



## RESEARCH ARTICLE

### Molecular Identification of *Sarcocystis* Species in Sheep (*Ovis aries*) and Goats (*Capra hircus*) of Duhok Province, Iraq

Shivan N Hussein\*, Assel A Ibrahim and Mohammed S Shukur

Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Duhok University, Duhok, Kurdistan Region of Iraq

\*Corresponding author: shivan.hussein@uod.ac

#### ARTICLE HISTORY (22-390)

Received: November 23, 2022  
Revised: January 15, 2023  
Accepted: January 29, 2023  
Published online: February 16, 2023

#### Key words:

*Sarcocystis* spp.  
18S rDNA  
Sheep  
Goats  
Iraq

#### ABSTRACT

*Sarcocystis* is the most common intracellular cyst-forming protozoan that infects the cardiac, smooth and striated muscles of animals and humans. This parasite has a significant economic, veterinary and medical impact in many regions of the world. The current study aimed to identify *Sarcocystis* species in domestic sheep and domestic goats slaughtered at the slaughterhouses of Duhok province in Iraq, from May 2021 to June 2022 by using molecular study. A total of 250 tissue samples collected from 147 sheep and 103 goats' different organs (79 esophagi, 105 skeletal muscles and 66 hearts) were examined for detection of sarcocysts through microscopic examination of fresh muscle tissues. Consequently, the isolated zoitocysts of sarcocysts and/or their fragments from each infected host were further used for molecular identification. Overall, 98.32% of the examined sheep and 98.06% of the examined goats were microscopically found to be positive for *Sarcocystis* species. Molecular identification of *Sarcocystis* spp. isolates based on analysis of the 18S rRNA gene sequences revealed the existence of *S. tenella* in both sheep and goats and *S. capracanis* in goats only. Alignment and phylogenetic analysis of the isolates in this study showed a very close relationship and close matching with other *Sarcocystis* DNA sequences which were available in the GenBank database. Results of the phylogenetic analysis showed sequences of *S. tenella* as a monophyletic cluster to the sequences of *S. capracanis*. As the 18S rRNA sequences of *S. tenella* and *S. capracanis* showed high similarity with each other, further molecular studies based on the cox1 and ITS1 regions are required for the exact characterization of species identified in the present study. Due to cross-infection and high incidence of *Sarcocystis* spp. in both sheep and goats in the world and in Iraq, development of disease control and prevention measures would be essential.

**To Cite This Article:** Hussein SN, Ibrahim AA, Shukur MS, 2023. Molecular identification of *Sarcocystis* species in sheep (*Ovis aries*) and goats (*Capra hircus*) of duhok province, Iraq. Pak Vet J, 43(2): 248-254. <http://dx.doi.org/10.29261/pakvetj/2023.016>

#### INTRODUCTION

*Sarcocystis* species (Apicomplexa; Sarcocystidae) are intracellular cyst-forming coccidian and zoonotic parasites that infect a wide variety of livestock and humans, with about 200 available species. Most species of the genus *Sarcocystis* have an obligatory two host life cycle; herbivorous, omnivorous or carnivores, in which muscular sarcocystosis develops as intermediate host and omnivorous or carnivorous in which, intestinal sarcocystosis occurs as definitive host (Dubey *et al.*, 2016).

There are four common *Sarcocystis* spp. infecting domestic sheep; *Sarcocystis tenella* and *S. arietanis* are known as pathogenic and microscopic cyst-forming which transmitted by canids as well as, *S. gigantea* and *S. medusifformis* are known as non-pathogenic and macroscopic cyst-forming that transmitted by felids. Furthermore, goats usually become infected with three different species of *Sarcocystis*, including *S. capracanis* and *S. hercicanis* regarded as pathogenic and microscopic cyst-forming for which canines act as definitive hosts, and *S. moulei* considered as non-pathogenic and macroscopic cyst-forming for which felines act as definitive hosts

(Dubey, 2015; Fayer *et al.*, 2015). Generally, *Sarcocystis* spp. are more specific to their intermediate hosts than to their final hosts. In addition, *Sarcocystis* spp. that are transmitted by canines are found to be more prevalent and more pathogenic than those species that are transmitted by felines (Lindsay and Dubey, 2020).

The prevalence of cyst forming *Sarcocystis* spp. in domestic and wild sheep and goats in different countries across the world were ranged from 45% to 100% (Dubey *et al.*, 2016; Anvari *et al.*, 2020). In Iraq, sarcocystosis is an endemic disease with infection rate reached 90-100% (Latif *et al.*, 1999; Zangana and Hussein, 2017). In Iran, the prevalence ranged from 36.83% to 100% (Mirzaei and Rezaei, 2016; Hooshyar *et al.*, 2017; Pestehchian *et al.*, 2021). In Egypt, it was 40-100% (Elmishmishy *et al.*, 2018). And in Turkey, the prevalence was 55-100% (Beyazit *et al.*, 2007). Depending on the morphological characteristics using light microscopy for detection of *Sarcocystis* spp. in Iraq, Barham *et al.* (2005), reported the infection of goats with *S. capracanis* and *S. moulei* in Baghdad, and Abdullah (2021), reported the infection of sheep with *S. tenella* and *S. arieticanis* in Sulaymaniah.

The morphological characters of sarcocysts based on observation of wall types of the cysts such as thick or thin and palisade-like villar protrusions or hair-like villar protrusions by light and transmission electron microscopy (TEM) are usually used for identification of *Sarcocystis* spp. by many authors (Dubey *et al.*, 2016). Although, morphological methods are beneficial for detection of parasitic infection, they are not precisely reliable to distinguish the specific or closely related *Sarcocystis* species. Therefore, several molecular diagnostic techniques such as Polymerase Chain Reaction (PCR) and its variants have been developed for specific identification of *Sarcocystis* spp. using different molecular markers, such as 18S rRNA and 28S rRNA gene regions as well as internal transcribed spacer 1 (ITS1) and mitochondrial cytochrome c oxidase subunit 1 (cox1) sequences (El-Morsey *et al.*, 2019; Gjerde *et al.*, 2020). Although the mitochondrial cox1 sequence is considered as a more precise molecular marker for Sarcocystidae, the 18S rRNA gene region has extensively been used to distinguish apicomplexan as well as the genus *Sarcocystis* from other eukaryotic species due to its hyper-variable regions interspersing within highly-conserved DNA sequences (Fischer and Odening, 1999; Stojceki *et al.*, 2012). Furthermore, the databases comprise more 18S rRNA sequences of the genus *Sarcocystis* which are ready to be used for species identification than other molecular gene sequences (El-Morsey *et al.*, 2019; Imre *et al.*, 2019). These characters make the 18S rRNA gene a valuable diagnostic marker.

Meanwhile, no molecular study was conducted for identification of *Sarcocystis* spp. in Iraq. Hence, the present study was conducted to identify *Sarcocystis* spp. in domestic sheep and goats using PCR, sequencing and phylogenetic analysis based on the 18S rRNA gene.

## MATERIALS AND METHODS

**Sample collection:** Two hundred fifty tissue samples were randomly collected from 147 slaughtered sheep (46 esophagi, 37 hearts, and 64 skeletal muscles) and 103

slaughtered goats (33 esophagi, 29 hearts, and 41 skeletal muscles) aged from 1 to 2 years in the slaughterhouses (Sumail, Zakho, and Amedia) of Duhok province, Kurdistan region of Iraq, from May 2021 to June 2022. Each heart, skeletal muscle (25-30 g) and whole esophagus (70-100 g) sample was individually placed in a self-sealing plastic bag, transported in an ice box to Parasitology Research Center at College of Veterinary Medicine, University of Duhok and then stored at 4°C for further investigations.

**Macroscopic examination:** The macroscopic examination was performed immediately after collection of the tissue samples. Several cross-cuts were made using a clean scalpel blade in the heart and skeletal muscle for observation of macroscopic sarcocysts. The whole tissue of the esophagus was longitudinally segmented in order to expose its lumen; additionally, its external and internal walls were macroscopically examined for the existence of macrosarcocysts (Bittencourt *et al.*, 2016).

**Microscopic analysis of fresh tissues:** The esophagus, heart and skeletal muscle tissues were microscopically examined for detection of microscopic sarcocysts using squash and grinding methods. In squash preparation, the tissue samples (2-3 mm) were compressed between two glass slides and observed under a light microscope at 10× magnification power (Latif *et al.*, 2015). In tissue grinding method, approximately 15-20 g of each tested sample was minced thoroughly in a blender, mixed with 30 ml of phosphate buffered saline (pH; 7.2), and then stirred on a magnetic stirrer with a magnetic bead for 10 minutes until a complete homogenization. Moreover, the mixture was filtered by a strainer mesh, collected in a clean test tube and then centrifuged at 600 ×g for at least 10 minutes. Moreover, the supernatant was removed, and the sediment was re-suspended with 15 ml of PBS buffer. Then, the mixture was transferred into a clean petri-dish for visualization and measurement of sarcocysts under an optical microscope at 10× and 40× magnifications (Imre *et al.*, 2019). Eventually, several complete and/or fragments of the cysts (5-10 cysts/ sample) of each positive sample were put in 1.5 ml DNase and RNase free microtubes containing 80-100 µl of free-ionized water, and then stored at - 20°C for molecular analysis.

**DNA extraction and PCR amplification:** The genomic DNA of the collected sarcocysts was extracted through a commercial DNA extraction Kit (Jena-Bioscience, Germany) according to the manufacturer's instructions with a modification in the final addition of buffer by adding 10 µl to the extracted DNA instead of 100 µl (Bittencourt *et al.*, 2016). PCR analysis was carried out for 100 positive samples (34 esophagi, 36 skeletal muscles and 30 hearts) of collected microcysts from 50 sheep and 50 goats. A 900 base pair (bp) fragment of the 18S rRNA gene of *Sarcocystis* spp. was amplified by PCR using the primer pair; 2L (5'- GGATAAACCGTGGTAATTCT ATG -3') as forward and 3H (5'- GGCAAATGCTTTC GCAGTAG -3') as reverse (Imre *et al.*, 2019). PCR reaction was performed in 20µl of the reaction mixture containing 10 µl of 2× Master Mix (Taqpolymerase, PCR buffer, MgCl<sub>2</sub>, and dNTPs), 1µl (10pmol) of each primer,

2 µl (200ng) of DNA sample, and 6 µl of free-ionized water. The reaction was performed in a thermocycler (ABI, Gene-Amp, 9700 PCR, 96-well thermal cycler). The cycling conditions applied consisted of initial denaturation for 2 minutes (min) at 95°C, followed by 35 cycles of denaturation for 45 seconds (s) at 94°C, annealing for 30 s at 55 °C, extension for 1 min at 72°C, and final extension for 7 min at 72 °C. Finally, 1 µl of 11× SYBER DNA stain (Jena Bioscience, Germany) was added into 9 µl of each amplified PCR product to be analyzed on 1% agarose gel and visualized under UV transilluminator. A good quality of genomic DNA extracted from sarcocysts (accession no. ON564602) of sheep from a previous study was used as a control positive, and ultra-pure water was used as a negative control in this study.

**Sequencing and phylogenetic analysis:** A total of six positive amplicons (2 esophagi, 2 skeletal muscles and 2 hearts) of the infected sheep and goats were purified using a column-based purification kit (Thermo-scientific; USA), and then sequenced by Sanger sequencing method through an automated sequencer (Perkin-Elmer ABI; USA, Macrogen LTD Korea). Sequencing of the nucleotides was performed by using 10 µl of the same reverse primer which was used for the PCR reaction. The obtained nucleotide sequences were searched and matched with other published *Sarcocystis* DNA sequences in the GenBank database by the BLAST search tool on the NCBI website to obtain *Sarcocystis* spp. genotypes from the samples. Furthermore, the partial sequences of the 18S rRNA regions of *Sarcocystis* isolates were submitted to the NCBI submission portal under accession numbers; PO363930, PO363951, OP363953, OP363955, OP363963, and OP376570. Moreover, the sequences of *Sarcocystis* isolates and other related GenBank-retrieved *Sarcocystis* sequences across the world were deposited and analyzed using MEGAX software to determine their phylogenetic relationships based on the 18S rRNA gene sequences. Multiple sequence alignment was performed by applying muscle alignment method. Furthermore, the sequences of the particular genes were cut at both ends to provide the homologous nucleotide positions. The phylogenetic tree was constructed through applying the neighbor joining (N-J) method based on evolutionary distances computed by the Kimura two-parameter model with the 1000 replicates bootstrap value. *Eimeria tenella* was used as an out-group species.

## RESULTS

In the present study, no macroscopic sarcocysts were observed in the hearts and skeletal muscles of the 101 slaughtered sheep and 70 slaughtered goats. Furthermore, macroscopic sarcocysts were also not detected on the outer and inner layers of the 79 examined esophagi of the hosts. Out of 250 examined samples, 247 (98.8%) samples were found to be infected with microscopic sarcocysts in sheep and goats similarly through both of the fresh tissue microscopic-examination techniques. Moreover, microscopic sarcocysts were found in 146 (99.32%) out of 147 examined sheep and 101 (98.06%) out of 103

examined goats. The microscopic cysts were predominantly obtained from 67 (100%) samples out of 67 hearts, followed by 77 (98.72%) esophagus tissues (n=78), and 103 (98.10%) samples out of 105 skeletal muscles. In general, the cysts isolated from esophagus, skeletal muscle and heart of sheep and goats were found to be elongated ovals, measuring; 198-595 µm × 50-150 µm (363±107 µm × 86±38 µm, n=30) and slender, measuring; 625-1,188 µm × 30-70 µm (750±235 µm × 50±14.5 µm, n =30) in shape with thick palisade-like protrusions or finger-like protrusions wall, measuring; 2.5-7.4 µm (4.3±1.2 µm, n=30) enclosing numerous banana shaped bradyzoites, measuring; 7.5-15 µm × 2.5-3.5 µm (12.80±1.9 µm × 3.55±0.6 µm n=30) among and/or in striated, non-striated and cardiac muscle fibers of sheep and goats (Fig. 1a, Fig. 1b).

PCR analysis of microscopic sarcocysts from the infected sheep and goats showed a specific band (900 bp) on gel electrophoresis, which was indicating the presence of *Sarcocystis* spp. No band was found in the lane of negative control. The sequencing analysis and alignment results showed that the genotypes of the isolates from sheep and goats in this study were 99% to 100% identical to *S. tenella* and *S. capracanis* which were reported in different countries across the worldwide (Table 1). In the present study, the sequences of *S. tenella* and *S. capracanis* in both sheep and goats shared 99.30–99.95% nucleotide homology with themselves under 84–99% of query covers based on the 18S rRNA gene level.

The phylogenetic relationship's analysis of the isolates in this study showed that, *S. tenella* haplotypes of sheep and goats in the phylogenetic tree placed in the same cluster with haplotypes originated from other countries such as Norway, Egypt, Poland, Iran, Spain, India, China, and Argentina, and Hungary. Similarly, the *S. capracanis* isolate of goats in this study was closely placed with isolates of the same host from China and Iran (Fig. 2). The consequences of the phylogenetic analysis showed sequences of *S. tenella* as a monophyletic cluster to sequences of *S. capracanis* in both of the hosts.

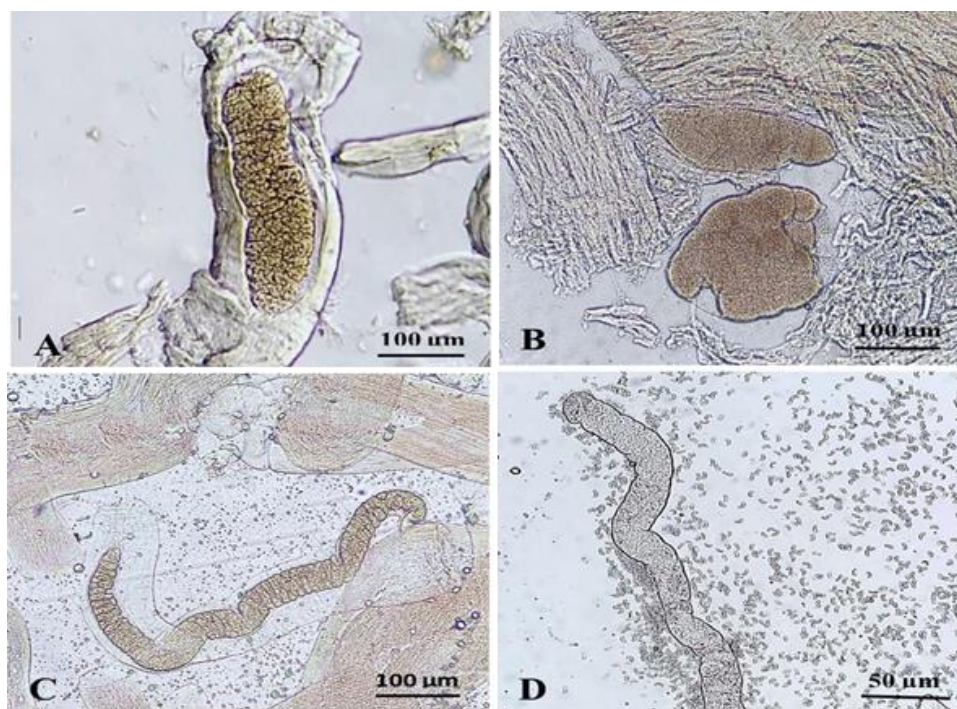
## DISCUSSION

The macroscopic sarcocysts which are characteristic of *S. medusiformis* and *S. gigantea* in sheep and *S. moulei* in goats were not observed in any of the examined animals. In Iraq, domestic sheep and domestic goats intended for production of meat are commonly reared on grass and in close contact with stray dogs more than with stray cats; this could be one of the main causes for the absence of macrosarcocysts in the examined animals. *Sarcocystis tenella* in sheep and *S. capracanis* in goats are the most predominant pathogenic species inducing microscopic cysts. Macroscopic sarcocysts are visible by the naked eye and can easily be removed by veterinarians in the slaughterhouses, while the microscopic sarcocysts are neglected because they are invisible in nature; hence it remains in the meat, repeat its life cycle and lead to enormous economic losses (Lindsay and Dubey, 2020). Therefore, promoting prevention and control strategies for parasitic infection are essential. Tissue cysts of *S. tenella* and *S. capracanis* are typically found in the esophagus, diaphragm, heart, skeletal muscle, liver, and brain of



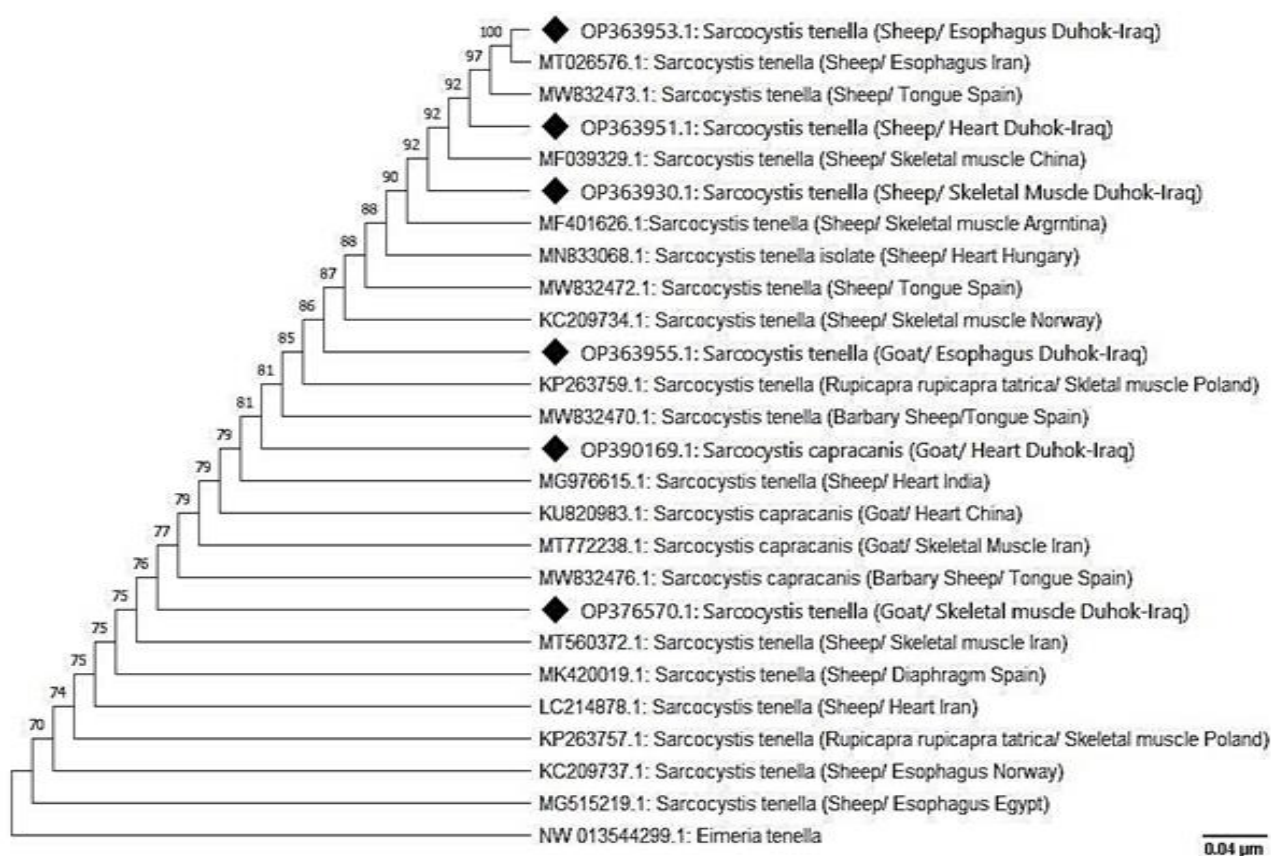
**Table 1:** *Sarcocystis* spp. isolates of sheep and goats targeting the 18S rRNA regions compared with the most related *Sarcocystis* DNA sequences present in the GenBank database.

| Animals | Organs          | <i>Sarcocystis</i> spp. | GeneBank Numbers | Highest identities with other isolates | Differences from the closest isolates                         |
|---------|-----------------|-------------------------|------------------|--|---|
| Sheep   | Esophagus       | <i>S. tenella</i>       | OP363953         | 100%                                   | no difference   |
| Sheep   | Skeletal muscle | <i>S. tenella</i>       | OP363930         | 100%                                   | no difference   |
| Sheep   | Heart           | <i>S. tenella</i>       | OP363951         | 99.70%                                 | (A) insertion at 641 position                                 |
| Goat    | Esophagus       | <i>S. tenella</i>       | OP363955         | 100%                                   | no difference   |
| Goat    | Skeletal muscle | <i>S. tenella</i>       | OP376570         | 100%                                   | no difference   |
| Goat    | Heart           | <i>S. capracanis</i>    | OP390169         | 99.17%                                 | substitutions at 145 (G-A), 161 (G-T) and 170 (C-T) positions |

**Fig. 1a:** Microscopic examination of tissue cysts from sheep and goats. A and B) Elongated oval microcysts in and out of the muscle fibers of skeletal and esophagus after tissue grinding and squash preparation (scale bar = 100  $\mu$ m). C) A slender shaped microcyst detected by tissue compression of skeletal muscle (scale bar = 100  $\mu$ m). D) Numerous bradyzoites surrounding the cyst of heart muscle after rapture by tissue grinding method (scale bar = 50  $\mu$ m).**Fig. 1b:** Light microscopic appearance of *Sarcocystis* spp. in wet mounts. A) Fragment of a slender shaped sarcocysts of *S. tenella* in skeletal muscle of sheep with several internal septa forming compartments enclosing bradyzoites (scale bar = 20  $\mu$ m). B) A portion of *S. tenella* wall with abundant villus or finger-like protrusions (arrow), (scale bar = 10  $\mu$ m). C) A mature spindle shaped sarcocysts of *S. capracanis* released from heart muscle of goat containing large number of bradyzoites with irregularly shaped wall (scale bar = 50  $\mu$ m). D) A head of *S. capracanis* wall with densely packed palisade-like protrusions (arrow), (scale bar = 10  $\mu$ m).

sheep and goats (Hong *et al.*, 2016). The cysts morphology was ranged from elongated oval to slender in shape with recorded dimensions of 140-1690  $\mu$ m in length and 30-130  $\mu$ m in width (Barham *et al.*, 2005; Hu *et al.*, 2016; Marandykina-Prakienė *et al.*, 2022). In addition, bradyzoites of the cysts were found to be banana in shape

with measurement size 8-13.5  $\mu$ m x 1.5-3.5  $\mu$ m (Kutty *et al.*, 2015; Latif *et al.*, 2015; Hong *et al.*, 2016). The severity of clinical symptoms of sarcocystosis in sheep and goats depends on the ingested dose of sporocysts, which were shed by carnivores, and the immune status of the hosts (Fayer *et al.*, 2015). In acute sarcocystosis, the



**Fig. 2:** Phylogenetic tree showing relationship of 18S rRNA gene sequences of *Sarcocystis* spp. identified in the present study with those available in GenBank. The percentage value of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Bootstrap values below 50% are not shown. Names in parenthesis next to the *Sarcocystis* spp. names and their accession numbers are referred to animal species, organs and countries of the isolates. Black symbols referred to isolates of *Sarcocystis* spp. identified in the present study.

clinical symptoms usually include anorexia, fever, anemia, muscle weakness, abortion, encephalitis, encephalomyelitis and even death. Chronic sarcocystosis can lead to economic losses due to emaciation and a decrease in animal productions such as meat, milk and wool (Fayer *et al.*, 2015; Lindsay and Dubey, 2020). Clinical symptoms such as inflammation, myocarditis and hepatitis were also reported in experimentally inoculated sheep with sporocysts of *S. tenella* from canine feces (Dubey *et al.*, 2016). The incidence of microsarcocysts in sheep and goats in this study was compatible with results reported in Turkey, Iran, Saudi Arabia, and Egypt ranged from 80-100% (Beyazit *et al.*, 2007; Dehaghi *et al.*, 2012; Al-Quraishy *et al.*, 2014; Elmishmishy *et al.*, 2018). The high infection rate of the parasite could be related to close contact of hosts with carnivores or might be due to persistent keeping of dogs with sheep and goats' herds for safety issues, in addition to rapid maturation (within 1 to 2 months) of the cysts in muscle tissues of the intermediate hosts after ingestion of sporocysts (Dubey *et al.*, 2016).

In this study, *S. tenella* and *S. capracanis* were identified in sheep and goats through molecular analysis of 18S rRNA gene sequences. *Sarcocystis tenella* was isolated from the esophagus, skeletal muscle and heart of sheep, in addition to the esophagus and skeletal muscle of goats, and *S. capracanis* was isolated from heart of goats. The cysts of *S. tenella* were globally regarded as a specific species for sheep and they have been isolated from the cardiac, smooth, and striated muscles of the hosts by

several authors from various countries (Hu *et al.*, 2017). While, in a similar study in Korea, *S. tenella* was isolated from muscle tissues of goats rather than of sheep (Hong *et al.*, 2016). In a case report from Iran, *S. tenella* was also isolated from brain of sheep (Selahi *et al.*, 2014). Furthermore, our finding was compatible to studies that reported *S. capracanis* in muscle tissues of goats and didn't find it in sheep (Kutty *et al.*, 2015; Metwally *et al.*, 2019). In contrast, *S. capracanis* was regarded as an alternate parasite for other herbivorous and was reported in the cerebrospinal fluid of two sheep with nervous clinical signs in United Kingdom (Formisano *et al.*, 2013). Moreover, *S. capracanis* was identified in wild sheep species in Spain and Austria (Delgado-de Las Cuevas *et al.*, 2021; Prakas *et al.*, 2021), and *S. tenella* was identified in goat-antelope species (*Rupicapra rupicapra*) in Poland (Kolenda *et al.*, 2015) based on the 18S rRNA and cox1 gene sequences. Nevertheless, the situation of the morphologically similar species pair *S. tenella*/*S. capracanis* in regard to their life cycle within the Caprinae intermediate host group appears to be not finally elucidated and further studies are needed to clarify the intermediate host status of the Caprinae species (Prakas *et al.*, 2021). Meanwhile, sheep and goats are regarded as alternate hosts for transmission of *Sarcocystis* species; *S. tenella* of sheep and *S. capracanis* of goats are genetically considered as sister sequences due to their close phylogenetic correlation at the 18S rRNA gene sequence level (El-Morsey *et al.*, 2019). *Sarcocystis capracanis* was



most closely related with *S. tenella* based on 18S rRNA, 28S rRNA, and mitochondrial *cox1* sequences, as it shares identities of 95.7-99.1, 95.3 and 92.3-93.2% with other *S. tenella*, respectively (Hu *et al.* 2016). However, using *cox1* as the preferable genetic marker for discrimination of sibling sequences of *Sarcocystis* spp. in sheep and goats could be significant (Gjerde *et al.*, 2020).

**Conclusions:** This is the first molecular study to identify and confirm *S. tenella* and *S. capracanis* in domestic sheep and domestic goats in Iraq. High incidences of infection by *Sarcocystis* species-forming microcysts in muscle tissues of slaughtered sheep and goats intended for human consumption were detected in the slaughterhouses of Duhok province, north of Iraq. The identified species of *Sarcocystis* (*S. tenella* and *S. capracanis*) are the most common pathogenic ones in sheep and goats which cause significant economic losses. Therefore, it's essential to prevent the contaminated food and water with sporocysts to livestock. In addition, keeping dogs out of livestock housing or away from the slaughterhouses is also essential for controlling the spread of infection. On the other hand, whole sequence analysis of the 18S rRNA gene or of other genetic loci is necessary to identify genetic diversity between the identified *Sarcocystis* species. Even though, the 18S rRNA gene region has extensively been used to discriminate apicomplexan as well as the genus *Sarcocystis* from other eukaryotic species due to its hyper-variable regions interspersing within highly conserved DNA sequences, applying mitochondrial *cox1* as the preferable genetic marker for discrimination of sibling sequences of *Sarcocystis* spp. in sheep and goats could be significant. Furthermore, a comparative study using genetic markers, such as 28S rRNA, *cox1* and ITS1 are also required for more precise molecular differentiation of *S. tenella* and *S. capracanis* in both sheep and goats.

**Acknowledgments:** The authors would like to express their gratitude to all of the staff at the Duhok Research Center, Veterinary Medicine College, University of Duhok, Iraq, for their technical support.

**Authors contribution:** Assistant Professors; Dr. Assel Anwar Ibrahim and Dr. Mohammed Shukri Shukur designed and supervised the current research study. Corresponding author (PhD Student; Shivan Nawzad Hussein) collected the samples and investigated them at the laboratory of Duhok Research Center, College of Veterinary Medicine, University of Duhok. The supervisors have also helped in the analysis of the data and proof writing of the manuscript.

**Conflicts of Interest:** No conflict of interest exists.

## REFERENCES

- Abdullah SH, 2021. Investigation of *Sarcocystis* spp. in slaughtered cattle and sheep by peptic digestion and histological examination in Sulaimani Province, Iraq. *Vet Worl* 14:468-74.
- Al-Quraishy S, Morsy K, Bashtar AR, *et al.*, 2014. *Sarcocystis arieticanis* (Apicomplexa: Sarcocystidae) infecting the heart muscles of the domestic sheep, *Ovis aries* (Artiodactyla: Bovidae), from K. S. A. on the basis of light and electron microscopic data. *Parasitol Res* 113:3823-31.
- Anvari D, Narouei E, Hosseini M, *et al.*, 2020. Sarcocystosis in Ruminants of Iran, as Neglected Food-Borne Disease: A Systematic Review and Meta-analysis. *Acta Parasitol* 65:555-68.
- Barham M, Stützer H, Karanis P, *et al.*, 2005. Seasonal variation in *Sarcocystis* spp. Infections in goats in northern Iraq. *Parasitology* 130:151-6.
- Beyazit A, Yazicioğlu O and Karaer Z, 2007. The prevalence of ovine *Sarcocystis* species in Izmir province. *Ankara Üniv Vet Fak Derg* 54:111-6.
- Bittencourt MV, Meneses ID, Ribeiro-Andrade M, *et al.*, 2016. *Sarcocystis* spp. in sheep and goats: frequency of infection and species identification by morphological, ultra-structural, and molecular tests in Bahia, Brazil. *Parasitol Res* 115:1683-9.
- Dehaghi MM, Fallahi M, Masoud S, *et al.*, 2012. Survey of *Sarcocystis* infection in slaughtered sheep in Kerman Abattoir, Kerman, Iran. *Comp Clin Pathol* 22:343-6.
- Delgado-de Las Cuevas GE, Prakas P, Rudaitytė-Lukošienė E, *et al.*, 2021. First description of *Sarcocystis* species infecting Barbary sheep (*Ammotragus lervia*). *Parasitol Res* 120(8):2881-6.
- Dubey JP, 2015. Foodborne and waterborne zoonotic sarcocystosis. *Food Waterborne Parasitol* 1:2-11.
- Dubey JP, Calero BR, Rosenthal BM, *et al.*, 2016. Sarcocystosis of animals and humans. 2nd ed. Boca Raton, CRC Press, USA pp:501.
- Elmishmishy B, Al-Araby M, Abbas I, *et al.*, 2018. Genetic variability within isolates of *Sarcocystis* species infecting sheep from Egypt. *Vet Parasitol Reg Stud Reports* 13:193-7.
- El-Morsey A, Abdo W, Sultan K, *et al.*, 2019. Ultrastructural and Molecular Identification of the sarcocysts of *Sarcocystis tenella* and *Sarcocystis arieticanis* Infecting Domestic Sheep (*Ovis aries*) from Egypt. *Acta Parasitologica* 64:501-13.
- Fayer R, Esposito DH and Dubey JP, 2015. Human Infections with *Sarcocystis* Species. *Clin Microbiol* 28:295-311.
- Fischer S and Odening K, 1999. Characterization of Bovine *Sarcocystis* Species by Analysis of Their 18S Ribosomal DNA Sequences. *J Parasitol* 84:50-4.
- Formisano P, Aldridge B, Alony Y, *et al.*, 2013. Identification of *Sarcocystis capracanis* in cerebrospinal fluid from sheep with neurological disease. *Vet Parasitol* 193:252-5.
- Gjerde B, Fuente C, Alunda J, *et al.*, 2020. Molecular characterization of five *Sarcocystis* species in domestic sheep (*Ovis aries*) from Spain. *Parasitol Res* 119:215-31.
- Hong EJ, Sim C, Chae JS, *et al.*, 2016. Ultrastructural and molecular identification of *Sarcocystis tenella* (Protozoa, Apicomplexa) in naturally infected Korean native goats. *Veterinárni Medicína* 61:374-81.
- Hooshyar H, Abbaszadeh Z, Sharafati-Chaleshtori R, *et al.*, 2017. Molecular identification of *Sarcocystis* species in raw hamburgers using PCR-RFLP method in Kashan, central Iran. *J Parasit Dis* 41:1001-5.
- Hu JJ, Liu TT, Liu Q, *et al.*, 2016. Prevalence, morphology, and molecular characteristics of *Sarcocystis* spp. in domestic goats (*Capra hircus*) from Kunming, China. *Parasitol Res* 115:3973-81.
- Hu JJ, Huang S, Wen T *et al.*, 2017. *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics. *Parasite* 24:30.
- Imre K, Dărăbuș G, Tîrziu E, *et al.*, 2019. *Sarcocystis* spp. in Romanian Slaughtered Cattle: Molecular Characterization and Epidemiological Significance of the Findings. *Biomed Res Int* 13:1-6.
- Kolenda R, Schierack P, Zieba F, *et al.*, 2015. First molecular characterization of *Sarcocystis tenella* in Tatra chamois (*Rupicapra rupicapra tatraica*) in Poland. *Parasitol Res* 114(10):3885-92.
- Kutty MK, Latif B, Muslim A, *et al.*, 2015. Detection of sarcocystosis in goats in Malaysia by light microscopy, histology, and PCR. *Trop. Anim Health Prod* 47:751-6.
- Latif B, Al-Delemi J, Mohammed B, *et al.*, 1999. Prevalence of *Sarcocystis* spp. in meat producing animals in Iraq. *Vet J Parasitol* 84:85-90.
- Latif B, Kannan KM, Muslim A, *et al.*, 2015. Light microscopy and molecular identification of *Sarcocystis* spp. in meat producing animals in Selangor, Malaysia. *Trop Biomed* 32:444-52.
- Lindsay DS and Dubey JP, 2020. Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants: An Update. *Am Food Anim Pract* 36:205-22.
- Marandykina-Prakienė A, Butkauskas D, Gudiškis N, *et al.*, 2022. Molecular Identification of *Sarcocystis* Species in Sheep from Lithuania. *Animals (Basel)* 12:2048.

- Metwally DM, Al-Damigh MA, Al-Turaiki IM, et al., 2019. Molecular Characterization of *Sarcocystis* Species Isolated from Sheep and Goats in Riyadh, Saudi Arabia. *Animal (Basel)* 9:256.
- Mirzaei M and Rezaei H, 2016. The role of sheep in the epidemiology of *Sarcocystis* spp. in Tabriz area northwest of Iran. *J Parasit Dis* 40:285-8.
- Pestehchian N, Yousefi H, Kalantari et al., 2021. Molecular and Microscopic Investigation of *Sarcocystis* Species Isolated from Sheep Muscles in Iran. *J Food Qual* 3:1-6.
- Prakas P, Rehbein S, Rudaitytė-Lukošienė E, et al., 2021. Molecular identification of *Sarcocystis* species in diaphragm muscle tissue of European mouflon (*Ovis gmelini musimon*) from Austria. *Parasitol Res* 120; 2695-702.
- Selahi M, Bahari P and Vatanchian M, 2014. First Molecular Identification of *Sarcocystis ovis* (Protozoa, Apicomplexa) in the Brain of Sheep in Iran. *Iranian J Parasitol* 9:286-91.
- Stojecki K, Karamon J, Sroka J, et al., 2012. Molecular diagnostics of *Sarcocystis* spp. infections. *Pol J Vet Sci* 15(3): 589-96.
- Zangana IK and Hussein SN, 2017. Prevalence of *Sarcocystis* species (*Sarcocystis ovis* and *Sarcocystis capricanis*) in tongue muscle of sheep and goats in Duhok province, Kurdistan Region, North Iraq. *Sci J Koya Univ* 5:36-40.