



Morphological and Molecular Investigations of *Sarcocystis* spp. in *Camelus dromedaries* in Kuwait

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ABSTRACT

Sarcocystosis is a disease caused by *Sarcocystis* parasites, which are widely distributed among mammals, birds, and reptiles and may cause myositis and encephalitis. The present study aimed to investigate the prevalence, morphological and histopathological characteristics, and molecular identification of *Sarcocystis* infection in dromedary camels (*Camelus dromedarius*) in Kuwait using pepsin digestion, histopathological examination, and molecular characterization based on the 18S rRNA gene. A total of 1,350 tissue samples from muscles, diaphragm, tongue, heart, and esophagus were collected from 270 camels slaughtered in Kuwait. The camels comprised 214 males and 56 females and were categorized into three age groups, including younger than 8 months (n = 99), between 8 months and 3 years old (n = 113), and older than 3 years old (n = 58). Microscopic examination revealed *Sarcocystis* infection in 194 of 270 camels (71.85%). Infection was not significantly associated with sex, whereas age and season were significantly associated with *Sarcocystis* prevalence. *Sarcocystis* prevalence was 63.3% in muscle, 63.0% in diaphragm, 62.2% in tongue, 62.0% in heart, and 56.3% in esophagus. Histopathological examination revealed three cyst morphologies, including spindle-shaped, ellipsoidal, and irregularly elongated. Pepsin digestion revealed three distinct bradyzoite morphologies, including banana-shaped, sausage-shaped, and round forms. Five tissue samples were selected for molecular analysis using 18S rRNA sequencing. The BLASTn analysis demonstrated the highest similarity to *Sarcocystis* spp. isolated from *Camelus dromedarius* in Iran (GenBank accession no. GU074011), with 69% query coverage and a sequence identity of 98.4-98.67%. The present study reported a high prevalence of *Sarcocystis* infection (71.85%) among dromedary camels in Kuwait, with infection rates significantly influenced by age and season. Three distinct cyst and bradyzoite morphologies, along with molecular data showing high sequence similarity to Iranian dromedary isolates, strongly suggested that *Sarcocystis* infections are widespread and diverse among camel populations in Kuwait.

Keywords: *Camelus dromedarius*, Molecular analysis, *Sarcocystis* spp., Season

INTRODUCTION

Sarcocystosis is a zoonotic parasitic disease caused by intracellular coccidian protozoa of the genus *Sarcocystis*, which belongs to the phylum Apicomplexa (Latif and Muslim, 2016; Muslim and Chin Heo, 2022). Members of the genus *Sarcocystis* are found worldwide and infect a broad range of intermediate and definitive hosts, such as mammals, birds, and reptiles (Latif and Muslim, 2016; Muslim and Chin Heo, 2022). Infections with *Sarcocystis* spp. are particularly common in dromedary camels (*Camelus dromedarius* [*C. dromedarius*]) and have been reported in several camel-producing countries. High infection rates have been documented in Saudi Arabia (Fatani et al., 1996), Iraq (Latif et al., 1999), Iran (Valinezhad et al., 2008; Motamedi et al., 2011), Egypt, and other endemic regions (Gareh et al., 2020). Valinezhad et al. (2008) identified *Sarcocystis* infection in 83.6% of 250 camels examined in Eastern Iran, illustrating the high prevalence of the parasite in camel populations.

Sarcocystis infection in camels was first documented in the early twentieth century, when Mason (1910) described the cysts, their morphology, and cyst-wall dimensions. Subsequent studies identified multiple *Sarcocystis* species in camels. Nonetheless, the classification of these parasites remained unclear because some species lacked detailed morphological characterization or had no type specimens. Dubey et al. (2015) conducted a comprehensive review of the available literature and provided information on the sarcocysts of *Sarcocystis cameli* (*S. cameli*) and *Sarcocystis ippeni* (*S. ippeni*) from *C. dromedarius*, utilizing light microscopy and transmission electron microscopy. In addition, Dubey et al. (2015) clarified key morphological features of *S. cameli* and *S. ippeni* and considered *S. camelicanis*, *S. camelocanis*, and *S. miescheri* as invalid species.

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Camels become infected with *Sarcocystis* by consuming food or water contaminated with sporulated oocysts or sporocysts from the definitive hosts. After ingestion, the parasite undergoes developmental stages in the intermediate host and ultimately forms sarcocysts in the muscles (Valinezhad et al., 2008). While many infections are asymptomatic or subclinical, severe diseases can arise. *Sarcocystis* infection can induce inflammatory responses, tissue damage, necrosis, and fibrosis, particularly when cysts degenerate (Valinezhad et al., 2008).

Sarcocystis infection significantly impacts camel production and meat quality. Macroscopic sarcocysts negatively impact the visual appeal and acceptability of meat, potentially leading to carcass or tissue condemnation. Although microscopic sarcocysts may remain undetected during routine meat inspection, their high prevalence can lead to economic losses and affect animal health and productivity. In animals exhibiting clinical signs, reported manifestations include fever, anemia, anorexia, muscle weakness, decreased productivity, abortion, and, in severe cases, mortality (Valinezhad et al., 2008; Metwally et al., 2020).

Diagnosing *Sarcocystis* infection in camels can be difficult, especially when there are no visible macroscopic cysts or clinical symptoms. Several diagnostic methods are employed, including muscle compression or squash techniques, pepsin and trypsin digestion, histopathological analysis, and molecular assays. Each method has distinct diagnostic capabilities, and the use of morphological, histopathological, and molecular techniques can improve the detection and characterization of *Sarcocystis* infections (Saeed et al., 2018a).

Although *Sarcocystis* infection has been extensively documented in camels in several countries, information on its prevalence, tissue distribution, morphological characteristics, and molecular identity in dromedary camels in Kuwait remains limited. Given the economic importance of camels in Kuwait as sources of meat and milk, it is essential to determine the epidemiological status of *Sarcocystis* infection. Therefore, the present study aimed to determine the prevalence and tissue distribution of *Sarcocystis* infection in camels slaughtered in Kuwait and to characterize the infection using pepsin digestion, histopathological examination, and molecular analysis.

MATERIALS AND METHODS

Ethical approval

This study was conducted in accordance with the regulations established by the Public Authority of Agriculture Affairs and Fish Resources (PAAFR), Rabia, Kuwait, for research activities, and received approval from the technical committee of the Animal Resources sector.

Study design and samples

A cross-sectional study was conducted over one year, from January to December 2020. Given the climatic conditions in Kuwait, the year has been divided into two distinct seasons, including a dry season from April to November, and a wet season from December to March (Halwagy et al., 1974). The study population comprised one-humped camels (*Camelus dromedarius*) of both sexes and different age groups, reared under an extensive husbandry system that allowed free grazing. Information on the specific diets and husbandry practices of individual camels was unavailable because the camels originated from different owners and farms. A total of 270 camels were slaughtered at two slaughterhouses in Kuwait, including Al-Jahra Slaughterhouse in Northern Kuwait (Al-Jahra Governorate) and Al-Dhahar Slaughterhouse in Southern Kuwait (Mubarak Al-Kabeer Governorate). The sample size in the present study was determined based on previous studies by Gareh et al. (2020) and Metwally et al. (2020). Camels were selected from animals that were presented for slaughter during the study period.

Information on sex, age, body condition, and breed were recorded at the time of sampling. Age was determined through data obtained from owners and through dentition assessment. In accordance with the classification outlined by Bello et al. (2013), the camels were categorized into three groups, including less than 8 months, between 8 months and 3 years, and over 3 years.

Microscopic examination

Pepsin digestion technique

In the present study, over a period of one year and through bi-weekly visits to two slaughterhouses, approximately 20 grams of tissue samples from five organs, namely the diaphragm, esophagus muscles, skeletal muscles, tongue, and heart, were collected, minced, and subsequently incubated in 100 mL of digestion solution at 40°C for 30 minutes. The digestion solution contained pepsin (1.3 g), HCl (3.5 mL), and NaCl (2.5 g) dissolved in 500 mL of distilled water (Dubey et al., 1989). Following digestion, samples were centrifuged at $3500 \times g$ for 3 minutes, stained with Giemsa, and examined under a light microscope (Hamidinejat et al., 2013).

Histopathological examination

Collected samples, each measuring $2 \times 1 \times 1$ cm³, were fixed in 10% formalin for histological preparation (Bancroft and Gamble, 2007). Paraffin embedding was performed, followed by sectioning of 5- μ m-thick slices and hematoxylin and eosin (H&E) staining. Cysts were measured using an ocular micrometer under a microscope and photographed with the AX10 microscope (Zeiss, Germany) connected to the high-resolution camera (HRC; FLIR, UK). All measurements were recorded in micrometers.

Molecular analysis

Eight positive samples, prepared using pepsin digestion, were chosen for the molecular analysis. The sediments were preserved in 80% alcohol until use and were transported by airplane to Iran. All molecular techniques were performed at the Parasitology Department, Medical Sciences Faculty, Tarbiat Modares University, Tehran, Iran. DNA from eight samples was extracted using a DNA purification kit (Yekta Tajhiz Azma, YTA Genomic DNA Extraction Mini kit for Tissue, Tehran, Iran) according to the manufacturer's instructions and stored at -20°C for molecular analysis. Polymerase chain reaction (PCR) was performed to identify *Sarcocystis* species by amplifying a partial sequence of the small subunit ribosomal RNA (18S rRNA) gene from *Sarcocystis* in camels. For amplification of *Sarcocystis* species 18S rRNA, primers SarcoF 5'-CGCAAATTACCCAATCCTGA-3' and SarcoR 5'-ATTTCTCATAAGGTGCAGGAG-3' were used (Moréet al., 2011; Bräunig et al., 2016). The 20 µL PCR mixture included 1 µL of template DNA, 10 µL of Taq DNA Polymerase 2x Master Mix RED (Ampliqon, 2 mM of MgCl₂), and 1 µM of each primer. The PCR process consisted of 35 cycles (95°C for 30 s, 57°C for 30 s, and 72°C for 1 min), starting with an initial denaturation at 95°C for 5 minutes and ending with a final extension at 72°C for 10 minutes.

Phylogenetic analysis

For sequence analysis, five isolates were selected from 8 positive tissues, including isolates 1 and 5 from skeletal muscle, isolates 2 and 3 from the tongue, and isolate 4 from the diaphragm. These five positive samples were sequenced on the ABI3730XL sequencer (Japan). The sequences were edited and aligned using Clustal W and compared with reference sequences from GenBank (NCBI). For *Sarcocystis* species, the phylogenetic tree was constructed using the maximum-likelihood algorithm in the Molecular Evolutionary Genetics Analysis (MEGA) software (version 7.0; USA). Sequences of representative *Sarcocystis* species infecting camels were included from NCBI GenBank. *Neospora caninum* (LC897915) was used as an outgroup owing to its classification within the family *Sarcocystidae*, despite belonging to a different genus (Table 1). The sequences from the five samples were deposited in GenBank (NCBI) with accession numbers PQ120680- PQ120684.

Table 1. Coccidian species employed in phylogenetic analysis, along with their corresponding GenBank accession numbers

Accession number	Parasite species	Host	Country
PQ120680	<i>Sarcocystis</i> sp. Isolate 1	<i>Camelus dromedarius</i>	Kuwait
PQ120681	<i>Sarcocystis</i> sp. Isolate 2	<i>Camelus dromedarius</i>	Kuwait
PQ120682	<i>Sarcocystis</i> sp. Isolate 3	<i>Camelus dromedarius</i>	Kuwait
PQ120683	<i>Sarcocystis</i> sp. Isolate 4	<i>Camelus dromedarius</i>	Kuwait
PQ120684	<i>Sarcocystis</i> sp. Isolate 5	<i>Camelus dromedarius</i>	Kuwait
GU074011	<i>Sarcocystis</i> sp.	<i>Camelus dromedarius</i>	Iran
OP959654	<i>Sarcocystis cameli</i>	<i>Camelus dromedarius</i>	India
OM462705	<i>Sarcocystis cameli</i>	<i>Camelus dromedarius</i>	Not shown
OM462704	<i>Sarcocystis cameli</i>	<i>Camelus dromedarius</i>	India
KU527109	<i>Sarcocystis masoni</i>	<i>Lama guanicoe</i>	Argentina
KU527110	<i>Sarcocystis masoni</i>	<i>Lama glama</i>	Argentina
KU527111	<i>Sarcocystis masoni</i>	<i>Lama glama</i>	Argentina
KU527113	<i>Sarcocystis masoni</i>	<i>Vicugna pacos</i>	Argentina
LC171832	<i>Sarcocystis fayeri</i>	<i>Equus caballus</i>	Canada
MH025628	<i>Sarcocystis bertrami</i>	<i>Equus caballus</i>	China
KT901104	<i>Sarcocystis sinensis</i>	<i>Bubalus bubalis</i>	Egypt
KF954731	<i>Sarcocystis hominis</i>	Cattle	Germany
KY019056	<i>Sarcocystis silva</i>	<i>Capreolus capreolus</i>	Italy
KR186117	<i>Sarcocystis fusiformis</i>	<i>Bubalus bubalis</i>	Egypt
KJ778019	<i>Sarcocystis cafferi</i>	<i>Syncerus caffer</i>	South Africa
KU247910	<i>Sarcocystis buffalonis</i>	<i>Bubalus bubalis</i>	Egypt
KC209741	<i>Sarcocystis hirsuta</i>	<i>Bos taurus</i>	Argentina
L76473	<i>Sarcocystis moulei</i>	Experimentally infected goats	Germany
KC209733	<i>Sarcocystis gigantea</i>	<i>Ovis aries</i>	Norway
KF831275	<i>Sarcocystis alceslatrans</i>	<i>Alces alces</i>	Canada
AF017123	<i>Sarcocystis aucheniae</i>	Not specified	Australia
JX110662	<i>Sarcocystis aucheniae</i>	<i>Lama glama</i>	Argentina
JX110663	<i>Sarcocystis aucheniae</i>	<i>Lama glama</i>	Argentina
KU527114	<i>Sarcocystis aucheniae</i>	<i>Lama guanacoe</i>	Argentina
KU527116	<i>Sarcocystis aucheniae</i>	<i>Lama glama</i>	Argentina
KU527118	<i>Sarcocystis aucheniae</i>	<i>Lama guanicoe</i>	Argentina
KF383267	<i>Sarcocystis aucheniae</i>	Not specified	Argentina
KF383268	<i>Sarcocystis aucheniae</i>	Not specified	Argentina
LC897915	<i>Neospora caninum</i> (outgroup)	<i>Columba livia domestica</i>	Not shown

Statistical analysis

Data were analyzed and compared statistically using IBM SPSS software (version 23). Associations between parasitism and different parameters were assessed using the Pearson chi-square test. A p-value less than 0.05 was considered significant.

RESULTS

No macroscopic *Sarcocystis* spp. were found in any of the examined camels. However, microscopic *Sarcocystis* spp. were detected in 194 of 270 camels, yielding an overall prevalence of 71.85% using the pepsin digestion method. The rate of infection was not significantly associated with gender ($p > 0.05$), although males had a higher infection rate (74.3%) than females (62.5%; Table 2). In contrast, age and season significantly influenced the prevalence of *Sarcocystis* infection ($p < 0.05$). The highest infection rates were observed among camels aged between 8 months and 3 years and during the wet season, compared to other age groups and the dry season, respectively (Tables 2 and 3). Thus, camels aged >8 months–3 years had a significantly higher prevalence than both other age groups. The groups 0–8 months and >3 years did not differ significantly. A total of 1,350 tissue samples, including skeletal muscles, diaphragm, tongue, heart, and esophagus, from 270 camels were analyzed for *Sarcocystis* spp. infection using the pepsin digestion method. The prevalence rates were 63.3% in skeletal muscles, 63.0% in the diaphragm, 62.2% in the tongue, 62.0% in the heart, and 56.3% in the esophagus.

Table 2. Prevalence of *Sarcocystis* species according to gender and age groups in Kuwait

Category		Number of negative (%)	Number of positive (%)	Total number (%)	P-value
Gender	Male	55 (25.7)	159 (74.3)	214 (79.3)	$p > 0.05$
	Female	21 (37.5)	35 (62.5)	56 (20.7)	
Age	0-8 months	40 (40.4)	59 (59.6)	99 (36.6)	$p < 0.05$
	> 8 months-3 years	16 (14.2)	97 (85.8)	113 (42)	
	> 3 years	20 (34.5)	38 (65.5)	58 (21.4)	
Season	Dry	70 (37.6)	116 (62.4)	186 (68.8)	$p < 0.05$
	Wet	6 (7.2)	78 (92.8)	84 (31.2)	
	Total	76 (28%)	194 (72%)	270	

Table 3. Prevalence of *Sarcocystis* species according to age groups in Kuwait using post-hoc pairwise comparisons with Bonferroni correction

Pairwise comparison	Positive prevalence	Raw P-value	Bonferroni-adjusted P	Interpretation
0-8 months versus > 8 months-3 years	59.6% versus 85.8%	< 0.001	<0.001	Significant
0-8 months versus > 3 years	59.6% versus 65.5%	0.461	1.000	Not significant
> 8 months-3 years versus > 3 years	85.8% versus 65.5%	0.002	0.006	Significant

Morphological results

Cysts

Three morphological types of cysts were observed in the examined tissues, including spindle, ellipsoid, and irregularly elongated. The length of the elongated shape was $2.75 \times 1.2 \mu\text{m}$ in esophagus samples and $286.9 \times 78.2 \mu\text{m}$ in muscle samples, with $144.8 \pm 200.9 \mu\text{m}$ length and $39.7 \pm 54.45 \mu\text{m}$ width. The measured ellipsoid shape was $113.4 \times 86.4 \mu\text{m}$ in muscle samples and $38.8 \times 31 \mu\text{m}$ in tongue samples. The spindle shape was observed only in a single diaphragm sample measuring $387.4 \times 148.8 \mu\text{m}$. The cyst wall was thin in elongated and spindle-shaped cysts, whereas it was thick in ellipsoid-shaped cysts (Figure 1).

Bradyzoites

Three morphological forms of bradyzoites were observed. The first form included banana-shaped bodies, measuring $15.5\text{--}29.6 \mu\text{m}$ in length ($20.62 \pm 5.84 \mu\text{m}$) and $3.5\text{--}18.5 \mu\text{m}$ in width ($10.46 \pm 7.0 \mu\text{m}$). Each bradyzoite had a pointed, arrow-like tip at one end and a rounded tip at the other end, producing a lancet-like shape (Figure 2A). The second form was sausage-shaped, measuring $9\text{--}26 \mu\text{m}$ in length ($17.64 \pm 6.87 \mu\text{m}$) and $7.2\text{--}13.2 \mu\text{m}$ in width ($9.86 \pm 2.3 \mu\text{m}$; Figure 2B). The third form of bradyzoites was spherical, with a diameter ranging from 7.8 to $16.5 \mu\text{m}$ ($12.34 \pm 3.7 \mu\text{m}$; Figures 2A and 2B).

Molecular results

The PCR assay yielded a specific amplicon of approximately 700 bp. Clear bands were observed in six samples, while the remaining two samples exhibited very weak bands (Figure 3). DNA fragments of approximately 655 bp were successfully obtained from all five isolates, and their sequences were subsequently deposited in GenBank under accession numbers PQ120680–PQ120684. Isolates 1 and 5 had identical nucleotide compositions, including 194 adenine (A), 113 cytosine (C), 153 guanine (G), and 195 thymine (T). Isolates 2 and 3 exhibited identical sequences with compositions of 194 A, 114 C, 152 G, and 195 T. Isolate 4 demonstrated minor variations in nucleotide composition, with 193 A, 114 C, 152 G, and 196 T. The five isolates exhibited a pairwise sequence identity of 99.69%. Phylogenetic

analysis of the 18S rRNA sequences revealed that all five *Sarcocystis* isolates obtained from camels in Kuwait clustered within the same clade as *Sarcocystis* sp. isolated from *Camelus dromedarius* in Iran (GenBank accession number GU074011; Figure 4). The BLASTn analysis of the 18S rRNA sequences against the GenBank database revealed that the *Sarcocystis* sp. from *Camelus dromedarius* in Iran (GU074011) had the highest similarity to the Kuwaiti isolates, with 69% query coverage and 98.4-98.67% sequence identity. In contrast, the sequences of *Sarcocystis* sp. from *Camelus dromedarius* in India (OP959654, OM462704, and OM462705) exhibited 68% query coverage and 95.11% sequence identity with the isolates of the present study.

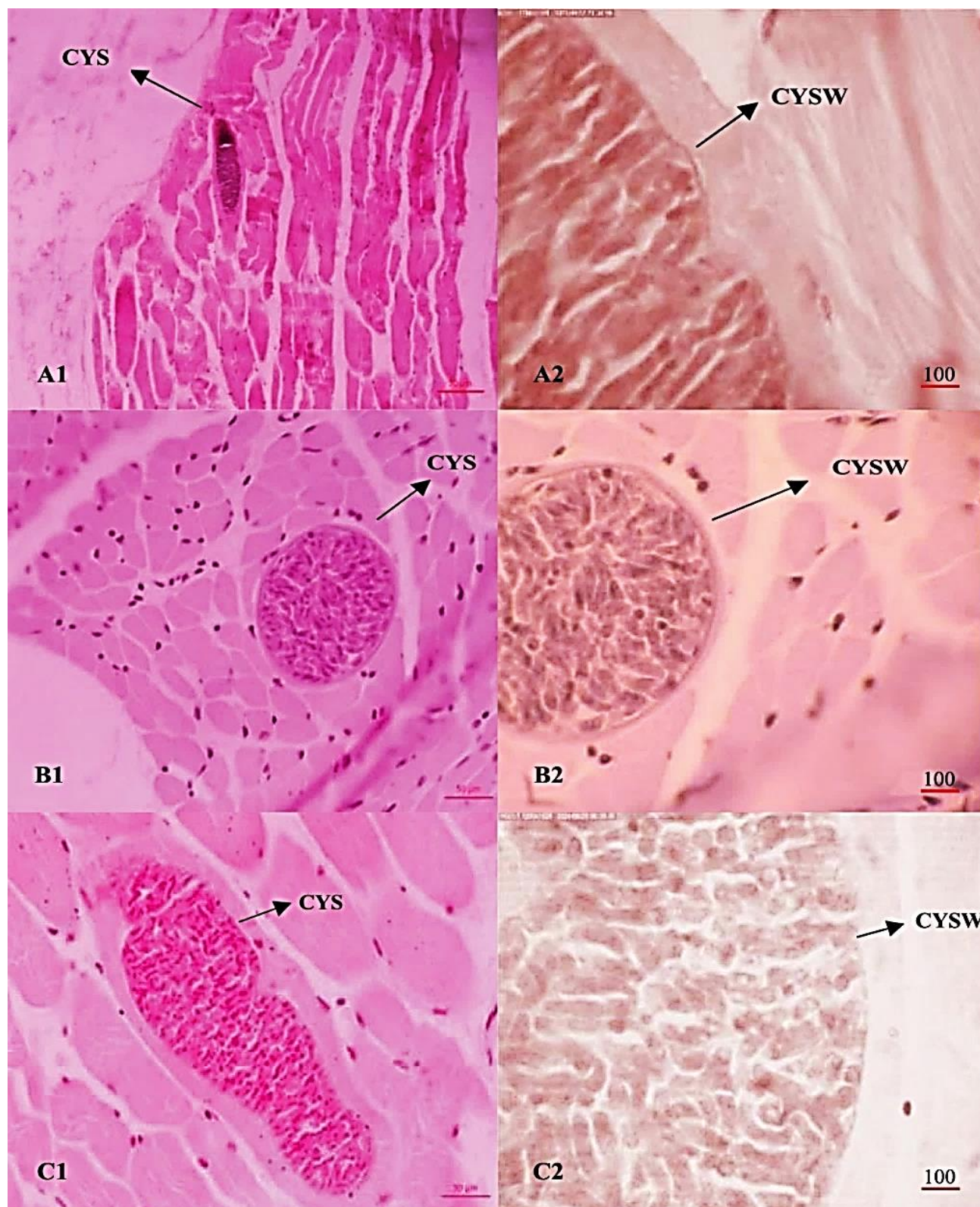


Figure 1. Muscle sections of *Camelus dromedarius* infected with *Sarcocystis* species showing different cyst types and their cyst walls. **A1:** Spindle-shaped cyst from the muscle tissue, **A2:** The cyst wall of A1. **B1:** An ellipsoid cyst from the tongue tissue, **B2:** The cyst wall of B1. **C1:** Elongated, irregular-shaped cyst from the diaphragm, **C2:** The cyst wall of C1. CYS: Cyst. CYSW: Cyst wall (100x magnification).

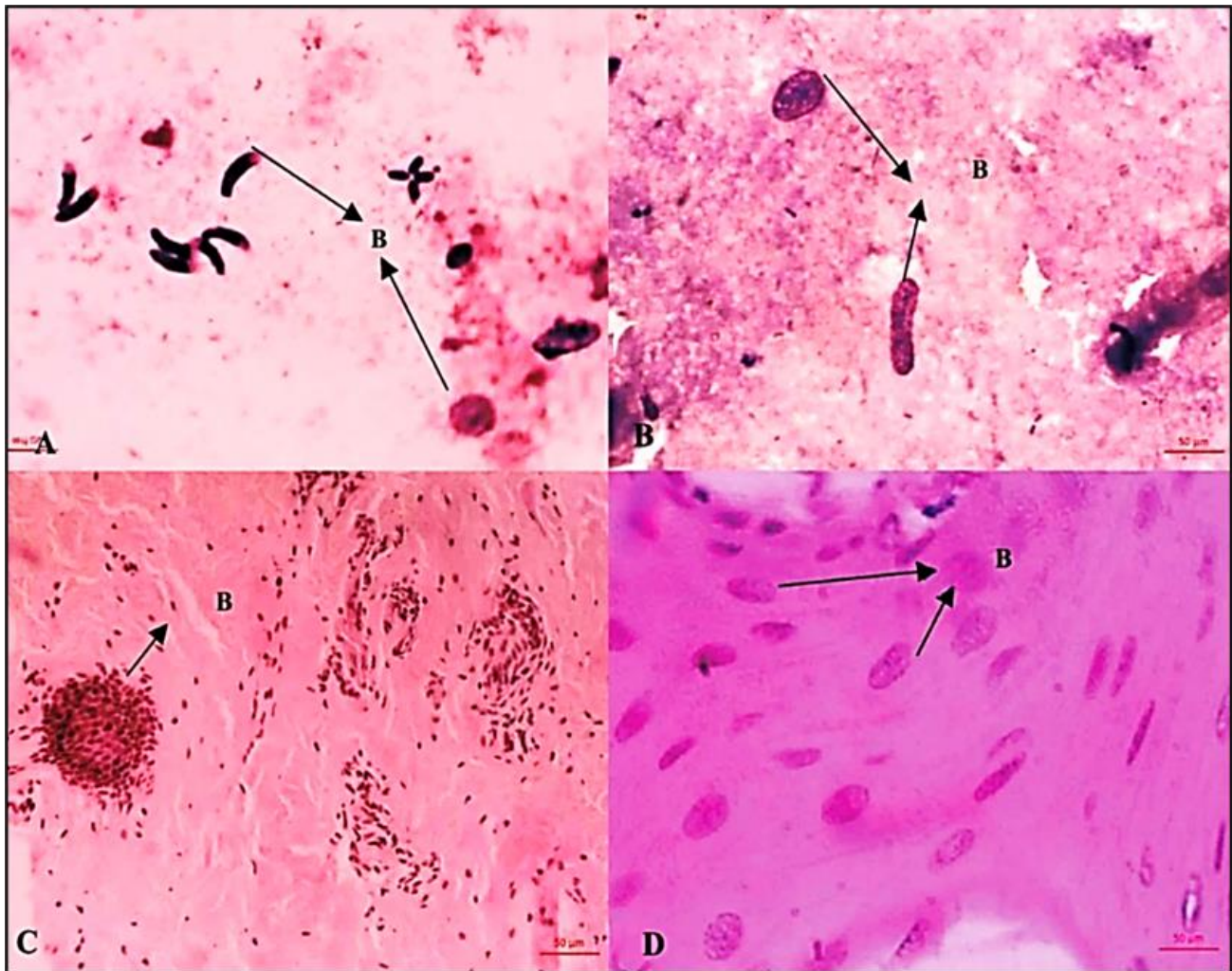


Figure 2. Pepsin digestion method used on muscles from *Camelus dromedarius* raised in Kuwait, with different types of bradyzoites. **A:** Banana and round-shaped bradyzoites, **B:** Round and sausage-shaped bradyzoites, **C and D:** Intensive distribution of bradyzoites, **B:** Bradyzoite.

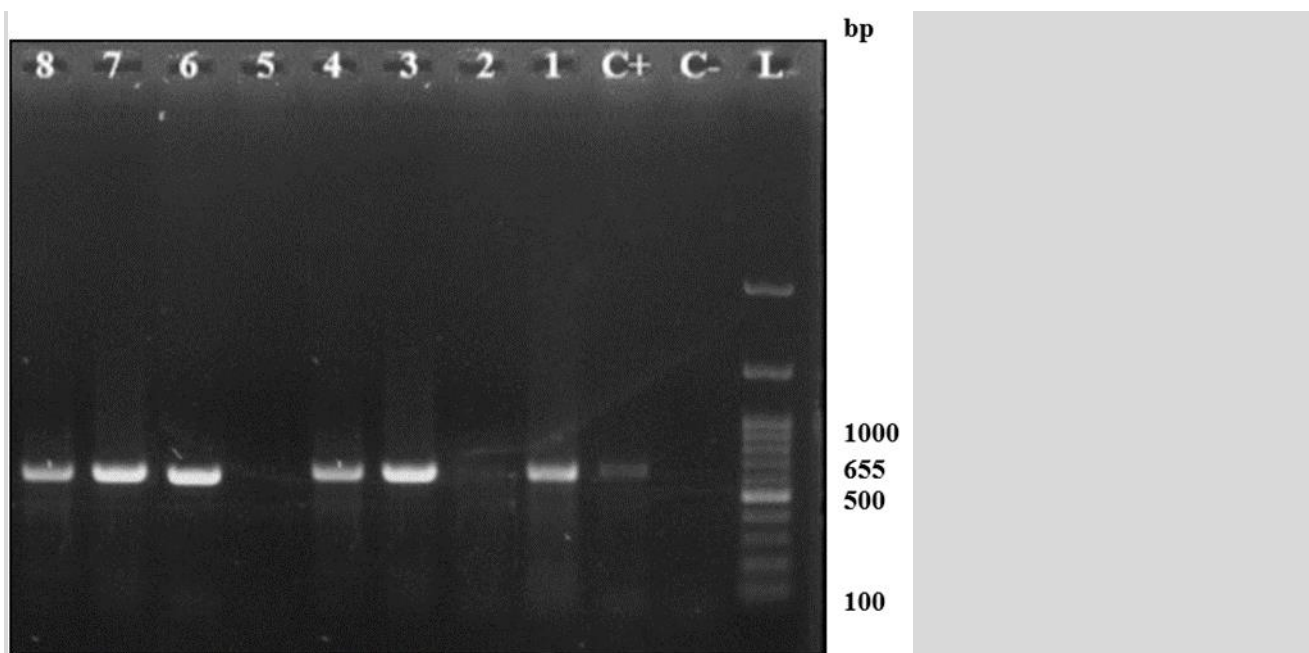


Figure 3. Agarose gel electrophoresis (1.5%) of 18S rRNA PCR products (700 bp) from *Sarcocystis*-positive tissues of *Camelus dromedarius*. Lanes 1 and 7: *Sarcocystis* DNA extracted from muscles, Lane 2: *Sarcocystis* DNA extracted from heart, Lanes 3 and 4: *Sarcocystis* DNA extracted from tongue, Lane 5: *Sarcocystis* DNA extracted from esophagus, Lane 6: *Sarcocystis* DNA extracted from diaphragm, Lane 8: *Sarcocystis* DNA extracted from esophagus of *Camelus dromedarius*, C+: Positive control, C: Negative control, L: Ladder 1000bp.

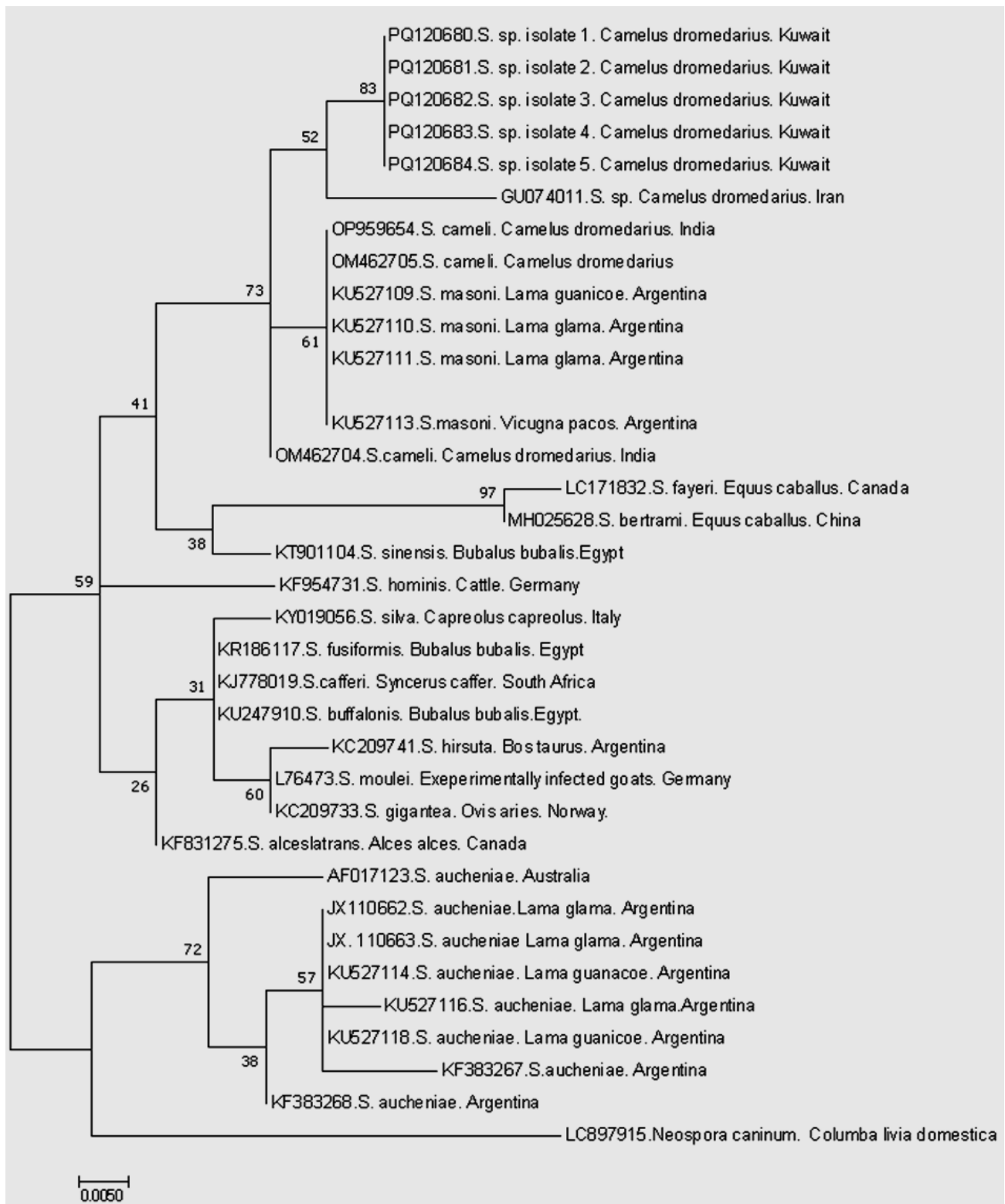


Figure 4. Maximum likelihood algorithm of the phylogenetic relationship between the obtained sequences (isolates 1-5) of *Sarcocystis* species and other sequences from GenBank (NCBI) based on 18S rRNA.

DISCUSSION

The present study is one of the initial investigations about the prevalence of *Sarcocystis* infection among camels in Kuwait. No macroscopic cysts were observed during the gross examination of the camel carcasses, in contrast to the findings reported previously in Iraq by [Dubey et al. \(2017\)](#).

In the present study, 194 of the 270 camels examined were positive for *Sarcocystis* infection by pepsin digestion, yielding a relatively high prevalence of 71.85%. This finding was consistent with the prevalence reported by [Gareh et al. \(2020\)](#). Additionally, high prevalence rates have been reported in previous studies using different diagnostic techniques,

including light microscopy, transmission electron microscopy, and histopathological examination (Valinezhad et al., 2008; Rabie et al., 2021). Enzymatic digestion methods, such as pepsin and trypsin, are reported to be more sensitive than muscle squash preparations or histological methods. This increased sensitivity might be due to their ability to examine larger tissue samples and effectively release bradyzoites from sarcocysts (Saeed et al., 2018a).

The prevalence of infection was higher in males (74.3%) than in females (62.5%), consistent with previous reports from Egypt (Gareh et al., 2020; Rabie et al., 2021). The higher number of infected male camels can be due to the higher number of males slaughtered and included in the study. The increased demand for tender camel meat could lead to more slaughtering of younger animals, particularly those aged between 8 months and 3 years. In the current study, camels aged between 8 months and 3 years demonstrated the highest infection rates (85.8%), indicating a notable association between age and *Sarcocystis* infection. In contrast, Shekarforoush et al. (2006) reported a higher prevalence among older animals, possibly due to longer exposure to infective oocysts or sporocysts.

Season was substantially associated with *Sarcocystis* infection, with a higher prevalence in the wet season (92.8%) than in the dry season (62.4%). This variation could result from differences in the number and traits of animals slaughtered across the two seasons. In Kuwait, the wet season is shorter than the dry season, which could influence the availability of animals for slaughter and affect the distribution of the examined samples.

Among the tissues examined, the esophagus had the lowest *Sarcocystis* infection rates, whereas the other tissues had relatively similar rates. Histopathological examination was useful for evaluating the size and shape of the sarcocysts. Saeed et al. (2018b) indicated that microscopic *Sarcocystis* cysts may differ in shape depending on the tissue, appearing as round, spherical, elongated, ellipsoid, spindle-shaped, or spiral forms. However, cyst shape alone is insufficient for species identification. Rabie et al. (2021) identified *S. cameli* and *S. ippeni*, exhibiting similar thin-walled cyst morphology. Factors including cyst age, location within the body, and tissue processing methods can affect the morphological features of sarcocysts. *Sarcocystis cameli* and *S. ippeni* can be differentiated based on the shape of their villar protrusions, which are finger-like at one end and conical at the other (Saeed et al., 2018a). Therefore, the morphology of the cyst wall is an important criterion for identifying and classifying *Sarcocystis* species. Dubey et al. (2015) stated that several species reported in camels, namely *S. camelicanis*, *S. camelocanis*, and *S. miescheri*, are invalid, with *S. camelicanis* considered similar to *S. cameli*.

Although sarcocystosis is generally considered asymptomatic or subclinical in camels, microscopic infection may be associated with pathological alterations. These pathological changes include pyrexia, anorexia, anemia, peripheral eosinophilia, hyperproteinemia, hyperglobulinemia, and hemorrhagic lesions affecting several organs, including the omentum, mesenteric lymph nodes, urinary bladder, myocardium, brain, lungs, and skeletal muscles. In the United States, eosinophilic myositis associated with natural *Sarcocystis* infection was reported in an alpaca that exhibited clinical signs including recumbency, hyperthermia, reduced responsiveness to external stimuli, dyspnea, marked muscle tremors, decreased milk production, and abortion. Furthermore, experimental infection of young alpacas with sporocysts resulted in reduced body weight gain and hematocrit levels compared to uninfected controls, along with a high mortality rate (Saeed et al., 2018 a,b). These observations indicated that microscopic *Sarcocystis* infection could have pathological implications under specific conditions.

It has been suggested that consuming raw or undercooked *Sarcocystis*-infected camelid meat may cause gastroenteritis due to exposure to sarcocyst-derived toxins, although no confirmed human cases have been reported. Experimental studies indicated that boiling, grilling, or frying infected meat inactivated the toxin, as assessed in rabbits inoculated with sarcocyst homogenates. In contrast, while charqui preparation prevented parasite transmission to dogs, it did not completely eliminate the toxic effects of sarcocysts in rabbits. Additionally, data on the impact of sarcocystosis on productivity parameters in Old World camels are scarce (Wieser et al., 2024).

Previous molecular investigations have used mitochondrial cytochrome c oxidase subunit I (*cox1*) sequences to detect *Sarcocystis* in Saudi Arabia (Metwally et al., 2020), Iraq (Aggar et al., 2023), and India (Narnaware et al., 2024). The 18S rRNA gene has been utilized to molecularly characterize *Sarcocystis* isolates from camels in Iran (Motamedi et al., 2011), Saudi Arabia (Omer et al., 2017), Egypt (Rabie et al., 2021), and India (Narnaware et al., 2024). In a recent study, Wieser et al. (2024) used 18S rRNA gene sequences to construct a phylogenetic tree that included *Sarcocystis* species from New World and Old World camelids. Wieser et al. (2024) found that *S. masoni* and *S. cameli* grouped within the same clade, clearly separated from *S. aucheniae*, which aligns with the present findings, as the Kuwaiti isolates clustered with *S. masoni*, whereas *S. aucheniae* appeared more distantly related. These results indicated a strong genetic connection between the isolates identified in the present study and those reported by Wieser et al. (2024) for *S. cameli* and *S. masoni*.

The 18S rRNA fragment obtained in the present study was 655 bp, compared with fragments of 539 bp and 610 bp reported in studies from India and Iran, respectively. Sequence identities between the Kuwaiti isolates and those from Iran ranged from 98.4% to 98.67%, indicating a strong genetic connection. The transportation and importation of camels

across countries can facilitate the spread of genetically related *Sarcocystis* isolates. However, the current data did not identify the exact source of infection.

The discrepancies in prevalence, histopathological findings, and molecular characteristics between the present study and those from other countries (Fukuyo et al., 2002; Valinezhad et al., 2008; Metwally et al., 2020) could be explained by several factors, including differing diagnostic techniques, geographical settings, tissue selection, age of animals, sample sizes, herd management, sanitation, and the occurrence of definitive hosts, especially dogs (Saeed et al., 2018a). These factors are important to consider when comparing the epidemiology and molecular characteristics of *Sarcocystis* infection across different camel populations.

CONCLUSION

A high prevalence of microscopic *Sarcocystis* infection in camels was detected by pepsin digestion, whereas no macroscopic cysts were observed during gross examination. Histopathological analysis characterized sarcocyst morphology. Molecular analysis of the 18S rRNA gene revealed that the Kuwaiti isolates were genetically closely related to previously reported *Sarcocystis* isolates, especially those in Iran. Since the 18S rRNA gene is relatively conserved among closely related *Sarcocystis* species, relying on this marker alone may not yield reliable species-level identification. Further studies incorporating additional variable molecular markers, such as *cox1*, alongside comprehensive morphological and ultrastructural characterization, are required to attain more precise identification of *Sarcocystis* species infecting camels in Kuwait.

DECLARATIONS

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Author's contributions

Ameen Ashour, Hoda Taha, and Wessam Youssef conceived, supervised, and designed the study. Hoda Taha wrote and drafted the manuscript. Adawia Henedi and Abdolhossein Dalimi conducted the investigation and performed the formal analysis. All authors reviewed and approved the final edition of the manuscript.

Availability of data and materials

The data supporting the findings of this study are accessible from the first and corresponding authors upon reasonable request. The nucleotide sequences of the 18S rRNA obtained have been deposited in GenBank under accession numbers (PQ120680-PQ120684).

Competing interests

The authors declared no potential conflicts of interest with respect to the study, authorship, and/or publication of this article.

Ethical considerations

The authors confirmed that the present study is original, has not been previously submitted for publication or under review elsewhere, and adheres to ethical standards regarding data integrity and the avoidance of plagiarism. The authors affirmed that no artificial intelligence tools were used in the writing and preparation of the manuscript. The authors accept full responsibility for the originality of the data both prior to submission and throughout the peer review process.

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