

# Traditional Identification of *Sarcocystis* Spp. in Slaughtered Camels in Al-Najif Province, Iraq

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## Abstract

**Background:** *Sarcocystis* is one of the most important intracellular protozoan parasites which infect many domestic animals, including camels as an intermediate host resulting to variable economic losses in particular in asymptomatic cases.

**Aim:** This study was aimed for traditional detection of *Sarcocystis* spp. in tissue samples of slaughtered camels in Iraq.

**Materials and methods:** Totally, 200 slaughtered camels (*Camelus dromedarius*) of different ages and sexes were selected from the abattoir of Al-Najaf province (Iraq) during October (2021) to July (2022). After slaughter, fresh tissue samples collected from different organs were subjected for macroscopic inspection and then for microscopic examination using of trichinoscopy, squeezing and acid pepsin digestion test.

**Results:** Although, no positive samples were detected by macroscopic examination; the findings of microscopic examination reported that the infection rates with *Sarcocystis* spp. using the trichinoscopy, acid pepsin digestion method, and squeezing were 49%, 72.5% and 56%, respectively. Furthermore, the major risk factors related to the development of sarcocystosis in camels detected that the prevalence rate of *Sarcocystis* spp. was significantly higher in esophagus and diaphragm than skeletal muscle and heart; older aged (>4 years) than younger (≤4 years) camels, and in females more than males.

**Conclusion:** This study demonstrated the widespread prevalence of *Sarcocystis* spp. in slaughtered camels in Al-Najaf, Iraq. Considering the facts that the infection rate is massive, the impacts of *Sarcocystis* on musculoskeletal function, feeding, health, and productivity are necessary to study, especially its economical importance in the future. Also, the role of camel in transmission of the parasite between domestic animal and possibly to human requires to furthermore studying.

**Keywords:** Sarcocystosis, Acid pepsin digestion, Trichinoscopy, Squeezing, *Camelus dromedaries*

## Introduction

*Sarcocystis* is one of the most prevalent protozoal infections in domestic animals which caused by Sarcocystidae family, Eucoccidiorina order of Apicomplexa phylum. The parasite was first

observed by the Swiss scientist Friedrich Miescher as milky white threads in skeletal muscle of a deer mouse (*Peromyscus*) in 1843; and then, these threads were named "Miescher's Tubules" (More et al., 2016; Strazdaitė-Žielienė et al., 2022). After 124 years later (1967), *Sarcocystis* was first described by electron microscopy as spindle- or crescent-shaped bodies (bradyzoites) similar to those seen in other apicomplexan protozoa like *Toxoplasma* and *Eimeria*. In the 1970s, bradyzoites isolated from the sarcocysts of bird muscles were inoculated in mammalian cells to detect the development of asexual stage to sexual stages and oocysts (Al-Hyali et al., 2011; Nahed et al., 2014; Asal and Al Zubaidy, 2016; Verma et al., 2017). In camels, there were six different names have been used to describe the main *Sarcocystis* species including *S. cameli*, *S. ippeni*, *S. camelicanis*, *S. camelocanis*, *S. miescheri* and *S. meischeri*. However, these studies showed that the taxonomy of *Sarcocystis* spp. of camels still debatable as a result of restricted collected samples and weak descriptions of structural features (Dubey et al., 2015a, b). To date, only sporocysts have been discovered in dog feces indicating that this animal act as a potential final host to infecting camel; however, the complete life cycle of *Sarcocystis* spp. in camels need to more investigation (Al-Khalidi et al., 1988; Omar and Hussain 2021; El-Mahdi et al., 2023). Additionally, distribution of *Sarcocystis* in intermediate hosts has been influenced by a number of variables including immunological condition of a host, quantity of oocysts and sporocysts consumed, and the species of *Sarcocystis* involved (Wernery et al., 2014; Dubey et al., 2015a). Microscopic examination recorded that the cysts can be found in heart, tongue, skeletal muscles such as masseter and limb muscles, esophagus, diaphragm, and other tissues causing typically asymptomatic or subclinical form of disease in camels (Valentine, 2017; Gareh et al., 2020). Due to lack of commercially available standard diagnostic test, asymptomatic or unspecific clinical signs of disease with existence of microscopic sarcocysts embedded deeply within the muscles make the diagnosis of acute sarcocystosis in camels is difficult (Al-Taie and Abdulla, 2011; Saeed et al., 2018). Therefore, many serological assays such as Agar-gel diffusion tests (AGD), complement fixation tests (CFT), hemagglutination inhibition tests (HIT), indirect fluorescent antibody tests (IFA), sabin-feldman dye tests, isoenzymes electrophoresis, western blots, direct agglutination tests (DAT), and enzyme-linked immunosorbent assay (ELISA) have been developed in the past three decades to determine antibodies against the parasite; however, standardization of these techniques is greatly challenged (Zimmerman and Crisman, 2008; Nageib and Kuraa, 2018; Aghwan et al., 2021). Post slaughter, a variety of macroscopic and microscopic diagnostic methods were used to detect the presence of sarcocysts in inspected muscles (El-Dakhly et al., 2011; Bayati, 2021). In Iraq, neither studies nor information are available about the prevalence *Sarcocystis* spp. in camels. Hence, this represent the first Iraqi study that aimed to investigate the prevalence of *Sarcocystis* spp. in slaughtered camels by the traditional methods, with detection correlation between the rate of positive infections and different risk factors (age, sex, months and organ).

## Materials and methods

### *Ethical approval*

The current study was licensed by the Scientific Committee of the Department of Parasitology in the College of Veterinary Medicine (University of Baghdad, Baghdad, Iraq).

### *Study animals and sampling*

Totally, 200 slaughtered camels (*Camelus dromedarius*) of different ages (< 2 years - ≤ 5 years) and sexes (107 males and 93 females) were selected randomly from the abattoir of Al-Najaf province (Iraq) during October (2021) to July (2022). Approximately, 100 grams of fresh tissue samples were collected from different organs (each esophagus, diaphragm, skeletal muscle and heart) of each slaughtered camel into individual plastic labeled containers for the macroscopic and microscopic examinations.

### *Traditional examination*

#### *Macroscopic inspection*

All fresh meat samples that collected from the esophagus, diaphragm, skeletal muscle and heart were tested by naked eye to detect the presence of sarcocysts in these organs (Gareh *et al.*, 2020).

#### *Microscopic examination*

1. Trichinoscopy: Small piece of each esophagus and diaphragm samples was crushed strongly between two glass slides and examined microscopically to detect the sarcocystes (Kamil and Faraj, 2020; Castro-Forero *et al.*, 2022).
2. Acid Pepsin Digestion Test: According to method described by different researchers, a total 20 grams of each muscle sample were crushed, digested in digestion fluid, incubated, filtered through gauze, and finally centrifuged. The sediment was smeared on a slide, stained by Giemsa stain, and examined microscopically (Mavi *et al.*, 2020).
3. Squeezing: This method was carried out using approximately 3-5 grams of each sample which ripped and pressed to extract the meat juice. A drop of meat juice of each sample was transferred on a slide, stained with Giemsa stain and examined microscopically to detect the presence of bradyzoites (Al-Saadi *et al.*, 2020).

### *Statistical analysis*

The findings of present study were analyzed using the GraphPad Prism version 6.0.1.298 (GraphPad Software Inc., USA) Software. Chi-square ( $\chi^2$ ) was applied to detect significant differences between values of traditional diagnostic methods; while, Odds ratio was applied to estimate statistical association between age, sex and month with the positive traditional. Values were represented as percentage (%), and statistical differences in obtained results were considered significant at  $P < 0.05$  (Gharban, 2023).

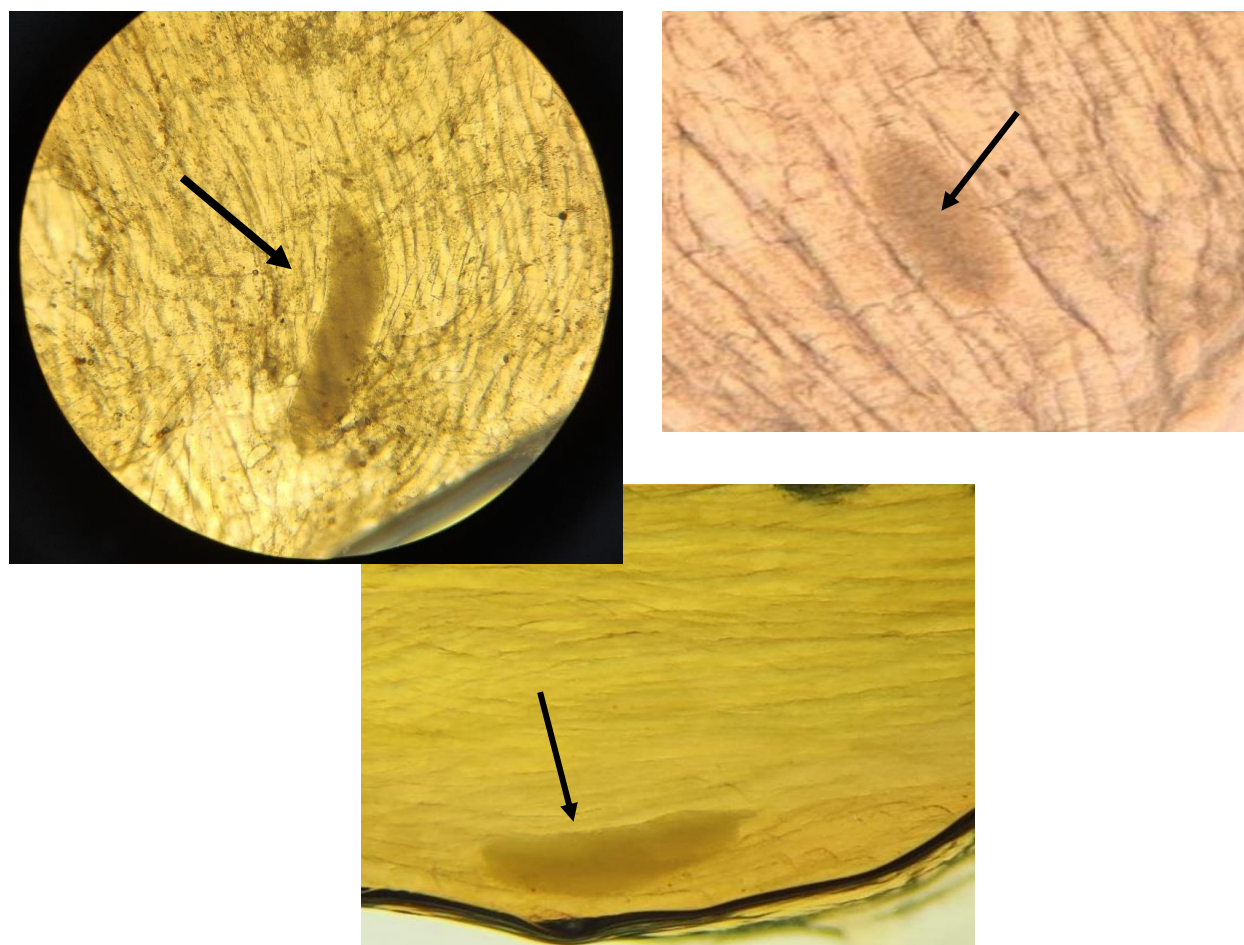
## Results

Among totally 200 meat samples collected from the slaughter camels, no positive samples were detected by macroscopic examination. However, the infection rate with *Sarcocystis* spp. using the acid pepsin digestion method was 72.5% (145/200) which significantly ( $P < 0.05$ ) higher than detected by trichinoscopy [49% (98/200)] and squeezing [56% (112/200)] (Table 1). The

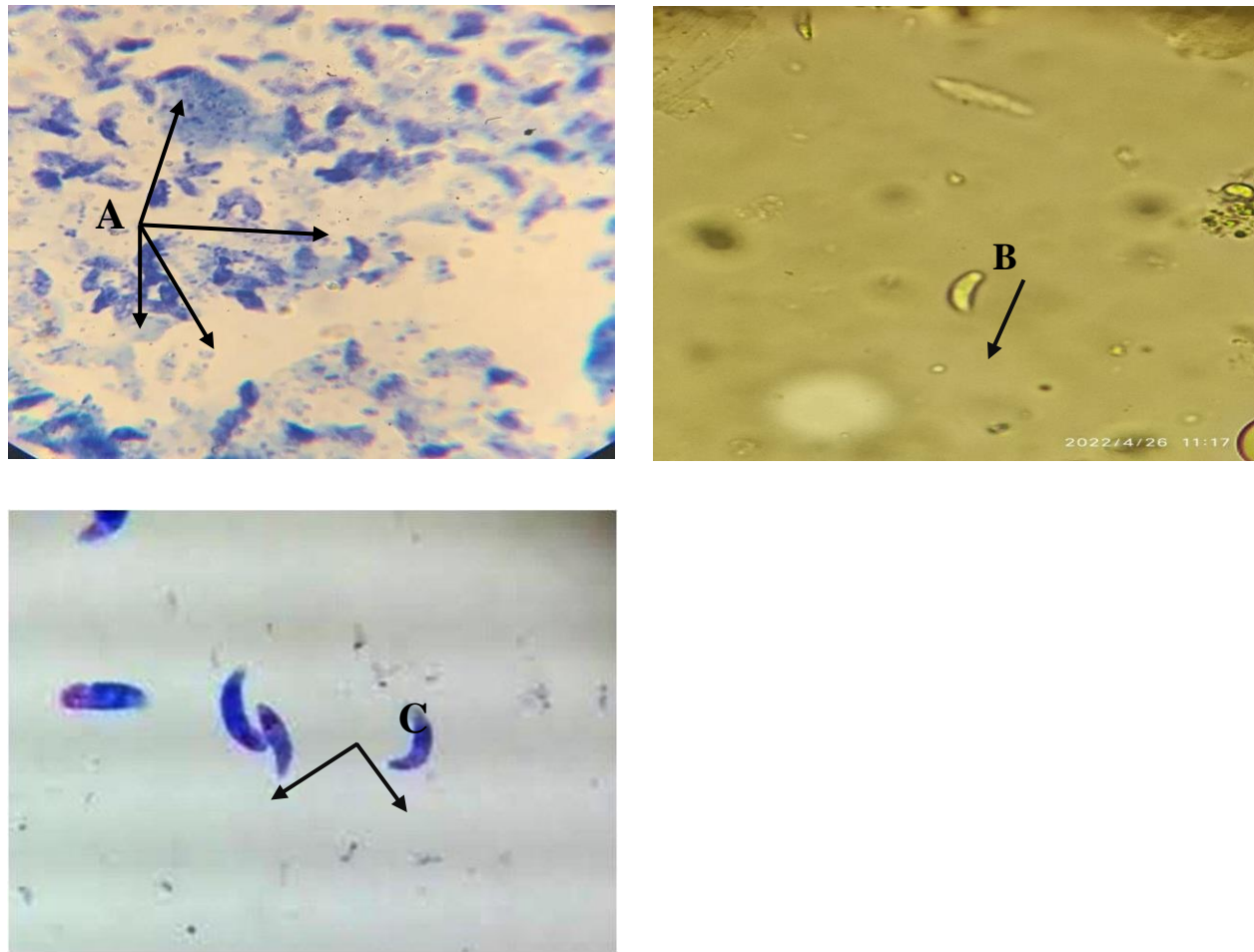
morphometric description of *Sarcocystis* spp. using the microscopic trichinoscopy technique revealed that the presence of elliptical form of the parasite in esophagus, which showed the cyst septa dividing the internal compartments that appeared as dark structures (Figure 1). Morphologically, bradyzoites (cystozoites) stained by the Giemsa stain were appeared having the characteristics of dark blue banana-shape with different sizes and little pointed anterior end with rounded posterior end. Nucleus was located near the last third part in close to the posterior end were not clear obviously (Figure 2).

**Table (4.1): Total infection rate of sarcocystosis in camel's meat by traditional methods**

Sample	Total No.	Trichinoscopy	Squeezing	Acid pepsin digestion
Meat	200	98 (49%)	112 (56%)	145 (72.5%)
Chi-Square ( $\chi^2$ )		6.153 *		
Significance * (P<0.05)				



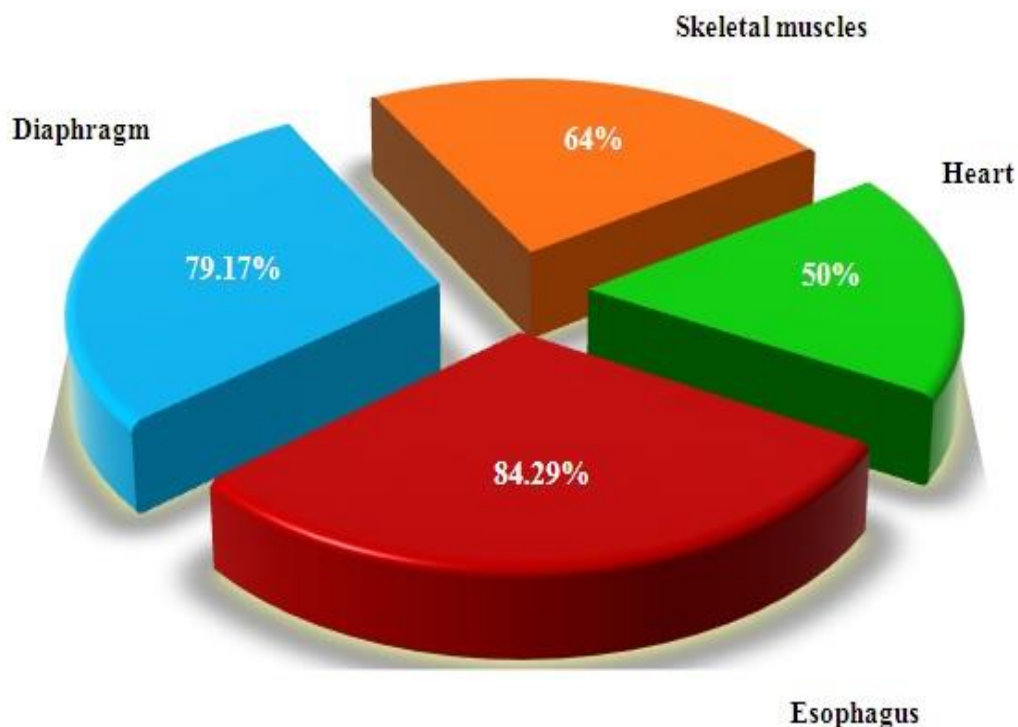
**Figure (1): *Sarcocystis* spp. in tissue samples of esophagus by trichinoscopy (10 X)**



**Figure (2): (A and B): Bradyzoites (Cystozoites) of *Sarcocystis* spp. in tissue samples stained with Giemsa stain; (C): Nucleus**

#### **B. Infection rate according to organ**

Microscopically, the high infection rate were showed in esophagus (84.29%) and diaphragm (79.17%) when compared to values of other organs; skeletal muscle (64%) and heart (50%), (Figure 3).



**Figure (3): Total infection rate of sarcocystosis in camels by traditional method according to organ**

### C. Infection rate according to age

Concerning age, camels aged > 4 years old were revealed significantly ( $P < 0.05$ ) a higher infection rate (89.6%) of *Sarcocystis* spp. than those aged  $\leq 4$  years old (44%), (Table 2).

**Table (2): Total infection rate of sarcocystosis in camels by traditional method according to age**

Age (year)	Total No.	Positives	
		No.	%
$\leq 4$	75	33	44
$> 4$	125	112	89.6 *
<b>Total</b>	200	145	72.5
Chi-Square ( $\chi^2$ )	-	-	5.793
Vertical comparison between values (%) refers to significant differences at ( $P < 0.05$ ) *			

### D. Infection rate according to sex

In comparison between both sex groups, significant prevalence ( $P < 0.05$ ) of sarcocystosis in camels was higher in females (76.67%) than males (69.09%), (Table 4.3).

**Table (3): Total infection rate of sarcocystosis in camels by traditional method according to sex**

Sex	Total No.	Positives	
		No.	%
Male	110	76	69.09
Female	90	69	76.67 *
Total	200	145	72.5
Chi-Square ( $\chi^2$ )	-	-	4.002
Vertical comparison between values (%) refers to significant differences at (P<0.05) *			

## Discussion

Among the most common parasites in domestic ruminants, *Sarcocystis* spp. can generate significant economic losses when causing clinical and subclinical disease. Up to now, at least five species of *Sarcocystis* have been named in camel (Dubey et al., 2015a). Macroscopic examination of collected tissue samples revealed that no gross lesions were observed in all tested sample tissues as reported by many studies (Valinezhad et al., 2008; Motamedi et al., 2011; Hamidinejat et al., 2013; Gareh et al., 2020), but in contrast with others (Latif et al., 1999; Rabie et al., 2021).

As shown in the present study, the prevalence of *Sarcocystis* infection in slaughtered camels in Al-Najaf province using the microscopic examination was 72.5%, 56% and 49% by acid pepsin digestion, squeezing and trichinoscopy, respectively. In comparison with the results of other previous and current studies, the prevalence rate of *Sarcocystis* spp. was 91.6% in Iraq (Latif et al., 1999); 47.3-66.3% in Afghanistan (Kirmse and Mohanbabu, 1986); 42.3% (Mandour et al., 2011), 75% (Gareh et al., 2020) and 85.5% (Rabie et al., 2021) in Egypt; 52.3% (Rahbari et al., 1981), 52.6% (Shekarforoush et al., 2006), 83.6% (Valinezhad et al., 2008) and 51.5% (Hamidinejat et al., 2013) in Iran; 6.18% in Jordan (Al-Ani and Amr, 2017); 100% in Mongolia (Fukuyo et al., 2002); 47.32% in Morocco (Kirmse 1986); 88.4% (Fatani et al., 1996); 41.3-50% (Omer et al., 2017) and 40.54% (Metwally et al., 2020) in Saudi Arabia; 82.5% in Somalia (di Sacco, 1989); 45.45% in Southern Ethiopia (Woldemeskel and Gumi, 2001); 81% in Sudan (Hussein and Warrag, 1985); and 50% in United Arab Emirates (El-Afifi et al., 1963). Differences between the reported infection rates could be attributed to various factors, including degree of contact between camels and dogs since some camel pastoralists are not using dogs in camel rearing (camels are reared on a free-range basis in the desert). Furthermore, different husbandry management systems, as well as diagnostic methods could influence the infection rate. However, the high infection rate in intermediate hosts is attributed to the fact that the farm animals are raised in close association with guard dogs which contaminate pastures with *Sarcocystis* sporocysts. Latif et al. (1999) mentioned that the infected dogs could shed about 200 million sporocysts during the course of infection. The sporocysts are infective already when passed in the feces and this factor plays an important role in the epidemiology of sarcocystiosis (Dubey et al., 2015a, b).



Analysis of results on distribution of *Sarcocystis* spp. in different organs showed that researches on camel have reported dissimilar tissue patterns. The results of this study that *Sarcocystis* spp. was found in all tested organ, but significantly more prevalent in esophagus and diaphragm are in consistent with that reported other studies as *Sarcocystis* spp. can infect usually the muscular tissue of the heart, tongue, esophagus, and diaphragm (Wahba et al., 2014; Ahmed et al., 2016). Some studies reported that sarcocystosis is more prevalent in the cremaster muscle of an animal with orchitis, which observation encouraged us to investigate sarcocysts in testicular tissue samples (Bucca et al., 2011; SAĞLAM and KELEŞ, 2016). Meanwhile, some studies found the diaphragm of camels to be the most commonly affected site (Al-Ani and Amr, 2017), whereas another study identified the heart as the most commonly infected organ (Shekarforoush et al. 2006). Oryan et al. (2010) seen that the predilection sites for *Sarcocystis* spp. appear to be the esophagus, tongue, and heart. Gareh et al. (2020) observed that dissemination of *Sarcocystis* in different organs was especially in the esophagus with a prevalence rate of 49%; whereas, Rabie et al. (2021) observed that *Sarcocystis* was found in esophageal, heart and ocular muscles, with a higher infection rate in esophagus (85.5%). Asopa et al. (2023) show the prevalence of *Sarcocystis* spp. in dromedary camels from Bikaner district of Rajasthan was in tongue and esophagus. However, variations in distribution of *Sarcocystis* among camel organs could be explained by different *S. cameli* strains or differences in definite host species (Hamidinejat et al., 2013).

Age was another significant risk factor associated with infection. In this study, infection rate was increased significantly in old camels (>4 years) when compared to the younger ones ( $\leq 4$  years). Similar findings were reported by other studies in Egypt (El-Bahy et al. 2019), Iran (Hamidinejat et al. 2013), and Saudi Arabia (Omer et al. 2017). The higher prevalence of *Sarcocystis* infection in aged camels may likely reflect the higher rate of slaughtering of aged camels compared with younger animals, slow development of detectable cysts may explain the lower prevalence in young camels (Valinezhad, et al. 2008, Hamidinejat et al. 2013, Omer et al. 2017). Additionally, some owners kept the young camels indoor for breeding, and therefore, the young camels might be less exposed to infection than older ones (Valinezhad, et al. 2008, Hamidinejat et al. 2013).

The sex of the animal found to be a significant variable associated with infection. Our results reported the higher prevalence of females compared to males, which is in constable with that seen by other many studies in southern Ethiopia (Woldemeskel and Gumi 2001), Iran (Valinezhad et al. 2008), and Egypt (Rabie et al., 2021) as the male camels being at higher risk of infection than females. Omer et al. (2017) male camels were less than two years old when they were slaughtered while females were over 4 years old, and the prevalence of *Sarcocystis* infections in older camels was much higher than that of younger camels and the same applies for sex as the prevalence of parasite in females was much higher than in males. This difference might be attributed to the fact that most female animals are kept indoor for reproduction under good and clean management, whereas most of the males are left for grazing outdoor and used by owners for hard work; they may therefore be more exposed to the infection (Romero et al. 2017). No significant difference in frequency of sarcocystosis between male and female camels was



identified by Hamidinejat et al. (2013). Lack of relationship between sex and infection rates has shown in similar studies on camels (Woldemeskel and Gumi 2001; Shekarforoush et al. 2006; Valinezhad et al. 2008).

### **Conclusion**

This study demonstrated the widespread prevalence of *Sarcocystis* spp. in slaughtered camels in Iraq. Considering the facts that the infection rate is massive, the impacts of *Sarcocystis* on musculoskeletal function, feeding, health, and productivity are necessary to study, especially its economical importance in the future. Also, the role of camel in transmission of the parasite between domestic animal and possibly to human requires furthermore studying.

### **Authors' contribution**

OAA: Collection of tissue samples, extraction of DNAs and preparation of Mastermix tubes; MTSA: PCR analysis, sequencing and statistical analysis of obtained results. All authors have written of the manuscript and approved on the final copy of it.

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### **Competing interests**

No

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