., 2020). The disease spreads all over the world(Portella et al., 2021). causing a disease known as Sarcocystosis (Elmishmishy, 2017). Genus Sarcocystis 196 species are currently in force of Sarcocystis However(Dubey, 2015), the complete life cycles of most of them and only 26 have been discovered so far(Dubey, Calero-Bernal, et al., 2015).The host may be infected with one or more species of the genus Sarcocystis (Swar & Shnawa, 2021),There are several species that specialize in terminal hosts(Bittencourt et al., 2016). such as S.tenella, which is specialized in infecting sheep(da Silva et al., 2009). and S.hominis, which infects humans (Nimiri, 2014). There are also morphological changes in the parasite stages(Florin-Christensen & Schnittger, 2018). and due to the lack of knowledge of the hosts of most species and their life cycle (Fayer et al., 2015)some facts remain unclear (Abdullah, 2021).

Material and Method

Samples collection: During the current study period that started from October 2021 to May 2022, samples were collected regularly three times a week, including muscles of the esophagus, tongue, diaphragm and heart of 415 sheep, including 193 males and 222 females. Samples were collected from six different slaughterhouses in Al-Qadisiyah Governorate. And conduct the necessary examination before slaughter to ensure that all sheep have been studied in good health before slaughter. Then estimate the age of sheep based on dental examination to count the number of temporary teeth and 26 permanent incisors. The gender of each sample, the date of collecting the sample and the place from which it was taken were recorded and fixed on transparent plastic bags for transfer to the laboratory for laboratory study. Samples are examined

after slaughter to ensure the presence of visible injuries in the slaughtered sheep.

Table 1
Shows the number of macroscopic cysts by sex of sheep with sarcoidosis.

%	number of injuries	Number of samples	animal sex	
%2.73	5	193	male	**sheep**
%11.71	26	222	female	
% 7.46	31	415	**sum**	
Chi-square: 12.427 Degrees of freedom:2 p-value:0.00200222 * There are significant differences under the significance level (P<0.05)			**statistical analysis**	

Table 2
Shows the number of microscopic and macroscopic infections of sheep with sarcocystosis

%	number of injuries	Number of samples	Type of injury	Type
% 95.18	395	415	microscopic	**sheep**
%7.46	31	415	macroscopic	
Chi-square: 2.528 degrees of freedom:1 0.11184189: p-value *There are no significant differences under the level of significance (P>0.05).				**statistical analysis**

Macroscopic examinations

After washing and removing the impurities and the tunic from each esophagus very carefully to look for the large cysts of the Sarcocystis parasit .Large cysts were found Some bags were removed and released Collect esophageal tissue in a Petri dish Figure (1)It was classified on the basis of Physical attributes such as shape, color, and size. Cysts Their length varies between (4-13 mm) (11.7 ± 1.4 mm). they the colors of the macroscopic bags vary from pale white to pale yellow and Its shapes included both round and oval shapes, and their different sizes. Where it became clear that there are two types that are clear in the macroscopic

Figure 1. A- shows the large macroscopic cysts isolated from the esophagus of sheep infected with sarcocystosis in the slaughterhouses of Al-Qadisiyah Governorate Governorate – Iraq

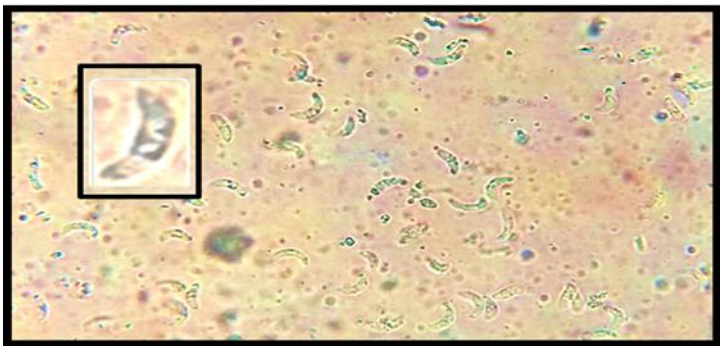


Figure 1. B- Positional of sheep esophagus samples showing the bradyzoite phase after digestion of the sample by the pepsin method. Under the microscope with a force of X40

After that, the macroscopic bags were placed from a petri dish to the endorof tube to be transferred to a laboratory for DNA isolation in order to perform the PCR and to know the genetic sequence, the type of Sarcocystis parasite and its genetic tree.

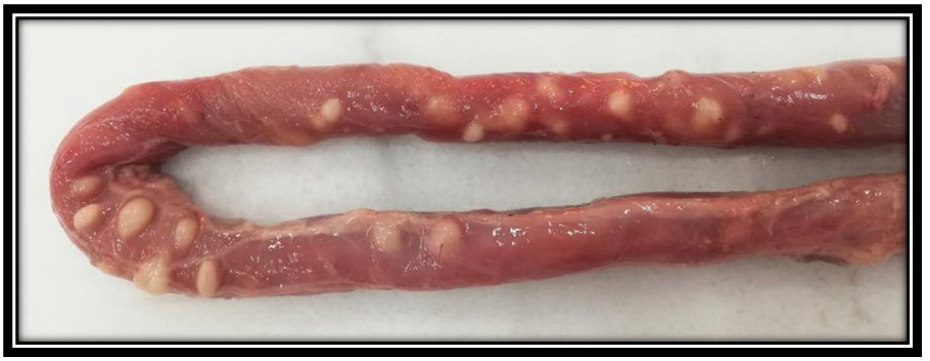


Figure 2. Represents the esophagus of sheep infected with macroscopic sarcoid. It shows the discrepancy in the sizes of the macroscopic

The study included the collection of 415 head of sheep, and the samples included 415 esophageal samples, 100 tongue samples, 50 diaphragm samples, and 50 hearts. All samples were macroscopically examined and it was confirmed that there were 27 macroscopic injuries to the esophagus, 3 macroscopic injuries to the tongue of sheep, and 1 In the diaphragm, and the absence of any visible injury to the heart of sheep. As shown in Table (3)

Table 3
Shows the percentage of gross injuries in some parts of the sheep's body

%	heart	%	diaphragm	%	tongue	%	esophagus	%	injuries	number	Type
-	-	0.24 %	1	0.72 %	3	%6.50	27	.746%	31	415	Sheep
Chi-square: 48.793 degrees of freedom: 4 p-value:0 There are significant differences under the level of significance p<0.05 between injuries of sheep members											statistical analysis

Molecular working methods

Table 4
The last solutions in the PCR work

NO	material used	manufacturer's	country
1	Primer (Forword& Reverse)	Add Bio	Korea
2	Nuclease free water	Add Bio	Korea
3	TaqMaster (2xConc.)	Add Bio	Korea

DNA Extraction Kits

The DNA extraction kit was used as shown in Table(3).

Table 5
Shows the kits used in the current- study, along with the name of the manufacturer and the country of origin

NO	country	manufacturer's	KIT
1	Korea	Add Bio	DNA Extraction Kit
2	Korea	Add Bio	Tissuees Lysis Buffer
3	Korea	Add Bio	binding Buffer
4	Korea	Add Bio	washing Buffer 1

5	Korea	Add Bio	washing Buffer 2
6	Korea	Add Bio	Proteinase K
7	Korea	Add Bio	Nuclease free water
8	Korea	Add Bio	Elution Buffer
9	Korea	Add Bio	Primer
10	Korea	Bioneer	TaqMaster (2xConc.)

Table 6
Shows the volumes of the reaction mixture used to amplify the SrRNA18 gene in the electrophoresis device

Sizes of materials	Materials to use		on
1microliter	Forwoed	Primer	1
microliter1	Reverse		
13microliter	Master max		2
4 microliter	DNA		3
6 microliter	Nuclease free water		5
25microliter	main reaction mixture		resulting

Molecular Study

Molecular Diagnosis of Polymerase Chain Reaction

Amplification of the Sarcocystis parasite 18SrRNA gene by PCR technique and electrophoresis process was used to confirm the presence of DNA bundles on the gelatinous material under current 40 and voltage difference70 within one hour, genetic sequence analysis, and tracing the evolution of strains to diagnose and classify the parasite and compare the molecular relationship of the parasite with the species It isolates from slaughtered animals and draws its genetic tree.

DNA Extraction from infected tissues

Special and equipped extractors kit from the Korean company consisting of a combination of different different materials and solutions and different machines were previously used to extract the parasite DNA. A view that was previously mentioned in the table

- DNA was extracted from the large bags found in the infected samples of slaughtered animals, according to the manufacturer's instructions
- A 1.5 ml Eppendorf tube was prepared and 20 ml/gm of the content of the macroscopic bags extracted from the affected tissues was added using a pipette. The bags were mashed well with a toothpick through a pipette and given their own serial number
- Then 200 microliters of Tissuees Lysis Buffer (CL) were added and the mixture was mixed using a Vortex hand mixer.
- Then 20 µl of Proteinase K solution was added and mixed with a Vortex rotary mixer.

- The formed mixture was incubated in a water bath for 10 minutes at a temperature of 60 C.
- Taking into account the use of the Vortex hand mixer device every 5 minutes in order to disperse the solution inside the Eppendorf tube.
- Add 200 ml of the binding buffer solution and gently re-mix it using the Vortex rotary mixer and then incubate in the water bath for 10 minutes at a temperature of 60°C.
- Then add 200 µl of absolute ethanol solution and re-mix with the Vortex device for 15 seconds. We rotate the Eppendorf down briefly so that the air droplets outside the solution cling to the cap.
- Carefully transfer the solution to the special collection tubes, with a capacity of 2.0 ml, by pipette, and transfer it to the Cool Of Centrifuge and close it tightly, and set it at a speed of 13,000 cycles for one minute.
- After completion, we get rid of the sedimentary material so that the DNA remains stuck to the cotton. Replace the tube with a new tube with a volume of 2 milliliters.
- Add 500 µl of washing Buffer 1 solution to the collection tube and return it to the cooled centrifuge at a speed of 13,000 d/min. Then we get rid of the deposit.
- Then add 500 µl of washing Buffer 2 solution to the collection tube again and return it to the refrigerated centrifuge. After the cycles are over, we get rid of the sediment and return it to the tube itself.
- Then we return the collecting tubes to the centrifuge again at 13,000 cycles for one minute in order to dry well from the remaining materials.
- Transfer the collection tube to a new tube with a volume of 2 ml and add 150 microliters of Elution Buffer using a pipette carefully to the cotton place to avoid touching the ends of the tube.
- Transferring the tubes after adding to the refrigerated centrifuge and at the same pre-time and after the completion of the rotation we take the extract of the parasite DNA from the tubes and keep it by freezing at a temperature of -20 C, thus ending the DNA isolation process and it is ready for use.

Preparation of PCR Reaction

Electrical migration of DNA extract .Special daily work solutions were prepared for the reaction, Using the components of the PCR kit and primer solutions according to the following steps:

- 5microliters of the DNA previously extracted from parasite bags were added to each 0.5 ml Eppendorf Tubes marked with the sample number.
- Then add 5 microliters of the Master Mix.
- Also add 1.5 µl of starters.
- The volume is supplemented with deionized distilled water to reach a total volume of 25 µl.
- Then mix the mixture using the hand mixer Vortex for ten seconds.

- The Alpendorf tubes are transferred to the Thermocycler-Cleavere scientific apparatus to start the multiplication reaction process according to the special program for amplification shown in Table (7)

Table 7
Shows the steps of the PCR program to amplify the DNA extracted from the cysts of the parasite Sarcocystis

Sarcocystis	Primer		Size	Time	tem per atu re
S. tenella (Bahari et al .,2014)	F	5-GCACTTGATGAATTCTGGCA-3	622 bP	5 M 30 S 35 S 55 s 10 s 32RPM for2 hours	95 95 55 72 72
	R	5-CACCACCCATAGAATCAAG-3			

Primers

Primers of the Small subunit ribosome RNA gene were used to detect Sarcocystis species using PCR technique. The primers are the sequences of a group of specific and targeted genomes on the sequence amplification of the 18SrRNA gene, which were previously prepared from the Korean company AddBio, as shown in Table (8) (Bahari et al., 2014); (Anemia, 2021).

Table 8
Shows the primers used to detect the 18SrRNA genetic sequence of two types of Myomycysts

TYPE	Primer		S
S. tenella (Bahari et al .,2014)	F	5-GCACTTGATGAATTCTGGCA-3	622 bP
	R	5-CACCACCCATAGAATCAAG-3	

Preparation and dilution of the primer

First, we dissolve the primer with a Nuclease free water solution by adding 250 ml to the primer and shake it well with the Vortex hand spinner, then we work for it in a Centrifuge and then transfer it to the incubator at a temperature of 37C for five minutes so that the primer mixes with the solution well, after extracting it from the incubator, shaking it well and re-working the Centrifuge for it For one minute, I returned it to the incubator again and for 2 minutes, and here the primer

is ready with a concentration of 100 pcm for each primer, then we dilute the primer to a concentration of 10 p.m. by placing 10 of primer with 90 nucleases free water to become a concentration of 10, then we shake it well and we work for it Centrifuge and then we give the Eppendorf symbol Tubes front R and rear F so that the primer is ready for use.

Preparation of gelatin agarose gel:

The gelatinous material is prepared according to the instructions (Sambrook et al., 1989). It was mentioned in (Elmishmishy, 2017) .7 ml of TBE is mixed with 90 ml of pure water, then 1.5 g of agar is added to it as in Table No. (9). Then the solution is shaken and transferred to a water bath at a temperature of 100 °C for 3-5 minutes to mix with heat and the solution becomes clear (aqueous), then we take it out and add 4 microliters of Ethidum bromide dye to it at a concentration of 2 microliters per 50 ml as prepared in Table (10). (taking into account the avoidance of steam from the gel because of its danger. We pour the gel after its temperature decreases at (50-60) C into the TRAY moulds and install its comb to make holes for the samples.

Table 9
Shows the materials and amounts of preparation of the gelatinous substance (Acarose).(Elmishmishy, 2017)

ON	Subject	Size
1	Agarose powder	1.5 g
2	TBE	7 ml
3	Pure.distilled water	90 ml

Electrophoresis

The solutions used in the electrophoresis process: The mixture was prepared from mixing several solutions, which are the following:

Ethylene Diamine Ttetraacetic acid 0.5M)(EDTA):

This solution was prepared by dissolving 18.612 g of EDTA in 80 ml of pure distilled water, completing the volume in the beaker to 100 ml, and adding Na0H to adjust the pH to 8 using a PH meter. The preparation was done as follows in Table (10).

Table 10
Shows the steps for preparing the buffer solution (EDTA) (Al-Saadi et al., 2020)

ON	Subject	Size
1	Ethylene,Diamine Ttetraacetic acid	18.612 g
2	distilled water	80 g
3	Na0H	PH

Regulating solution

The gelled TBE was prepared by dissolving 10 mL/L of TBE (10x) and the volume was completed to 100 mL by adding pure, deionized distilled water, keeping the solution under 5°C until use.

EDTA buffer (TBE- 10X) T-borate

The buffer solution was prepared by dissolving 5.5 g of Boric acid, 10.78 g of Tris-base and 0.82 immersion of pre-made EDTA at a concentration of 0.5 ml, adding 48 ml of pure distilled water, pH adjusted to 8, autoclave sterilized and kept until use. It was prepared as follows in Table (11).

Table 11
Shows the methods and volumes of the preparation solution (trisborate EDTA) TBE (WHO, 2002)

ON	Subject	Size
1	Tris buffer	10,88 g
2	boric acid	5.7 g
3	Edta Dina Salt	0.85 g

DNA Loading Buffer

The solution was prepared by dissolving 40 g of sucrose sugar with 100 ml of water and adding 0.25 g of bromophenol blue dye, to stain the DNA, and keeping it until use at room temperature. As indicated in the table (12)

Table 12
Shows the materials used in the preparation of BVR solution

ON	Subject	Size
1	sucrose sugar	40 g
2	Pure, deionized water	100 ml
3	Bromophenol blue dye	0.25 g

Safety Red Dye Dealer in interaction

Prepared by dissolving 5 g of red dye with 100 ml of pure water and collected in a sterile flask, bringing the final concentration of the solution to 0.5 mg/ml.

DNA Electrophoresis

An electrical relay process was carried out using a BIOBASE relay to detect parasite DNA after amplification and the solutions were prepared

according to the manufacturer's instructions (Tasara et al., 2005). As follows:

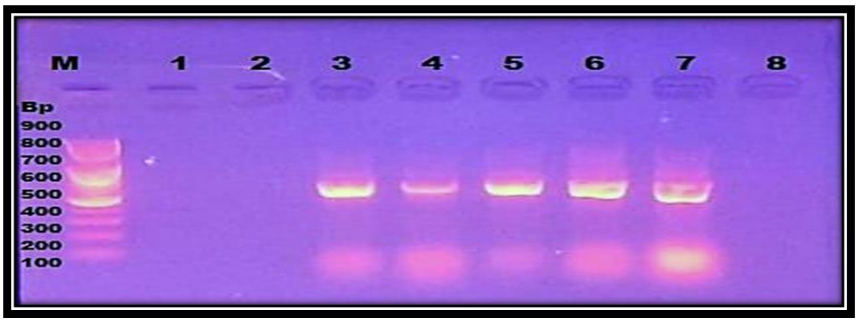
- Inject 100 DNA Ladder bp into the pits of the gelatin mold carefully with 5 µl of PCR products from each sample.
- Then the gel was transferred to the electric relay device after it was injected with the samples and the lid and immersed in the buffer.
- Close the device well and connect the wires, taking into account the electrodes carefully, and pass an electric current of 70 volts, which is calculated for every 1 cm 10 volts and remains for one hour.
- After the end of the period, the electric current is cut off, the electrodes are lifted, and the template is transferred to the TRANSILLUMINATOR device, which acts as a light source for it, which is ultraviolet (UVA) rays, which are specialized to determine the parasite's DNA bundles.
- In the case of the appearance of bands or bundles of sizes 622 base pairs of the parasite gene SrRNA18 on the gelatinous segment, indicating that the result is positive in the presence of DNA, otherwise the result is considered negative.
- Photographing the packages using a photographic camera to document the situation.-

DNA Sequencing, Sequence Analysis for Sarcocystis spp

When the electrophoresis process was completed and it was confirmed that clear packets appeared in the PCR process, the DNA products were sent to each sample with 25 microliters of Primer Forward at a concentration of 10% for each iteration of the products, and transferred to a refrigerated container according to the instructions of the Korean company Macrogen. The genetic sequence of the nitrogenous bases of the parasite was found after purification of the PCR products, using the Sanger technique sequencing. Then the sequence was edited using Finch TV, and then it was compared with the similar sequence in the gene bank using the Basic Local Alignment Tool Search program located in the National Center for Biotechnology and Information. And drawing of the genetic tree of the parasite muscular dystrophy.

Molecular description of the parasite gene SrRNA 18

The results of the electrophoresis process showed the DNA bundles of the genus Sarcocyst, especially the genus. S. tenella is specialized in sheep infection with the genetic 5-GCACTTGATGAATTCTGGCA-3, 5-CACCACCCATAGAATCAAG-3, by. PCR technique using the special primer targeting the SrRNA18 gene extracted from the cysts that infect sheep, and there is a large difference in the size and shape of the cysts in sheep and goats infected with sarcostasis, and With oval, spherical or elongated shapes resembling a grain of rice, it was observed that one personalized bundle of the SrRNA18 gene at 556 bp, the molecular size of the leader bundle was 1100 bp, base pair. as in picture 3.



Picture No. (3-4) shows the packages in the electrophoresis of PCR products of the 18SrRNA gene of Sarcocystis Spp. On the gel, some samples show bands of bp600 in size that are confirmation of the DNA of the Sarcocystis parasite. Also, no bands in places 1, 2, and 8 did not show any indication of the absence of the Parasite Mycysticergo DNA in these samples.

Evidence by PCR technique that specializes in myocytic infection in sheep

The results of the PCR test conducted on the DNA extracted from the muscular sacs of the parasites of the specific Myomycysts target gene confirmed 18SrRNA using a forward and reverse primer. To detect the type of specialist for the infection of the intermediate hosts according to the studies recorded in the gene bank. The sequence analysis and genetic sequence of the bundles appearing on the gel material confirmed that the infection is specific to Sarcocystis, with a rate of 99.64% for the isolates, as shown in Table (12).

Phylogenetic Tree

By reading and analyzing the genetic tree of the parasite and the genetic sequence of the DNA of the small subunt rRNA18, which is specific to comparing the types of the parasite Myomycystis spp. isolated from the macrocysts in sheep with the sequence of the same gene for the species of the parasite Sarcocystis Spp. Registered in NCBI and using the MEGA X program using the TAMURA-NEI model, the genetic tree of the types of Myomycysts was drawn in the current study, and the results showed the presence of stamping in the genetic sequence between the samples isolated in this study with the globally registered species of the same parasite in the gene bank.

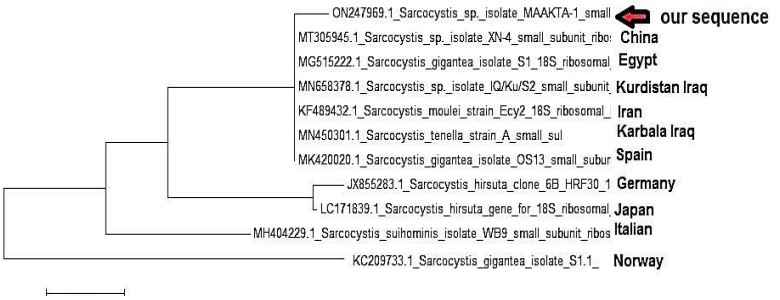


Figure (5) shows the evolutionary tree of Sarcocystis spp. By sequencing the nitrogenous bases of the rRNA 18 gene and comparing it with the species of Sarcocystis, its most closely related isolates, by the MEGA6 method.

Table 12
Alignment of local isolates with reference sequences of the NCBI database by gene bank

	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Sarcocystis sp. isolate XN-4 small subunit ribosomal RNA gene, partial sequence	1022	1022	100 %	0.0	99.82 %	615	MT305945.1
Sarcocystis sp. isolate XN-3 small subunit ribosomal RNA gene, partial sequence	1022	1022	100 %	0.0	99.82 %	615	MT305944.1
Sarcocystis sp. isolate XN-1 small subunit ribosomal RNA gene, partial sequence	1022	1022	100 %	0.0	99.82 %	615	MT305942.1
Sarcocystis gigantea isolate OS13 small subunit ribosomal RNA gene, complete sequence	1022	1022	100 %	0.0	99.82 %	1910	MK420020.1
Sarcocystis gigantea isolate S1 18S ribosomal RNA gene, partial sequence	1022	1022	100 %	0.0	99.82 %	582	MG515222.1
Sarcocystis gigantea isolate S1.1 18S ribosomal RNA gene, partial sequence	1022	1022	100 %	0.0	99.82 %	1868	KC209733.1

Sarcocystis sp. isolate MAAKTA-1 small subunit ribosomal RNA gene, partial sequence	1016	1016	100 %	0.0	99.64 %	610	ON247969.1

Sarcocystis sp. isolate MAAKTA-1 small subunit ribosomal RNA gene, partial sequence

Sequence ID: ON247969.1 Length: 556 Number of Matches: 1

Range 1: 1 to 556 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1027 bits(556)	0.0	556/556(100%)	0/556(0%)	Plus/Plus

```
Query  1    TTATATTAGGTTGATTCCTAGTATAATAATATTTATTATTATGGGGATGACAGTTACTTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTATATTAGGTTGATTCCTAGTATAATAATATTTATTATTATGGGGATGACAGTTACTTT  60

Query  61   GAGAAAATTAGAGTGTTTGAAGCAGGCTTTTGTTGCCTTGAATACTGCAGCATCGAATAA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GAGAAAATTAGAGTGTTTGAAGCAGGCTTTTGTTGCCTTGAATACTGCAGCATCGAATAA  120

Query  121  CAATATAGGATTTCGGTTCTATTATTTTGTTGGTTTTTTCTGTAGGACTGAAATAATGAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CAATATAGGATTTCGGTTCTATTATTTTGTTGGTTTTTTCTGTAGGACTGAAATAATGAT  180

Query  181  TAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTGT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  TAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTGT  240

Query  241  TAAAGACGAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TAAAGACGAACTACTGCGAAAGCATTTGCCAAAGATGTTTTCATTAATCAAGAACGAAAG  300

Query  301  TTAGGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  TTAGGGGCTCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCCGACTAG  360

Query  361  AGATAGGAAAATGTCACATTGTTGACTTCTCCTGCACCTTATGAGAAATCAAAGTCTTTG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  361  AGATAGGAAAATGTCACATTGTTGACTTCTCCTGCACCTTATGAGAAATCAAAGTCTTTG  420

Query  421  GGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  421  GGTTCTGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCA  480

Query  481  CCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  481  CCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGAC  540

Query  541  ATGGGAAGGATTGACA  556
            ||||||||||||||||
Sbjct  541  ATGGGAAGGATTGACA  556
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Comparing the results of PCR tests with local cities and neighboring countries

The results showed the closeness of the sequence analysis of the nitrogenous bases of the genetic sequence with the record number (ON247969.1) with the sequences of the nitrogenous bases in the local strain and the neighboring country strain, as the study sequence showed that there is convergence in the sequence of the nitrogen base sequence with each of Karbala Iraq, and Iraqi Kurdistan , Iran, Egypt, China and Spain with the index (MN450301.1, MN658378.1, KF489432.1, MG515222.1, MT305945.1) respectively .The local sample with the index number (ON247969.1) with the sequences of nitrogenous bases in other countries moved away by 1Node with the sequence of the nitrogenous bases of Germany and Japan with the record number (JX855283.1, LC171839.1) respectively.

The studied strain moved away by 2Node from the State of Italy with the record number (MH409733.1) .It also completely and differently departed

from the nitrogenous base sequence of Norway with the record number (KC209733.1). As shown in the following genetic sequence analysis tables

Table 13
Shows the local and global converging and different strains of the myocytic parasite

s	The index of the strain compared to its source
A	ON247969.1_Sarcocystis_sp._isolate_MAAKTA-mall_subunit_ ribosomal_RNA_gene_ partial_sequence
B	MT305945.1_Sarcocystis_sp._isolate_XN-4_small_subunit_ribosomal _RNA_gene_ partial_sequence
C	MG515222.1_Sarcocystis_gigantea_isolate_S1_18S_ribosomal_RNA_ gene_partial_sequence
D	JX855283.1_Sarcocystis_hirsuta_clone_6B_HRF30_18S_ribosomal_ RNA_gene_complete_sequence
E	MN658378.1_Sarcocystis_sp._isolate_IQ/Ku/S2_small_subunit_ribo somal_RNA_gene
F	KF489432.1_Sarcocystis_moulei_strain_Ecy2_18S_ribosomal_RNA_g ene_partial_sequence
G	MH404229.1_Sarcocystis_suihominis_isolate_WB9_small_subunit_ri bosomal_RNA_gene_complete
H	LC171839.1_Sarcocystis_hirsuta_gene_for_18S_ribosomal_RNA_part ial_sequence_isolate
I	MN450301.1_Sarcocystis_tenella_strain_A_small_subunit_ribosomal _RNA_gene_partial_sequence
J	KC209733.1_Sarcocystis_gigantea_isolate_S1.1_18S_ribosomal_RNA _gene_partial_sequence
K	MK420020.1_Sarcocystis_gigantea_isolate_OS13_small_subunit_ribo somal_RNA_gene.

S	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
B	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
C	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
D	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
E	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
F	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
G	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
H	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
I	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
G	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A
K	G	T	T	A	C	T	T	T	G	A	G	A	A	A	A	T	T	A	G	A

Table No. (14), which starts from 1 to 20, corresponds to the genetic sequences of the nitrogenous bases of the index (NO247969.1) by 100%

with each of China (MT305945.1), Egypt (MG515222.1), and Germany (JX855283.1), Iraqi Kurdistan (MK420020.1), Iran (KF489432.1), Italy (MH404229.1), Japan (LC171839.1), Karbala, Iraq with the record (MN450301.1), Norway with the record (KC209733.1), and Spain with the record (KC209733.1)

Table No. (15) shows that there is a congruence in the numbers of nitrogenous bases from 21 to 40, and no significant differences were observed in this figure between the standard moths, except for the country of Norway with the index number (KC209733.1) and there is a difference in two bases with the site (37, 29). In site 38, there was no rule except for Norway with the index number (KC209733.1). There was a difference in the presence of adenine. The difference was at site 29 with the presence of cytosine instead of adenine, and at site 37 the presence of adenine in place of thymine, and no other match was observed between countries.

S	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
A	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
B	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
C	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
D	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
E	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
F	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
G	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
H	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
I	G	A	T	G	C	C	T	T	G	A	T	T	A	C	T	C	G	A	G	C
G	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C
K	G	T	T	G	C	C	T	T	G	A	A	T	A	C	T	G	C	A	G	C

Table (16) The results of the current study of the genetic sequence sequencing showed that there is a clear match between the local strain by comparing it with the rest of the countries, as it was noted that there is a different nitrogen base in Karbala with the index number (MN450301.1) from the rest of the countries where it indicated at site 51 the presence of thymine locale Adenine and Norway differed with the record number KC209733.1 with three nitrogen bases at site 42 to replace thymine with the base adenine, and at site 56 the guanine was replaced by the base of cytosine and in the corresponding site No. 57 replaced by guanine in the place of cytosine.

S	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
A	A	T	C	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
B	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
C	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
D	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T

E	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
F	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
G	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
H	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
I	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
G	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T
K	A	T	G	G	A	A	T	A	A	C	A	A	A	T	T	T	C	G	G	T

Table (17) the results of the current study showed that there are only two different bases at site 99, as cytosine was replaced by thymine in the strain of the state of Germany with the index number (JX855283.1) and for the state of Egypt, where cytosine replaced the base of thymine in site 99. No other difference was observed between the studied strains, as we note that there is a sequence of nitrogenous bases between Karbala, Kurdistan, Iran, Spain, China, Japan and Italy by 100%.

S	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
A	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
B	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
C	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
D	A	T	T	T	T	G	T	T	G	G	T	T	C	T	T	T	C	T	G	T
E	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
F	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
G	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	T	T	G	T
H	A	T	T	T	T	G	T	T	G	G	T	T	C	T	T	T	C	T	G	T
I	A	T	T	T	T	G	T	T	G	G	T	T	T	C	T	T	C	T	G	T
G	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T
K	A	T	T	T	T	G	T	T	G	G	T	T	T	T	T	T	C	T	G	T

The molecular study confirmed that there is an infection in sheep with Sarcocyst parasite, and the results matched the genetic sequences in the PCR work resulting from the sheep's esophagus with the genetic sequences in the countries' gene bank.

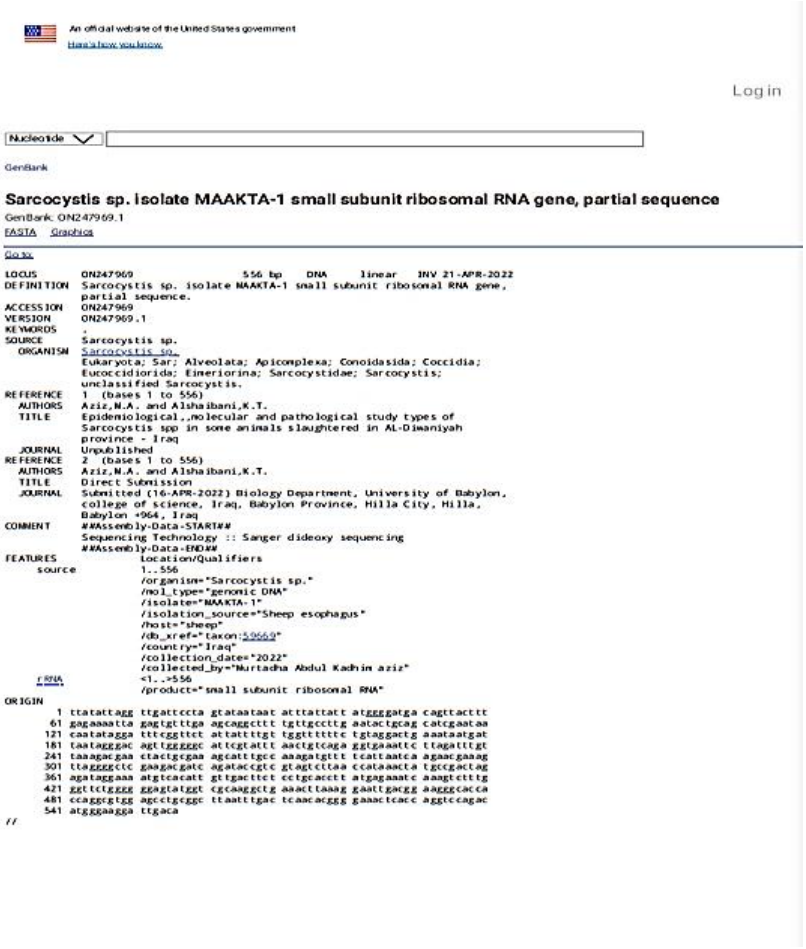

Figure 6. Shows the recording of the sequencing of the Myopycosis parasite extracted from the PCR process in the global gene bank and with a link https://www.ncbi.nlm.nih.gov/nuccore/ON247969

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