Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Prevalence, Morphology, and Genetic Relationship of *Sarcocystis* species in Naturally Infected Sheep

Ahmed M. Abdel-Hamied[*1,2], Bassiony Abdel-Hafez[2], Nosseur El-Sayed[2], Adel Abdelkhalek[3] and Refaat Ras[1,2]

[1]*Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Cairo, Badr City 11829, Egypt.*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.*
[3]*Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Cairo, Badr City 11829, Egypt.*

Abstract

Sarcocystosis is a parasitic disease caused by intracellular coccidian protozoan belonging to the genus *Sarcocystis*. While inducing substantial economic losses in host animals as well as neurological disorders and abortions, limited knowledge exists regarding the *Sarcocystis* species that infect sheep and their potential impact on mutton quality and human health owing to the presence of microscopic *Sarcocystis* in the flesh. The objective of the present study was to investigate the prevalence, morphological characteristics, and genetic relationship of *Sarcocystis* species identified during routine meat inspections in naturally infected sheep. A macroscopic infection rate of 0.35% was recorded, primarily involving *Sarcocystis gigantea* and *Sarcocystis medusiformis*, with a microscopic infection rate of 55.9%, and a high prevalence in diaphragmatic (72%), esophageal (57.1%), and cardiac (31.4%) muscles. Molecular analysis of Cytochrome oxidase COX1 and 18S rRNA genes highlighted high genetic similarity between *S. tenella* isolates from Egypt and other regions, including Spain, Norway, and China, with genetic identities ranging from 96.82% to 100%. Phylogenetic analysis indicated minimal host-specific variation, as Egyptian sheep and goat isolates clustered together. *S. arieticanis* also showed close genetic relationship with isolates from diverse locations. These findings demonstrate broad genetic consistency within *S. tenella* and *S. arieticanis* populations across geographic regions, suggesting limited regional variation and providing insights into *Sarcocystis* species phylogenetics in small ruminants. The findings of this study revealed a wide array of *Sarcocystis species* that infect livestock, highlighting the need for enhanced surveillance and control measures to mitigate their impact on animal health and food safety

Keywords: Sheep, *Sarcocystis* species, Prevalence, Morphology, Phylogenetic analysis.

Introduction

Sarcocystis species are intracellular cyst-forming protozoan parasites that infect humans and various animal species, thereby presenting a potential risk to public health and food safety [1, 2]. *Sarcocystis* requires two obligatory hosts to complete its life cycle: a carnivorous animal acting as the definitive host and either an omnivorous or herbivorous animal serving as the intermediate host [3, 4]. Consequently, carnivorous animals serve as the definitive hosts for intestinal *Sarcocystis* species, acquiring infections through the ingestion of fully developed sarcocysts in muscle tissue of the intermediate hosts, which may be either macroscopic or microscopic cysts. [5, 6]. Furthermore, humans served as definitive hosts for *S. hominis* and *S. suihominis*, or as incidental intermediate hosts for other species, such as *S. lindemanni* [7, 8]. Animals heavily infected with sarcocystosis typically manifest symptoms including fever, weakness, emaciation, and weight loss, diminished wool quality, hair loss, and reduced milk production. Moreover, the meat industry sustains significant economic losses due to the rejection of heavily infected animal carcasses containing visible *Sarcocystis* cysts [9, 10]. Sheep are susceptible to infection by six species of *Sarcocystis*. Four of these parasites, *S. tenella* (*S. ovicanis*), *S. arieticanis*, *S. microps*, and *S. mihoensis*; as their definitive hosts are canids. The other two, *S. gigantea* (*S. ovifelis*) and *S. medusiformis*, as felines are their final hosts. It

*Corresponding authors: Ahmed Mahmoud Abdel-Hamied, E-mail: vetahmed34@gmail.com, Tel.: 002 01010154725
(Received 30 October 2024, accepted 02 January 2025)
DOI: 10.21608/EJVS.2025.332244.2461

is noteworthy that *S. tenella* and *S. arieticanis* are classified as pathogenic and form microscopic cysts, whereas *S. gigantea* and *S. medusiformis* are non-pathogenic and produce macroscopic cysts in sheep [7, 9, 11, 12]. While most species of *Sarcocystis* exhibit host specificity or infect closely related host species, however, *S. gigantea* and *S. tenella* that commonly infect sheep, were recorded in goats [6, 13, 14], while *S. moulei* that normally detected in goats, was identified in sheep in Saudi Arabia and Iran [15, 16]. Researchers conducted multiple investigations across various Egyptian provinces to determine the prevalence of *Sarcocystis* in sheep. These studies revealed a high infection rate, with one report documenting a prevalence of 95.37% [17]. Differentiation among *Sarcocystis* species is achievable by examining their morphological characteristics, with particular emphasis on the *Sarcocystis* walls. This process can be performed using histopathological examination and electron microscopy. However, this method is inadequate for species-level identification [18]. Hence, transmission electron microscopy (TEM) serves as a valuable tool for descriptive research through cyst wall ultrastructural, its high cost and time-intensive nature preclude its incorporation into standard diagnostic procedures [19]. As a result, molecular assays have developed as the most efficacious techniques for conducting epidemiological studies and identifying specific *Sarcocystis* species in the intermediate hosts [20]. Given the scarcity of molecular research on *Sarcocystis* in Egyptian sheep, it is essential to perform molecular identification of the various *Sarcocystis* species infecting sheep in Egypt [21]. Therefore, the present study aimed to provide updated information on the prevalence of *Sarcocystis* species in Egyptian sheep (*ovis aries*) at the Central Abattoir of Cairo (the capital of Egypt). Additionally, the study involved analyzing the morphological and histopathological features of the collected samples, along with conducting molecular identification of *Sarcocystis* species using sequence analysis of the 18S rRNA gene and the mitochondrial Cytochrome c oxidase subunit I (COX1) gene.

Material and Methods

Sample collection and visual inspection

During May 2023 to April 2024, a total of 872 sheep carcasses, comprising samples from both sexes and various age groups, slaughtered at the Central Abattoir of Cairo, were examined for the presence visible macroscopic cyst-forming *Sarcocystis* spp. Thus, muscles samples of the fore and hind limbs, esophagus, tongues, diaphragms, abdominal muscles, skeletal muscles, and hearts were grossly inspected by direct observation in the abattoir according to Veterinary Services Authority of Egypt during routine meat inspection [3]. The detected macroscopic samples were morphologically described. On the other hand, 288 random fresh meat samples of the esophagus (n=210), heart (n=53) and diaphragm tissues (n=25) were obtained for microscopic examination.

Microscopic examination of fresh tissues

The microscopic examination was conducted on 288 fresh meat samples, specifically targeting the esophagus, heart, and diaphragm tissues, to detect the presence of microscopic *Sarcocystis*. Two methods were employed: Squash and digestion techniques as follows:

The muscle squash method

The technique, alternatively referred to as impression smear, involves squeezing tissue specimens of 2–3 mm in size between a pair of glass slides using a robust, slender thread. Following this procedure, the prepared slides were very thoroughly examined under a light microscope (10×) [6, 22]. Furthermore, the specimens were subjected to Giemsa staining and subsequently analyzed using microscopy [23].

The digestion method technique

Acid-pepsin digestion was employed to observe bradyzoites of *Sarcocystis* cyst. Approximately 20 g of infected meat was minced using scissors in a sterile petri dish, and the minced infected meat was transferred to 50 ml of digestion solution for 30 min at 40°C. The digested material was filtered through two layers of gauze to remove the undigested portion. The filtered solution was centrifuged at 3000 rpm for 5 min, the supernatant fluid was discarded, and the sediment sample was resuspended in 5 ml of phosphate-buffered saline (PBS, pH 7.2), fixed, stained with Giemsa stain, and examined microscopically [9, 24].

Histological examination

Positive samples were subjected to a histological examination. Following fixation of the tissue samples in 10% neutral-buffered formalin, a paraffin block was prepared. Subsequently, 4-5 µm sections were cut and stained with hematoxylin and eosin. The slides were then examined under a light microscope at 10× as well as further analysis at 40× magnification to identify *Sarcocystis* tissue cysts [25, 26].

Molecular identification of Sarcocystis species

DNA extraction

DNA was extracted from four *Sarcocystis* positive isolates from tissue using the DNeasy Blood & Tissue mini-Kit (Qiagen, Hilden, Germany) following manufacturer's recommendations [27]. Eluted DNA was stored at -20°C for further analysis.

DNA amplification by PCR

Extracted DNA were amplified by conventional PCR using two sets of primers, which the mitochondrial COX1 gene was amplified using SF1 (5′-ATGGCGTACAACAATCATAAAGAA-3′) and SR9 (5′-ATATCCATACCRCCATTGCCCAT-3′) [28]. Moreover, the second set of primers targeting 18S rRNA fragment were forward primer SarcoFext (5′-GGTGATTCATAGTAACCGAACG-3′) and reverse primer SarcoRext (5′-GATTTCTCATAAGGTGCAGGAG-3′) [29, 30]. Each PCR reaction was carried out in a 50 µL mixture containing 25 µL of GeneDireX OnePCR™ Master Mix (Cat# MB203-0050), 1 µL of DNA template, 1 µL of each 10 µM forward and reverse primers and 22 µL nuclease-free water. The cycling conditions of PCR were set up as follows: initial denaturation for 4 min at 95°C followed by 40 cycles of at 94°C for 40 seconds, annealing at 58°C for 1 min, extension for 1 min at 72°C, final extension step at 72°C for 6 min. The reactions were performed on a Benchmark Gradient thermal cycler (Benchmark Scientific, Inc., USA). The amplicons as well as a 100 bp ladder (Cat# DM003-R500) were separated on 1.5% agarose gel containing 0.4 µg/mL of ethidium bromide in 1x TAE buffer and visualized under UV transilluminator.

Sequencing and phylogenetic analysis

For sequencing, PCR products of *Sarcocystis* were purified with Thermo Scientific GeneJETPCR Purification Kit according to the manufacturer's instructions. The purified products were sequenced using the primers for PCR amplification performed by the Macrogen company, South Korea (https://dna.macrogen.com/) on an automated Sanger sequencer. The sequences in the current study, the generated sequences of mitochondrial COX1 gene were deposited in Genbank with the accession numbers PQ187423-PQ187426. On the other hand, the obtained sequences of 18S rRNA gene were deposited in Genbank with the accession numbers PQ182256-PQ182258 and PQ182594. The resultant mitochondrial COX1 and 18S rRNA gene sequences were identified using BLASTn (http://blast.ncbi.nlm.nih.gov/). To compare the taxonomic relationship between isolates and related species of ruminant *Sarcocystis* (sequence data were obtained from NCBI, http://ncbi.nlm.nih.gov/), a neighbor-joining phylogenetic tree with the evolutionary distances were computed by the maximum composite likelihood model using Molecular Evolutionary Genetics Analysis software (MEGA version 11) [31]. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies. *Hammondia heydorni* (JX473250) and Toxoplasma gondii (EF472967.1) as outgroup [9].

Statistical analysis

The prevalence of *Sarcocystis* infections was determined in sheep from the positive ratio to the total number of sheep examined. The variations in *Sarcocystis* prevalence between tissue examined, season, age and sex, were analysed using SPSS chi-square (χ^2) test (ver. 21). $P \leq 0.05$ was considered statistically significant.

Result

Prevalence of Sarcocystis spp. in sheep

The current study demonstrated that the prevalence of macroscopic *Sarcocystis* spp. in naturally infected sheep during routine meat inspection was 0.35%(3/872). In contrast, the prevalence of microscopic *Sarcocystis* spp. infection was 55.9%(161/288). Moreover, the highest prevalence rates of microscopic sarcocysts were observed in diaphragmatic muscles (72%), followed by oesophagus (57.1%) and heart (31.4%) (Table 1).

The results revealed a statistically significant variation (P values ≤ 0.05) in infection rates across different age groups. Animals older than two years exhibited a higher infection rate (83.3%) compared to their younger ones (53.4%) (Table 2). The findings of this investigation revealed statistically significant correlation between gender and infection rates; although females had higher percentage (70.59%) than males (53.94%) (Table 3). Furthermore, no statistically significant seasonal variation was observed in the prevalence of *Sarcocystis* spp. among examined sheep (Table 4).

Morphological descriptions of isolated macrosarcocysts and microsarcocysts

During macroscopic examination, two species of *Sarcocystis* were identified: *S. gigantea* and *S. medusiformis* which detected in oesophagus and diaphragm respectively. *S. gigantea* was characterized by its substantial dimensions, measuring 5-8.3 mm in length and 3-5.2 mm in width. It appeared as an oval to spherical shape and had a coloration ranging from creamy to yellowish or orange, resembling the appearance of cooked chickpeas (Fig. 1A). In contrast, *S. medusiformis* was small sized, measuring 1-2.5 mm in length and 0.2 - 0.4 mm in width, these sarcocysts appeared a slender, thin fusiform spindle-like shape and displayed an opaque white coloration. Their appearance beneath the connective tissue was reminiscent of rice grains (Fig. 1B). This distinct size and colour of macroscopic sarcocysts make it easily identifiable during a gross examination. Additionally, two microscopic species of *Sarcocystis* were detected, *S. tenella* and *S. arieticanis*. *S. tenella* exhibited a spindle-like shape (fusiform and cigar-shaped), with tissue cysts embedded parallel to muscle fibers (Fig. 2B). While, *S. arieticanis* displayed a ribbon-like structure (Fig. 2A).

Histomorphology examination

The histological microscopic examination of the infected esophagi and heart revealed multiple microsarcocysts of varying dimensions interspersed among muscle fibers in both examined tissues. These microsarcocysts contained banana-shaped bradyzoites, separated by internal septae, which appeared white when stained with hematoxylin and eosin. *S. arieticanis* was characterized by thin-walled sarcocysts (Fig. 3), whereas microsarcocysts with clearly visible likely a thick walls were identified as *S. tenella* (Fig. 4). For histopathological findings, there was no any tissue reactions.

Molecular and Phylogenetic analyses

The amplified PCR products of a partial *18S rRNA* and COX1 gene were successfully sequenced. The obtained four COX1 gene sequences and four *18S rRNA* gene sequences were aligned and compared with the data of *Sarcocystis* spp. previously deposited in the GenBank. Regarding to COX1 sequences, the current study sequences showed consistently high genetic identities with those of *S. tenella* across different countries, ranging from 96.82% to 99.7%. The highest genetic identity (99.7%) was observed in sheep from Spain, while the lowest (96.82%) was from sheep in China (Table 5). Furthermore, the *S. tenella* isolate in goats from Egypt shows a genetic identity of 99.5%, nearly identical to isolates from sheep in other countries.

The phylogenetic analysis elucidates the genetic relationship among *S. tenella* isolates obtained from sheep and goats across diverse geographical regions, including Egypt, Spain, China, Norway, and India. *Hammondia heydorni* was utilized as the outgroup to root the tree and provide a comparative context for *S. tenella*. No significant variation was observed among the four COX1 sequence gene isolates in this study. *S. tenella* isolates from sheep in this investigation (accession no., PQ187424.1, PQ187425.1, PQ187426.1, and PQ187423.1) formed a distinct clade with 100% bootstrap support, indicating a high degree of genetic similarity among these samples (Fig. 5). In addition, the newly sequenced *S. tenella* isolates clustered with *S. tenella* (KC209725.1) from sheep in Norway and *S. tenella* (MK419984.1, MW848316.1) from sheep in Spain as well as other halotypes from China and India (Fig. 5). Although the majority of isolates were derived from sheep in the present study, the phylogenetic tree includes a goat isolate from Egypt (PP668132.1). Notably, this goat isolate forms a close cluster with sheep isolates from the same geographical area. This observation suggests that the *S. tenella* population in Egypt, which infects both sheep and goats, exhibits genetic similarity. Such findings indicate minimal host-specific genetic variation within this particular region.

On the other hand, analysis of 18S rRNA gene sequences using blastn alignment revealed that three isolates were identified as *S. tenella*, while one isolate was determined to be *S. arieticanis*. For three identified *S. tenella* in the present study, multiple *S. tenella* isolates from various countries, including Norway (KC209734.1), Spain (MW832470.1), Egypt (PP657635.1), and Argentina (MF401626.1), exhibited 100% identity, indicating that they are either genetically identical or extremely closely related to one another (Table 6). Also, isolates from Spain (MK420019.1) and China (MF039329.1) have a 99.88% identity. This very small difference indicates these populations were nearly identical with only slight genetic variation. This is supported by phylogenetic tree which showed the evolutionary relationship among the representative three *Sarcocystis* spp. isolates and other *Sarcocystis* spp. The current study revealed that the majority of the isolates belong to *S. tenella*, which are clustered together with high bootstrap support (100% confidence) as well as isolates from Spain, Egypt, Norway, Argentina, and China are tightly clustered, showing that the *S. tenella* species from these regions were highly similar.

They form clusters with other *S. tenella* strains, suggesting close evolutionary relationship within the Egyptian isolates and between these isolates and those from other regions. There is a clear separation between *S. tenella* isolates and *S. capracanis* isolates, with *S. capracanis* (from Germany and China) forming a distinct cluster below *S. tenella*. This supports the idea that although both species infect different hosts, they are genetically distinct.

The evolutionary relationship among *Sarcocystis* species are demonstrated by the phylogenetic tree, which showed the relationship between the three representative *Sarcocystis* spp. isolates and other species within the genus. The present results suggest that *S. tenella* constitutes the majority of the isolates, forming a cluster with robust bootstrap support (100% confidence). Furthermore, *S. tenella* isolates from various countries, including Spain, Egypt, Norway, Argentina, and China, are closely clustered, indicating a high degree of similarity among these geographically diverse samples (Fig. 6). Additionally, the analyzed *S. tenella* shared the same clade suggesting close evolutionary relationship within the Egyptian samples and between these samples from other locations. A clear distinction was observed between *S. tenella* isolates and those of *S. capracanis*, with the latter (originating from Germany and China) forming a separate cluster below *S. tenella*.

The sequence analysis and alignment of partial 18s rRNA for the single *S. arieticanis* isolate obtained demonstrated a notably high genetic similarity (99.76%) with isolates from China

(MF039330.1) and Spain (MK420017.1). This finding indicates a close genetic relationship among these isolates despite their geographically distant origins (Table 7). Conversely, isolates from Egypt (MH413035.1 and MH413036.1) exhibited marginally lower similarity to the other isolates, with identity percentages ranging from 99.52% to 99.28%. This slight reduction in similarity may suggest subtle genetic variations in the Egyptian isolates. The phylogenetic analysis (Fig. 7) revealed that *S. arieticanis* isolates from Egypt (MH413035.1, MH413036.1, and PQ182594.1) form a closely related group with a bootstrap value of 99%, demonstrating their strong genetic similarity. These Egyptian samples are also closely related to *S. arieticanis* isolates from China (MF039330.1) and Spain (MK420017.1), as evidenced by equally high bootstrap values (99%). This suggests a significant level of genetic consistency among *S. arieticanis* strains across diverse geographical regions, including Egypt, China, and Spain. In contrast, *S. hircicanis* isolates from China (KU820984.1) and Egypt (OP430816.1) constitute a separate clade, distinct from the *S. arieticanis* group.

Discussion

Sarcocystosis, a parasitic disease in humans and animals, is caused by various *Sarcocystis* species. It is especially prevalent in small ruminants, causing detrimental effects and leading to substantial financial losses for local farmers and economies [32]. This study's results provide valuable information about the occurrence and distribution of *Sarcocystis* spp. infections in sheep, highlighting a notable difference between the parasite's macroscopic and microscopic forms. During routine meat examinations, macroscopic *Sarcocystis* spp. were observed at a comparatively low rate of 0.35%. In contrast, microscopic forms were found to be much more widespread, with a prevalence of 55.9%, demonstrating the extensive presence of microscopic sarcocysts in sheep tissues. Recent research has shown that the incidence of microscopic *Sarcocystis* spp. in sheep exceeds 80% in several countries. Studies report rates of 80.5% in Egypt [7], 96.1% in Brazil [33], 100% in Iran [25], and 98.3% in Iraq [6]. In contrast, the highest prevalence of macroscopic *Sarcocystis* spp. in sheep was observed in Egypt, reaching 13.2% [9]. The elevated rates are attributed to frequent contact between sheep and carnivores, resulting in contamination of sheep feed or water by faeces, as well as the practice of feeding raw or undercooked meat to dogs or cats [34, 35]. Definitive hosts play a crucial role in the spread of sarcocystosis as well as slaughtered or dead livestock carcasses should not be fed to or left accessible to dogs and cats [38]. In addition, variations between the prevalence rates may be explained by differences in diagnostic methods, geographical locations, and management practices [7].

The study further identified that the prevalence of microscopic *Sarcocystis* spp. infections varied significantly among different tissues, with the diaphragmatic muscles showing the highest infection rate (72%), followed by the oesophagus (57.1%) and the heart (31.4%). This distribution pattern is aligned with other research, which has frequently reported that *Sarcocystis* spp. tend to exhibit tissue tropism, with a particular affinity for striated muscles such as those found in the diaphragm [37]. The significant difference in infection rates among these tissues ($P \leq 0.05$) underscores the importance of tissue type as a factor in the pathogenesis and epidemiology of *Sarcocystis* spp. infections. In contrast, others reported that, the most affected organs were the oesophagus [7]. Meanwhile, other study reported that distribution of *Sarocystis* cysts among affected organs didn't follow a particular pattern [40].

The study findings indicate that sheep over two years of age demonstrated a considerably higher infection rate (83.3%) than their younger counterparts (53.4%). This observation is consistent with earlier studies suggesting that age is a key factor in the vulnerability and buildup of *Sarcocystis* infections in farm animals. Old sheep are more likely to have been exposed to the parasite's infectious stages, such as oocysts or sporocysts excreted by definitive hosts (typically dogs or cats), for a longer period, increasing the chances of persistent infection [17, 39]. Additionally, older sheep may have had multiple encounters with contaminated food or water sources, facilitating the growth and accumulation of sarcocysts over time [28, 44]. Therefore, animals slaughtered at an early age may not present the disease [35, 42]. On the other hand, the immune system of older animals might also be less capable of eliminating *Sarcocystis* spp. infections, particularly given the parasite's capacity to avoid host immune defenses during long-term infection phases. Once the cysts become embedded in muscle tissues, they can remain for extended periods without being effectively eliminated, which could explain the higher prevalence observed in older animals [10].

This study revealed a notable difference in infection rates between male and female sheep, with females exhibiting a higher prevalence (70.59%) than males (53.94%). This gender-based difference in *Sarcocystis* spp. infections has been noted in previous studies, although the underlying causes remain unclear. One potential explanation is that female sheep, especially those used for breeding purposes, are typically retained in production systems for longer periods than males, potentially increasing their exposure to the parasite and the likelihood of infection [12]. Furthermore, physiological and hormonal differences between male and female animals may influence their vulnerability to parasitic infections. For instance,

estrogen has been linked to the modulation of immune responses across various species, which could affect how female sheep react to *Sarcocystis* infection [43]. This might partly account for the higher prevalence observed in females in this study. It is worth noting that a limitation of this research was the sample size of small number of examined females. This research revealed no statistically significant seasonal differences in *Sarcocystis* spp. prevalence among the examined sheep. This aligns with the findings of other studies, which have shown that *Sarcocystis* spp. can be transmitted throughout the year given the widespread presence of definitive hosts of the parasite (such as domestic dogs and wild carnivores) that continuously shed infectious sporocysts [44]. Moreover, the hardiness of *Sarcocystis* sporocysts under various environmental conditions may reduce the effects of seasonal factors on parasite transmission patterns [17]. Although some studies have indicated slight seasonal trends in parasitic infection transmission due to environmental variations, such as temperature, humidity, and grazing habits, the absence of such variation in this study might be attributed to the consistent conditions of intensive sheep farming in the area, which likely diminishes the impact of external environmental influences [45]. Additionally, since *Sarcocystis* spp. infections are typically long-lasting and asymptomatic, the buildup of cysts in muscle tissues over time may mask any seasonal patterns in infection rates [46].

Macroscopic and microscopic examinations of *Sarcocystis* spp. in sheep tissues have elucidated the morphological characteristics of various species, facilitating their identification during routine inspections. Two macroscopic species, *S. gigantea* and *S. medusiformis*, were found in the esophagus and diaphragm, respectively. Additionally, two microscopic species, *S. tenella* and *S. arieticanis*, were identified in the muscle tissues. The clear morphological distinctions between *S. gigantea* and *S. medusiformis* enabled their identification during gross examination. The large sarcocysts of *S. gigantea* indicate an advanced developmental stage. Conversely, *S. medusiformis*, found in the diaphragm, is smaller, measuring 1–2.5 mm in length and 0.2–0.4 mm in width. Despite its smaller size, the spindle shape of *S. medusiformis* provides a reliable identification cue. These size variations align with previous reports indicating significant morphological diversity among *Sarcocystis* species, depending on the species and host tissue [10] as well as our results was nearly similar to the same macroscopic findings described by [9] in Egypt. In addition to the macroscopic species, the microscopic species, *S. tenella* and *S. arieticanis*, were identified. Their microscopic nature necessitates histological techniques for their detection, highlighting the importance of laboratory analysis in diagnosing *Sarcocystis* infections [47].

The analysis of *S. tenella* COX1 gene sequences revealed significant insights into this parasite's genetic diversity and phylogenetic relationship across different regions and host species. The high genetic identity (99.7%) with Spanish sheep isolates and the lowest (96.82%) with Chinese sheep isolates indicated minimal genetic variation in *S. tenella*, likely due to the conserved nature of COX1, a stable marker used in mitochondrial DNA studies [34, 48]. These results are consistent with those of other studies reporting low intraspecific variation in *Sarcocystis* species [10]. Phylogenetic analysis showed genetic similarity between *S. tenella* isolates from sheep and goats, with the Egyptian goat isolate (PP668132.1) clustered closely with the sheep isolates, suggesting minimal host-specific genetic variation. This implies a shared genetic lineage among the *S. tenella* population infecting ruminants in Egypt, likely because of environmental factors and host-parasite interactions in shared habitats [12, 49]. Examination of 18S rRNA gene sequences also highlights the genetic diversity and phylogenetic relationship among *Sarcocystis* species, particularly *S. tenella* and *S. arieticanis*. *S. tenella* isolates showed genetic identities of 99.88% to 100% compared to other isolates from Norway, Spain, Egypt, Argentina, and China, indicating strong genetic conservation. This homogeneity could be attributed to the slow evolutionary rate of the 18S rRNA gene, efficient parasite transmission, or livestock movement that facilitates gene flow [28].

The observed low genetic variation in *S. tenella* indicates a stable, well-adapted population, likely because of its ability to infect multiple hosts and thrive in diverse environments. This genetic stability suggests that control strategies for *S. tenella* can be applied universally, without region-specific adjustments [48]. Phylogenetic analysis supported the genetic similarity of *S. tenella* isolates from various regions. Isolates from our study, Spain, Egypt, Norway, Argentina, and China formed a tight cluster with 100% bootstrap support, indicating high confidence in their evolutionary relationship. This clustering suggests a common evolutionary lineage and minimal divergence over time, despite geographical separation. The distinct clustering of *S. capracanis* shows that, while *Sarcocystis* species share similar life cycles and infection mechanisms, host specificity can drive genetic divergence. *S. capracanis* has evolved as a separate lineage, potentially due to its adaptation to different host species, despite the geographic overlap with *S. tenella* [10]. The close evolutionary relationship between *S. tenella* isolates from sheep and goats in Egypt further support the hypothesis of a highly conserved genetic makeup, even when transmitted between different host species, suggesting that environmental factors play a significant role in shaping the *S. tenella* population structure [49]. Analysis of the partial 18S rRNA gene sequence of

the *S. arieticanis* isolate in this study revealed high genetic homogeneity among isolates from China, Spain, and Egypt. The *S. arieticanis* isolate showed 99.76% genetic similarity with samples from China (MF039330.1) and Spain (MK420017.1), indicating close genetic relationship despite the vast distances. This genetic uniformity implies considerable genetic consistency across the global distribution of *S. arieticanis*. Phylogenetic study corroborated the genetic similarity among *S. arieticanis* isolates from various locations. The grouping of Egyptian *S. arieticanis* isolates (MH413035.1, MH413036.1, and PQ182594.1) with those from China and Spain, supported by high bootstrap values (99%), reinforces strong genetic relationship, regardless of geographic origin. This consistency could be due to the conserved nature of the 18S rRNA gene and the movement of infected hosts or intermediate hosts between countries [49]. In contrast, *S. hircicanis* isolates from China (KU820984.1) and Egypt (OP430816.1) formed a clade distinct from the *S. arieticanis* group, reflecting the genetic distinctness of these species. The divergence between *S. arieticanis* and *S. hircicanis* may be driven by host specificity or adaptation to different environmental or host-related pressures [12].

Conclusion

This study revealed a significant prevalence of microscopic *Sarcocystis* infections in sheep, demonstrating considerable differences in infection rates across various muscle tissues, age categories, and sexes, although no seasonal influence was detected. Morphological and genetic examinations identified separate macroscopic and microscopic *Sarcocystis* species, with *S. tenella* and *S. arieticanis* exhibiting a strong genetic resemblance to samples from various geographical areas, indicating limited genetic diversity across countries. The phylogenetic results suggested minimal host-specific genetic variation among Egyptian samples from sheep and goats, underscoring the genetic consistency of *Sarcocystis* spp. in these animals. These findings provide important information regarding the epidemiology and phylogeny of *Sarcocystis* spp., potentially supporting the development of diagnostic and control methods for sarcocystosis in farm animals.

Acknowledgments: The authors would like to express their gratitude to the veterinarians of Central Abattoir of Cairo, Egypt for their help in the examination and collection of meat specimens. Also, we are grateful to Dr Ayman Elsayed, Department of Zoology, faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt for his invaluable assistance with the phylogenetic analysis in this study.

Funding statement: This study didn't receive any funding support.

Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval

Under protocol number ZU-IACUC/2/F/235/2023, all procedures conducted in this study were approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (ZU-IACUC committee).

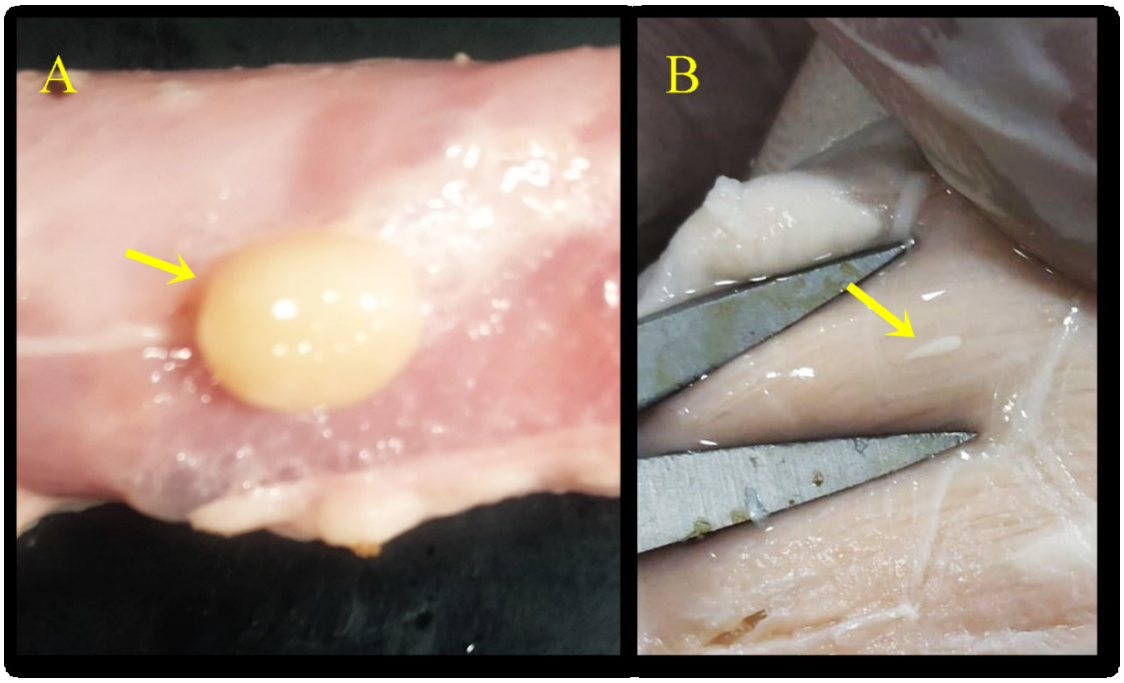

Fig. 1: Gross appearance of macroscopic *Sarcocystis* spp. from naturally infected sheep. (A)An oesophagus contains *S. gigantea,* (B) A diaphragm contains *S. medusiformis*

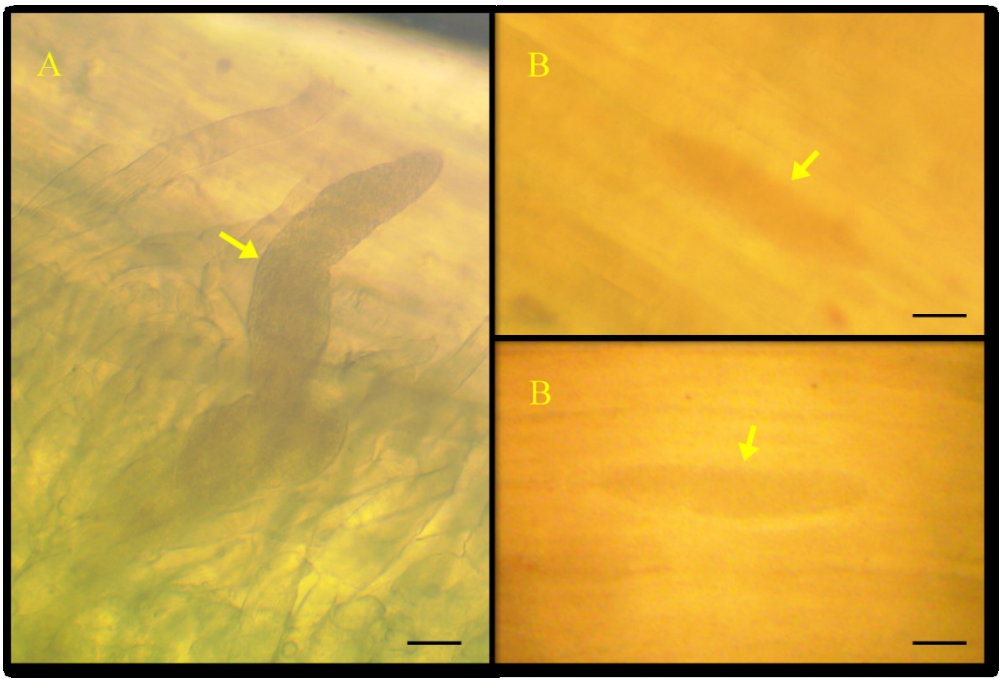

Fig. 2: Light microscopic appearance of microscopic *Sarcocystis* spp. from naturally infected sheep (Bar = 50 µm). A) Unstained *S. arieticanis* slide, B) Unstained *S. tenella* slide

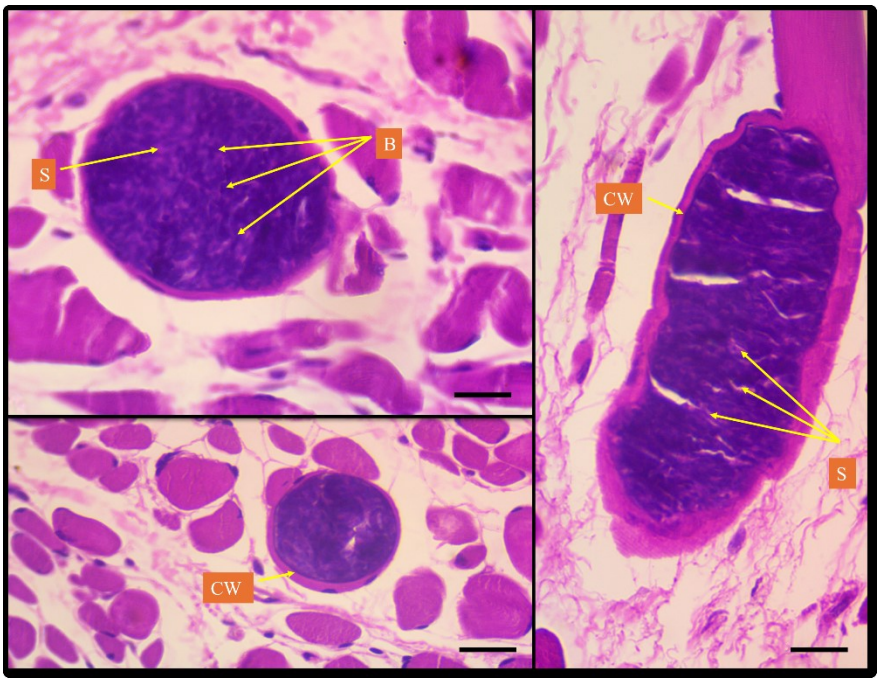

Fig. 3: Histological sections of oesophagus infected with microscopic *S. arieticanis* stained with H&E. Thin cyst wall (CW), Bradyzoites (B), and septae (S). Bar = 20 µm.

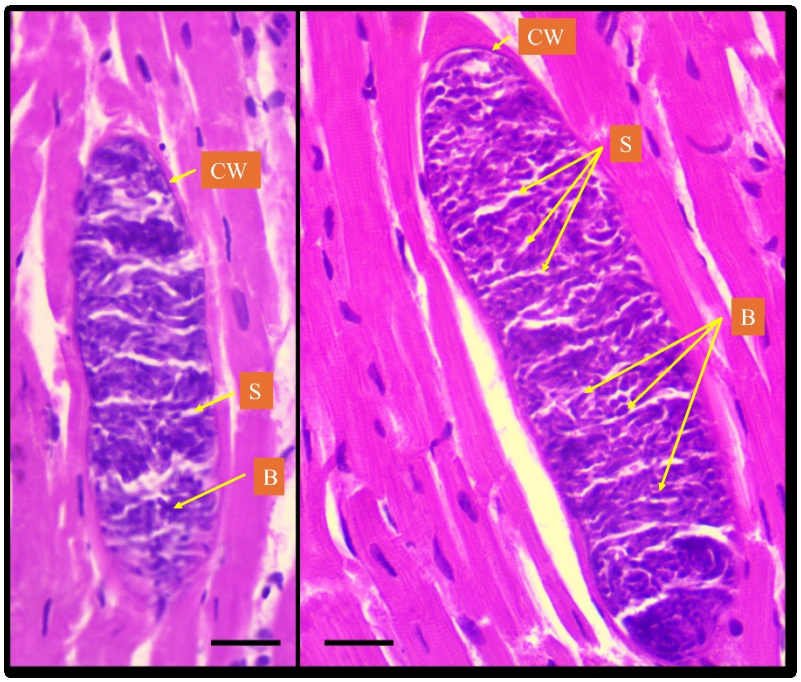

Fig. 4: Histological sections of heart infected with microscopic *S. tenella* stained with H&E. Thick cyst wall (CW), Bradyzoites (B), and septae (S). Bar = 20 µm.

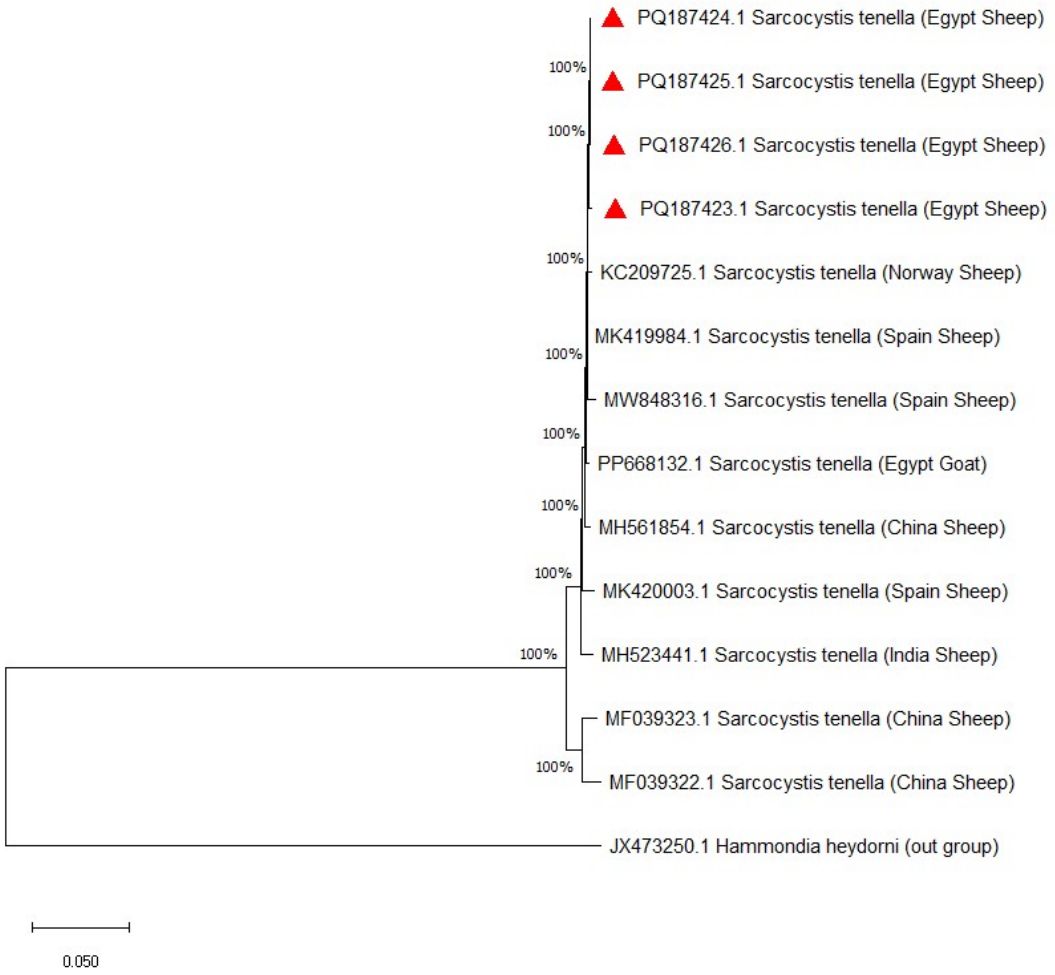

Fig. 5: Phylogenetic tree of selected *Sarcocystis* spp. based on the cytochrome oxidase subunit 1 (COX1) gene sequence showing genetic similarities to *S. tenella*. The accession numbers of *Sarcocystis* spp. isolates were followed by their country of origin and host. The current obtained sequences by this study were labelled with red triangle (▲). *Hammandia heyorni* was considered as outgroup branch.

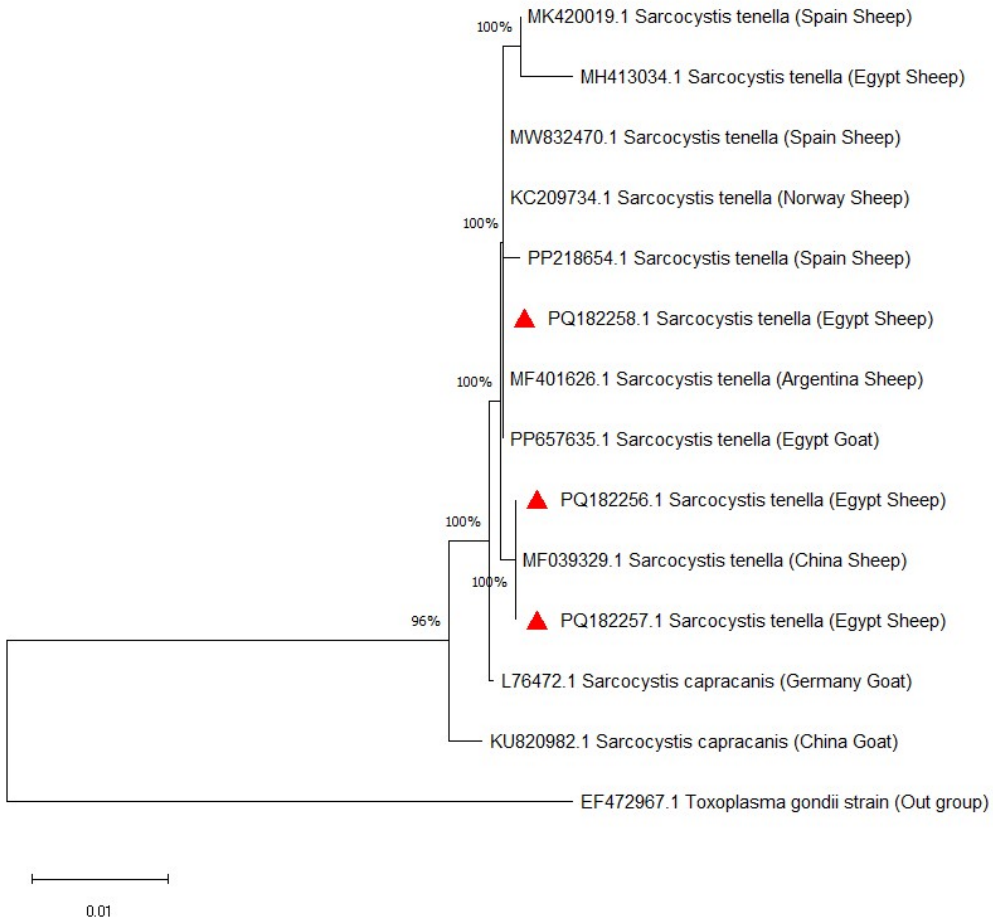

Fig. 6: Phylogenetic tree of selected *Sarcocystis* spp. based on 18S rRNA sequence showing genetic similarities to *S. tenella*. The accession numbers of *Sarcocystis* spp. isolates were followed by their country of origin and host. The current obtained sequences by this study were labelled with red triangle (▲). Toxoplasma gondii was considered as outgroup branch.

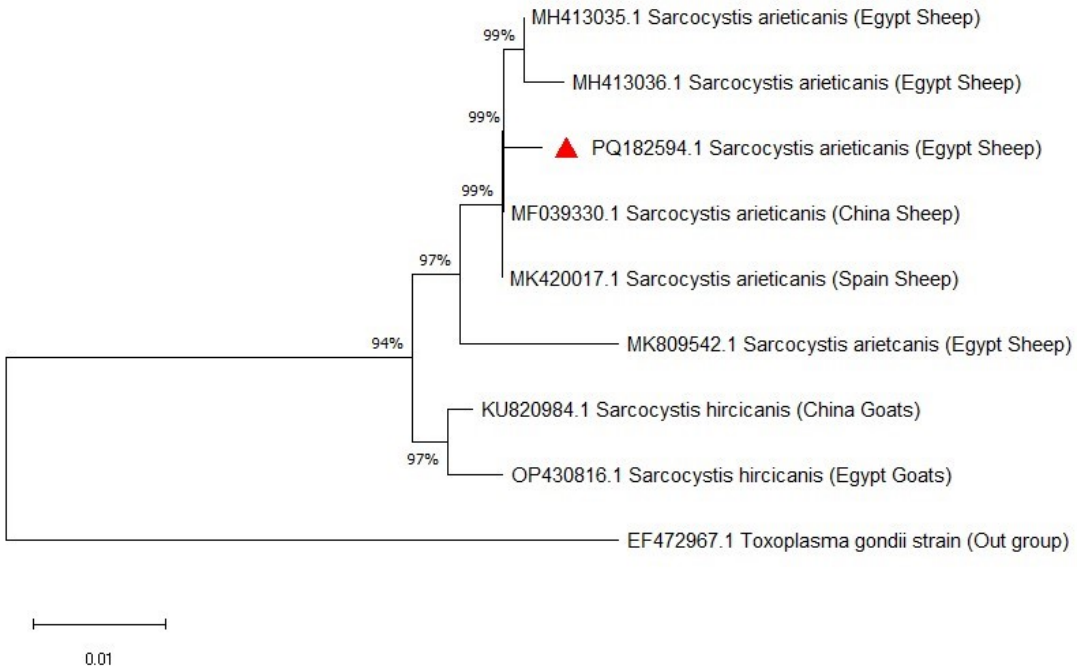

Fig. 7: Phylogenetic tree of selected *Sarcocystis* spp. based on 18S rRNA sequence showing genetic similarities to *S. arieticanis*. The accession numbers of *Sarcocystis* spp. isolates were followed by their country of origin and host. The current obtained sequences by this study were labelled with red triangle (▲). Toxoplasma gondii was considered as outgroup branch.

TABLE 1. Rate of infections of microscopic *Sarcocystis* spp. relative to examined organs

Organs	No. of examined organs	No. of infected organs	%	*P* value*
Oesophagus	210	120	57.1%	0.047
Heart	53	23	43.4%	
Diaphragm	25	18	72%	
Total	288	161	55.9%	

*A significant difference existed (P<0.05)

TABLE 2. Rate of infections of microscopic *Sarcocystis* spp. relative to age group

Age group	No. of examined animals	No. of infected animals	%	P value*
< two years	264	141	53.4%	0.005
≥ two years	24	20	83.3%	
Total	288	161	55.9%	

*A significant difference existed (P<0.05)

TABLE 3. Rate of infections of microscopic *Sarcocystis* spp. relative to sex

Sex	No. of examined animals	No. of infected animals	%	P value*
Males	254	137	53.94%	0.005
Females	34	24	70.59%	
Total	288	161	55.9%	

*A significant difference existed (P<0.05)

TABLE 4. Seasonal prevalence of microscopic *Sarcocystis* spp. in sheep

Season	No. of examined animals	No. of infected animals	%	P value**
Spring	50	32	64 %	0.076
Summer	91	41	45.1%	
Autumn	91	56	61.5%	
Winter	56	32	57.1%	
Total	288	161	55.9%	

**No significant difference existed ($P \geq 0.05$)

TABLE 5: Data extracted from the GenBank database pertaining to the sequence similarity percentages of *S. tenella* identified in sheep and goats hosts across various geographical regions based on COX1 gene sequences, accompanied by the corresponding GenBank accession numbers derived from BLASTn analysis results.

Animal	*Sarcocystis sp.*	Country	Accession No.	Identity %
Sheep	*Sarcocystis tenella*	Spain	MK419984.1-MK420003.1	99.7-98.91
sheep	*Sarcocystis tenella*	Norway	KC209725.1	99.6
Goat	*Sarcocystis tenella*	Egypt	PP668132.1	99.5
sheep	*Sarcocystis tenella*	Spain	MW848316.1	99.2
sheep	*Sarcocystis tenella*	China	MH561854.1	99.18
sheep	*Sarcocystis tenella*	India	MH523441.1	98.8
sheep	*Sarcocystis tenella*	China	MF039322.1-MF039323.1	96.92-96.82

TABLE 6: Data extracted from the GenBank database pertaining to the sequence similarity percentages of *S. tenella* identified in sheep and goats hosts across various geographical regions based on 18s rRNA gene sequences, accompanied by the corresponding GenBank accession numbers derived from BLASTn analysis results.

Animal	*Sarcocystis* sp.	Country	Accession No.	Identity %
Sheep	*Sarcocystis tenella*	Norway	KC209734.1	100
Sheep	*Sarcocystis tenella*	Spain	MW832470.1	100
Goat	*Sarcocystis tenella*	Egypt	PP657635.1	100
Sheep	*Sarcocystis tenella*	Argentina	MF401626.1	100
Sheep	*Sarcocystis tenella*	Spain	MK420019.1	99.88
Sheep	*Sarcocystis tenella*	China	MF039329.1	99.88
Goat	*Sarcocystis capracanis*	Germany	L76472.1	99.64
Sheep	*Sarcocystis tenella*	Spain	PP218654.1	99.62
sheep	*Sarcocystis tenella*	Egypt	MH413034.1	99.04
Goat	*Sarcocystis capracanis*	China	KU820982.1	99.04

TABLE 7: Data extracted from the GenBank database pertaining to the sequence similarity percentages of *S. arieticanis* identified in sheep and goats hosts across various geographical regions based on 18s rRNA gene sequences, accompanied by the corresponding GenBank accession numbers derived from BLASTn analysis results.

Animal	*Sarcocystis* sp.	Country	Accession No.	Identity %
Sheep	*Sarcocystis arieticanis*	China	MF039330.1	99.76
Sheep	*Sarcocystis arieticanis*	Spain	MK420017.1	99.76
Sheep	*Sarcocystis arieticanis*	Egypt	-MH413035.1 -MH413036.1	99.52 - 99.28
Goats	*Sarcocystis hircicanis*	China	KU820984.1	97.83
Goats	*Sarcocystis hircicanis*	Egypt	OP430816.1	97.71
Sheep	*Sarcocystis arieticanis*	Egypt	MK809542.1	97.46

References

1. Zhu, Z.,Ying, Z.,Feng, Z.,Liu, Q. and Liu, J. The Occurrence and Meta-Analysis of Investigations on *Sarcocystis* Infection among Ruminants (Ruminantia) in Mainland China. *Animals*, **13**(1), 149(2022).
2. Choli, R.R.,Mero, W.M.S. and Mohammed, A.B. The Prevalence and Morphological Studies of *Sarcocystis* Species in Slaughtered Ruminants in Zakho City Abattoir, Duhok Province, Kurdistan Region, Iraq. *Egyptian Journal of Veterinary Sciences*, **55**, 1-10(2024).
3. Ahmed, A.M., Elshraway, N.T. and Youssef, A.I. Survey on Sarcocystis in Bovine Carcasses Slaughtered at the Municipal Abattoir of El-Kharga, Egypt. *Vet World*, **9**(12), 1461-1465(2016).
4. Hussein, S.N., Ibrahim, A.A. and Shukur, M.S. Immunization of Rabbits against Whole Crude Antigen of Sarcocystosis (*S. gigantea*) Isolated from Macrocystis of Naturally Infected Sheep in Duhok Province, Iraq. *Egyptian Journal of Veterinary Sciences,* **56**(6), 1289-1299(2025).
5. Omar S., Hussain S. and Bushra. Recent Advances in Molecular Characterization of *Sarcocystis* Species in Some Meat Producing Animals: An Updated Review. *Asian Journal of Agriculture and Biology*, **1**, (2021).
6. Hussein, S.N., Ibrahim, A.A. and Shukur, M.S. Molecular Identification of *Sarcocystis* Species in Sheep (Ovis Aries) and Goats (Capra Hircus) of Duhok Province, Iraq. *Pakistan Veterinary Journal*, **43**(2), 248-254(2023).
7. Gerab, R.A.,Edris, A.-B.M.,Lamada, H.M. and Elrais, A. Prevalence and Distribution of *Sarcocystis* in Buffaloes and Sheep in Egypt. *Journal of Advanced Veterinary Research*, **12**(3), 302-307(2022).
8. Dubey, J.,Calero-Bernal, R.,Rosenthal, B.,Speer, C. and Fayer, R. *Sarcocystosis of Animals and Humans*: CRC Press. (2015).
9. El-Morsey, A.,Abdo, W.,Zaid, A.A.A. and Sorour, S.S.G. Morphologic and Molecular Identification of Three Macroscopic *Sarcocystis* Species Infecting Domestic Sheep (Ovis Aries) and Cattle (Bos Taurus) in Egypt. *Parasitology Research*, **120**, 637-654(2021).
10. Dubey,BR, C. and BM, R. *Sarcocystosis of Animals and Humans*. 2nd ed: CRC Press, USA. (2016).
11. Gjerde, B.,De la Fuente, C.,Alunda, J.M. and Luzón, M. Molecular Characterisation of Five *Sarcocystis* Species in Domestic Sheep (Ovis Aries) from Spain. *Parasitology Research*, **119**, 215-231(2020).
12. El-Morsey, A.,Abdo, W.,Sultan, K.,Elhawary, N.M. and AbouZaid, A.A. Ultrastructural and Molecular Identification of the Sarcocysts of *Sarcocystis tenella* and *Sarcocystis arieticanis* Infecting Domestic Sheep (Ovis Aries) from Egypt. *Acta Parasitologica*, **64**, 501-513(2019).
13. Ghaffar,Heydorn and Mehlhorn. The Fine Structure of Cysts of *Sarcocystis Moulei* from Goats. *Parasitology Research,* **75***,* 416–418(1989).
14. Hong,Sim, C.,Chae, J.,Kim, H.,Park, J.,Choi, K.,Yu, D.,Park, C.,Yoo, J. and Park, B. Ultrastructural and Molecular Identification of *Sarcocystis tenella* (Protozoa, Apicomplexa) in Naturally Infected Korean Native Goats. *Veterinární medicína*, **61**(7), 374-381(2016).
15. Al-Hoot,Al-Qureishy,K, A.-R. and AR, B. Microscopic Study on *Sarcocystis moulei* from Sheep and Goats in Saudi Arabia. *Journal of the Egyptian Society of Parasitology*, **35**, 295-312(2005).
16. Kalantari, N.,Khaksar, M.,Ghaffari, S. and Hamidekish, S.M. Molecular Analysis of *Sarcocystis* Spp. Isolated from Sheep (Ovis Aries) in Babol Area, Mazandaran Province, Northern Iran. *Iranian Journal of Parasitology*, **11**(1), 73(2016).
17. Abu El-Wafa, S.A.,Al-Araby, M.A.,Abbas, I.E. and and Elmishmishy, B.M.M. Prevalence of *Sarcocystis* Species Infecting Sheep from Egypt. *Egyptian Veterinary Medical Society of Parasitology Journal*, **12**(1), 74-90(2016).
18. Moré, G.,Abrahamovich, P.,Jurado, S.,Bacigalupe, D.,Marin, J.C.,Rambeaud, M.,Venturini, L. and Venturini, M.C. Prevalence of *Sarcocystis* Spp. In Argentinean Cattle. *Veterinary Parasitology*, **177**(1), 162-165(2011).
19. Helman, E.,Dellarupe, A.,Steffen, K.D.,Bernstein, M. and Moré, G. Morphological and Molecular Characterization of *Sarcocystis* Spp. In Pigs from Argentina. *Parasitology International*, **100**, 102859(2024).
20. El-Kady, A.M.,Hussein, N.M. and Hassan, A.A. First Molecular Characterization of *Sarcocystis* Spp. In Cattle in Qena Governorate, Upper Egypt. *Journal of Parasitic Diseases*, **42**(1), 114-121(2018).
21. Elmishmishy, B.,Al-Araby, M.,Abbas, I. and Abu-Elwafa, S. Genetic Variability within Isolates of *Sarcocystis* Species Infecting Sheep from Egypt. *Veterinary Parasitology: Regional Studies and Reports*, **13**, 193-197(2018).

22. Latif, B.,Kannan Kutty, M.,Muslim, A.,Hussaini, J.,Omar, E.,Heo, C.,Rossle, N.,Abdullah, S.,Kamarudin, M. and Zulkarnain, M. Light Microscopy and Molecular Identification of *Sarcocystis* Spp. In Meat Producing Animals in Selangor, Malaysia. *Tropical Biomedicine*, **32**(3), 444-452(2015).

23. Nematollahia, A.,Khoshkerdar, A.,Helan, J.A.,Shahbazi, P. and Hassanzadeh, P. A Study on Rate of Infestation to *Sarcocystis* Cysts in Supplied Raw Hamburgers. *Journal of Parasitic Diseases*, **39**, 276-279(2015).

24. Dubey, J.P.,Speer, C.A. and Charleston, W.A. Ultrastructural Differentiation between Sarcocysts of *Sarcocystis hirsuta* and *Sarcocystis hominis*. *Veterinary Parasitology*, **34**(1-2)153–157(1989).

25. Salehi, M.,Spotin, A.,Rostamian, M. and Adami, M. Prevalence and Molecular Assessment of *Sarcocystis* Infection in Livestock in Northeast Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, **80**, 101738(2022).

26. Abdullah, S.H. Investigation of *Sarcocystis* Spp. In Slaughtered Cattle and Sheep by Peptic Digestion and Histological Examination in Sulaimani Province, Iraq. *Veterinary World*, **14**(2), 468-474(2021).

27. Rubiola, S.,Chiesa, F.,Zanet, S. and Civera, T. Molecular Identification of *Sarcocystis* Spp. In Cattle: Partial Sequencing of Cytochrome C Oxidase Subunit 1 (Coi). *Italian journal of food safety*, **7**(4), 204-208(2018).

28. Gjerde, B. Molecular Characterisation of *Sarcocystis bovifelis*, *Sarcocystis bovini*, *Sarcocystis hirsuta* and *Sarcocystis cruzi* from Cattle (Bos Taurus) and *Sarcocystis sinensis* from Water Buffaloes (Bubalus Bubalis). *Parasitology Research*, **115**(4), 1473-1492(2016).

29. Peris, M.P.,Gracia, M.J.,Moreno, B.,Juan-Puente, P.,Morales, M.,Serrano, M.,Manzano, M.D.,Halaihel, N.,Badiola, J. and Castillo, J.A. Identification of *Sarcocystis* Spp. In Slaughtered Sheep from Spain and Evaluation of Bradyzoite Viability after Freezing. *Veterinary Sciences*, **11**(3), 103-115(2024).

30. Moré, G.,Schares, S.,Maksimov, A.,Conraths, F.J.,Venturini, M.C. and Schares, G. Development of a Multiplex Real Time PCR to Differentiate *Sarcocystis* Spp. Affecting Cattle. *Veterinary Parasitology*, **197**(1-2), 85-94(2013).

31. Tamura, K.,Stecher, G.,Peterson, D.,Filipski, A. and Kumar, S. Mega6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular biology and evolution*, **30**(12), 2725-2729(2013).

32. Amairia, S.,Amdouni, Y.,Rouatbi, M.,Rjeibi, M.R.,Awadi, S. and Gharbi, M. First Detection and Molecular Identification of *Sarcocystis* Spp. In Small Ruminants in North-West Tunisia. *Transboundary and Emerging Diseases*, **65**(2), 441-446(2018).

33. Minuzzi, C.E.,Cezar, A.S.,Bräunig, P.,Portella, L.P.,Rodrigues, F.d.S.,Sangioni, L.A. and Vogel, F.S. Occurrence of *Sarcocystis gigantea* Macrocysts and High Frequency of *S. tenella* Microcysts in Sheep from Southern Brazil. *Veterinary Parasitology: Regional Studies and Reports*, **15**, 100256(2019).

34. Kang, Y.,Lu, X.-S.,He, Y.-H.,Wang, C.,Wu, Z.-X.,Wang, L.,Wu, X.-J.,Hu, J.-J. and Zhu, X.-Q. First Molecular Identification and Prevalence of *Sarcocystis* Spp. In Sheep Intended for Human Consumption in Shanxi Province, China. *Veterinary Sciences*, **11**(10), 504(2024).

35. Zohra, B.F.,Mokhtaria, K.,Houari, H. and Ammar, S.S.M. Sarcosporidiosis of the Ovine Esophagus: Frequency and Histopathological Identification of the Species Involved in Tiaret, Algeria. *Journal of Applied Veterinary Sciences*, 81-85(2024).

36. Ajaj, E.A. and Taha, A.H. Detection of Sarcocystosis in Dogs in Nineveh Province. *Egyptian Journal of Veterinary Sciences*, **55**(6), 1531-1536(2024).

37. Fatani, A.,Hilali, M.,Al-Atiya, S. and Al-Shami, S. Prevalence of *Sarcocystis* in Camels (Camelus Dromedarius) from Al-Ahsa, Saudi Arabia. *Veterinary Parasitology*, **62**(3-4), 241-245(1996).

38. El-Dakhly, K.M.,El-Nesr, K.A.,El-Nahass, E.-S.,Hirata, A.,Sakai, H. and Yanai, T. Prevalence and Distribution Patterns of *Sarcocystis* Spp. In Buffaloes in Beni-Suef, Egypt. *Tropical Animal Health and Production*, **43**, 1549-1554(2011).

39. Fayer R,Esposito DH and JP, D. Human Infections with *Sarcocystis* Species. *Clinical Microbiology Reviews*, **28**(2), 295-311(2015).

40. Fayer, R. *Sarcocystis* Spp. In Human Infections. *Clinical Microbiology Reviews*, **17**(4), 894-902(2004).

41. Nourani, H.,Matin, S.,Nouri, A. and Azizi, H. Prevalence of Thin-Walled *Sarcocystis cruzi* and Thick-Walled *Sarcocystis hirsuta* or *Sarcocystis hominis* from Cattle in Iran. *Tropical Animal Health and Production*, **42**, 1225-1227(2010).

42. Sciarra, F.,Campolo, F.,Franceschini, E.,Carlomagno, F. and Venneri, M.A. Gender-Specific Impact of Sex Hormones on the Immune System. *International journal of molecular sciences*, **24**(7), 6302(2023).

43. Ahmed, A.M.,Elshraway, N.T. and Youssef, A.I. Survey on *Sarcocystis* in Bovine Carcasses

Slaughtered at the Municipal Abattoir of El-Kharga, Egypt. *Veterinary World*, **9**(12), 1461(2016).

44. Altizer, S.,Dobson, A.,Hosseini, P.,Hudson, P.,Pascual, M. and Rohani, P. Seasonality and the Dynamics of Infectious Diseases. *Ecology Letters*, **9**(4), 467-484(2006).

45. Rathish, B. and K, R. *Sarcocystis*. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing (2023). Accession date: Aug. 2024 https://www.ncbi.nlm.nih.gov/books/NBK574577

46. M Ibrahim, H.,El Sabagh, R.,A Wahba, A. and Abd El Rahman, E.S.A. The Incidence of *Sarcocystis* in Slaughtered Food Animals. *Benha Veterinary Medical Journal*, **35**(1), 106-122(2018).

47. Hu, J.-J.,Huang, S.,Wen, T.,Esch, G.W.,Liang, Y. and Li, H.-L. *Sarcocystis* Spp. In Domestic Sheep in Kunming City, China: Prevalence, Morphology, and Molecular Characteristics. *Parasite*, **24**, 1-8(2017).

48. Tenter, A.M.,Barta, J.R.,Beveridge, I.,Duszynski, D.W.,Mehlhorn, H.,Morrison, D.A.,Thompson, R.A. and Conrad, P.A. The Conceptual Basis for a New Classification of the Coccidia. *International Journal for Parasitology*, **32**(5), 595-616(2002).

49. Robertson, L.J.,Clark, C.G.,Debenham, J.J.,Dubey, J.P.,Kváč, M.,Li, J.,Ponce-Gordo, F.,Ryan, U.,Schares, G. and Su, C. Are Molecular Tools Clarifying or Confusing Our Understanding of the Public Health Threat from Zoonotic Enteric Protozoa in Wildlife? *International Journal for Parasitology: Parasites and Wildlife*, **9**, 323-341(2019).

دراسة نسبة الاصابة والوصف المورفولوجي والعلاقات الوراثية لأنواع الساركوسيست في الأغنام

احمد محمود محمد علي عبد الحميد[*1,2]، بسيوني عبد الحافظ احمد[2]، نصير محمد السيد[2]، عادل عبد الخالق سيد[3] ورفعت عاطف رأس[1,2]

[1]قسم الميكروبيولوجيا والطفيليات ، كلية الطب البيطري ، جامعة بدر بالقاهرة ، مدينة بدر ، القاهرة ،11829 مصر.

[2]قسم الطفيليات ، كلية الطب البيطري ، جامعة الزقازيق ، الزقازيق ،44511 مصر.

[3]قسم صحة وسلامه وتكنولوجيا الغذاء ، كلية الطب البيطري ، جامعة بدر بالقاهرة ، مدينة بدر ، القاهرة ،11829 مصر.

الملخص

الحويصلات الصنوبريه (الساركوسيست) يسببها كائن أولي ينتمي إلى جنس الساركوسيستس، يسبب هذا الطفيل اضطرابات عصبية وإجهاض بالإضافة إلى خسائر اقتصادية كبيرة في الحيوانات المصابه. أجريت هذه الدراسة لتحديد نسبه الاصابه والوصف المورفولوجي وكذلك العلاقات الجينية والوراثيه لأنواع الساركوسيستس وتأثيرها المحتمل على جودة لحم الضأن وصحة الحيوان وسلامه الغذاء أثناء عمليات الفحص والكشف عن اللحوم في الأغنام في المجازر في القاهره - مصر. لوحظ معدل إصابة بالانواع التي تري بالعين المجردة بنسبة 0.35٪، وكانت هذه الانواع *S. gigantea* و *S. medusiformis* ، مع معدل إصابة للانواع المجهرية بنسبة 55.9٪، وانتشار مرتفع للانواع المجهرية في عضلات الحجاب الحاجز (72٪)، والمريء (57.1٪)، والقلب (31.4٪). وقد أبرز التحليل الجزيئي لجينات (COX1) و (rRNA 18S) تشابهًا وراثيًا كبيرًا بين عزلات *S. tenella* من مصر ومناطق أخرى، بما في ذلك إسبانيا والنرويج والصين، حيث تراوحت الهويات الوراثية من 96.82٪ إلى 100٪. وأشار التحليل التطوري إلى تباين ضئيل بين العائل، حيث تتجمع عزلات الأغنام والماعز المصرية معًا. كما أظهرت *S. arieticanis* علاقات وراثية وثيقة مع عزلات من مواقع متنوعة. وتوضح هذه النتائج اتساقًا وراثيًا واسع النطاق داخل مجموعات *S. tenella* و*S. arieticanis* عبر المناطق الجغرافية، مما يشير إلى تباين إقليمي محدود ويوفر رؤى حول علم الوراثة لأنواع *Sarcocystis* في المجترات الصغيرة. كشفت نتائج هذه الدراسة عن مجموعة واسعة من أنواع *Sarcocystis* مما يؤكد على الحاجة إلى تدابير مراقبة معززة للتخفيف من تأثيرها على صحة الحيوان وسلامة الغذاء.

الكلمات الدالة: معدل انتشار، الساركوسيست، الأغنام، الوصف المورفولوجي، العلاقات الوراثية.