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Study on *Sarcosystis* Sp. By Light and Electron Microscopy in Camel Muscles at Assiut Governorate

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Abstract

Aims

It is the 1st time to study *sarcosysts meisheri* of camels in Assiut Govemorate; throughout the study different parts from camels in different locations were examined for *Sarcosystis meisheri.* (Abdominal, esophagus and tongue muscles).Therefore, the present study aimed to investigate the prevalence of *Sarcocystis meisheri*. Infection among slaughtered camels in Assiut, Upper Egypt and to examine the ultrastructural characteristics of the *sarcocystis meisheri* inside the different muscles of camels.

Main methods

Random samples of different regions of camel muscles (0esophagus, tongue and abdominal region) were collected for examination of any *Sarcocystis meisheri* in muscles.

Key findings

One hundred and ninety five (195) specimens were examined. Only one hundred and eight (108) were found to be infected (55.5%).The infectious rates was highest in esophagus (62%), followed by abdominal muscles (52.2%) and tongue muscles (51.25%).

Significance

Generally the infectious rate with the parasite was higher in males than in females. Morphological and ultrastructures of muscle stage (sarcocyst) have been reported and illustrated in this study by both light and electron microscope. TEM showed that there are two types of *Sarcocystis meisheri* in camels in Assiut Governorate.

The cyst consists of two types of cells these are metrocytes and merozoites and contained a large numbers of micronemes, rhopries, conoid and and mitochondrial organelles.

Keywords: Metrocytes; Merozoites; Micronemes; Rhoptries; Sporozoite; Mitochondria; Ground substance; *Sarcocystis*

Introduction

Sarcosystis cysts were first described by Miescher as early as 1843 and first named by Lankester [1] however; their coccidian life cycle as members of Phylum Apicomplexa was first established by Hedron and Rommel [2]. An obligatory heteroxenous life cycle was elucidated. The genus *Sarcosystis* comprises 130 species with differences in life cycle,pathogenicity and represents important members of the cyst, forming coccidia. Many reports on *Sarcosystis* infections among different vertebrates including even man were recorded [3-8]. *Sarcosystis* species are common parasites that infect many skeletal muscles in mammals, birds and reptiles.

Sarcosystis is a known parasite of considerable veterinary economic and public health importance. Intermediate hosts become infected when they ingest oocysts or sporocysts [9]. The parasite produces tissue cyst in muscles of intermediate host. The muscle cysts may be macroscopic or microscopic in size. Rahbari et al. and Shekarforoush et al. [10,11] examined the camel muscles by impression smear method, and they reported infection rates of 52.6% and 52.3%, respectively Valinezhad et al. examined the muscle by histopathological method found only microcysts in camel muscles and reported infection rates of 83.6% [12]. Eating raw or undercooked beef and pork containing mature cysts of *S. hominis* and *S. suihominis*, respectively has resulted in humans acquiring intestinal *Sarcosystis* and this coccidian parasite causes intestinal and muscular *sarcocystosis* in immunocompetent patients [13]. So that Seid, et al. studied the determination of the prevalence in cattle in Kerman, Iran [14]. *Sarcosystis* has been reported in goat from various countries such as

Iran [15], India [16,17] , Slovakia [18],China [19], Ethiopia [20],Nigeria [21], Sudan [22] and Jordan [23] so that, *Sarcosystis* infection in slaughtered goats in Kerman, Iran., was studied by Mohammed et al. [24].

In recent years, molecular technique has been applied for *Sarcosystis* characterization isolated from different animals by some investigators [25-32]. As a noticeable point there is no molecular report conducting camel *Sarcosystis* in the world [33] had been studied full description and distinguish the *Sarcosystis* species isolated from camel by studying the ultrastructure of the cyst wall by electron microscopy and combining these data with information on DNA sequence and restriction fragment length polymorphism (PCR-RELP) characters. Mandour et al. had studied the ultrastructure of *Sarcosystis meisheri* that infect camels in Qena Governorate and the oocysts passing with feces of experimentally infected dogs [34].

Therefore, the present study aimed to investigate the prevalence of Sarcocystis meisheri. Infection among slaughtered camels in Assiut, Upper Egypt and to examine the ultrastructural characteristics of the sarcocystis meisheri. inside the different organs of the camels.

Material & Methods

Random samples of different regions of camel muscles (oesophagus, tongue and abdominal region) were collected for examination of any Sarcocystis in muscles. The infected muscles were subjected to the following procedures.

Preparation of whole mounts (trichinoscopy)

Pieces of the infected muscles of about (0.5 × 0.5 cm) were compressed between two slides to elucidate the cysts to be stained with glacial acetic acid allum carmine.

Preparation of paraffin sections

Pieces of the infected muscles of about (0.5 × 0.5 cm) were taken and fixed in 10% formal saline.

Transmision Electron Microscopy (TEM). Knoll 1932

Camel muscles which were highly infected with *Sarcosystis* sp. immediately fixed in 3 ml. of 3% glutaraldehyde solution in phosphate buffer (PH 7.2), for 24 hr and Kept at 40°C in refrigerator. The samples were post fixed in 1% Osmium tetroxide in phosphate buffer (PH 7.2, 300 mom), for 30 minutes. They were washed several times with phosphate buffer solution.

The samples were then embedded in Epon which can preserve in structure from distortion during processing then ultra-thin sections were cut by an Ultra microtome and examined by JEOL, 100 CXII operating at 80 KV (TEM).

Results

Only microscopic *Sarcosystis meisheri* were observed in the examined camels muscle. *Sarcosystis meisheri* measured (179 µm - 2.5 |mm) in length and (70-112.5 µm) in width in whole mount specimens as in Figures 1 and 2 and in transverse sections as in Figures 3 and 4.

The incidence of the infection rates in different parts of both sex (males & females) were shown in table 1 and histogram 1. Some photomicrographs showed the presence of more than one cyst in microscopy field Figure 5.

The cysts located within muscle fibers were surrounded by a spiny primary cyst wall which was thickness.

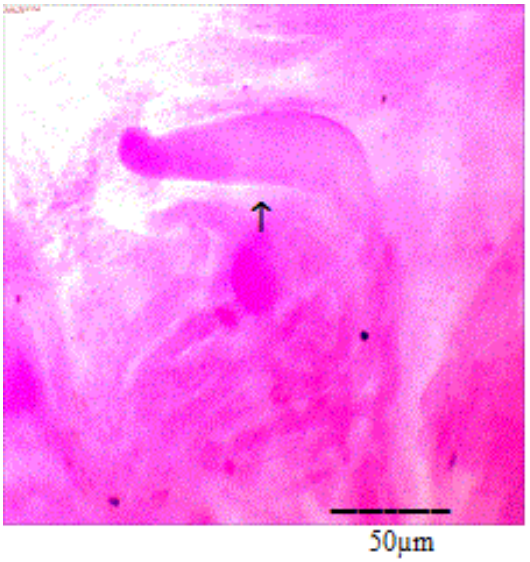

Figure 1: Photomicrograph showing Sarcocystis cyst (arrow) in skeletal muscle of Camels stained with aceto-acetic alum carmine x bar=50 µm.

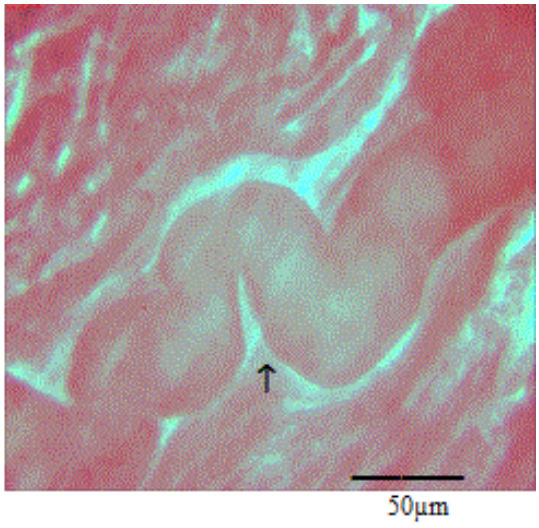

Figure 2: Photomicrograph showing spiral shaped Sarcocystis cyst (arrow) in muscles stained with aceto- acetic alum carmine x bar=50 µm.

Organ or part of muscles	Esophagus		Abdominal muscles		Tongue muscles	
	Males	Females	Males	Females	Males	Females
No. of examined specimens	159	36	159	36	159	36
No. of infection specimens	109	20	100	15	88	17
Percent of infection rate in each sex	68.50	55.50	62.80	41.60	55.30	47.20
Total	62		52.20		51.25	

Table 1: Showing the infection rate with *Sarcosystis* sp. in different muscles in both males and female camels.

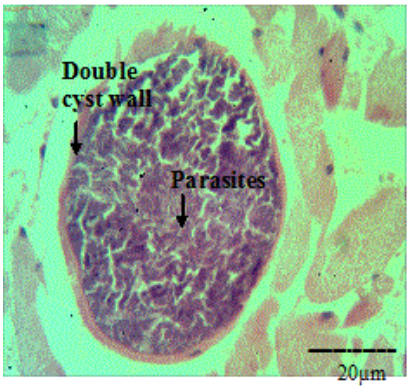

Figure 3: Photomicrograph showing cyst with double wall and divided into compartments filled with many parasites in muscles stained with H & E. x bar=20 µm.

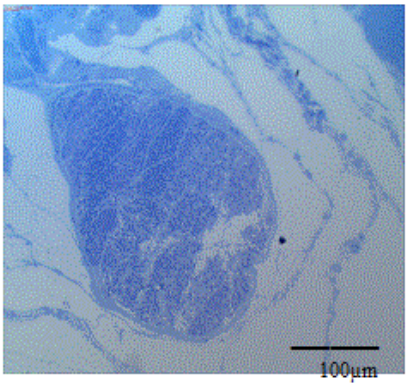

Figure 4: Photomicrograph showing cyst of Sarcosystis in muscles stained with toluidene bluexbar=100 µm.

Microscopic study of *Sarcosystis meisheri*

The microscopic *Sarcosystis meisheri* are elongated in shape as shown from the whole mounts, or the sections that stained with H & E. stain Figure 6.

The cyst wall

By using the light microscope: Cysts were bounded by a thick primary cyst wall which has numerous projections that appear to be spiny with pointed ends and some were spiny with blunt end Figure 7.

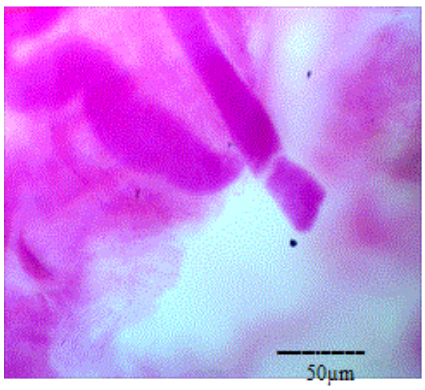

Figure 5: Photomicrograph showing two cysts of Sarcosystis in the same field in muscles stained with aceto-acetic alum carmine x bar=50 µm.

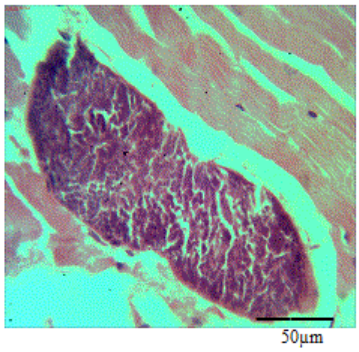

Figure 6: Photomicrograph showing whole mounts elongated shape of Sarcosystis cyst in muscles stained with H & E. x bar=50 µm.

The parasites: The interior of the cyst is occupied by a large number of parasites which are of two types, these are metrocytes and merozoites. The metrocytes were ovoid or irregular in shape usually

located beneath the cyst wall measuring about (4.5-7.5 µm) in length and (3.7-4.5 µm) in width. The merozoites were banana shaped with one end more pointed than the other; the nucleus was located centrally or shifted towards the less pointed end, these measured (20.95-32 (im) in length and (7.5-14.2 µ m) in width.

Transmission electron microscope: Examination of ultrathin sections of different cysts showed that, the primary cyst wall appeared as a thick electron dense layer and two types of cyst walls were described as follow:

Cysts showed small papillae, projecting outwards from the small cyst wall as in figure 8 or villi-like protrusions within which fine fibrils and also presence of schizont in parasitiphorous vacuole as in Figure 9.

Cysts showed italic spine-like protrusions more or less blunt apex, the core of these protrusions contained longitudinally extended microfibrils throughout the whole length of the protrusion appeared as they originated from the ground substance as showed in Figure 10.

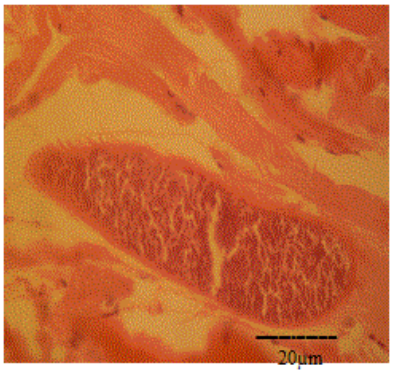

Figure 7: Photomicrograph showing spiny cyst wall of Sarcosystis in skeletal muscles of Camelus dromedarius stained with H & E. x bar=20 µm.

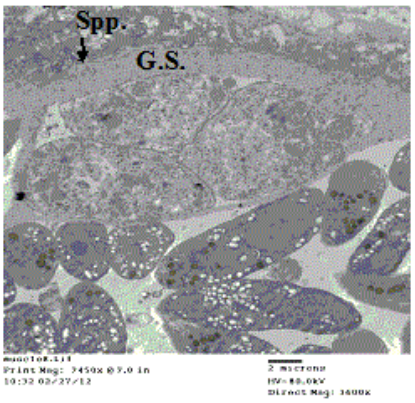

Figure 8: Transmission electron micrograph of cyst (sarcosystis sp.) showing small papillae (Spp) and ground substance (G.S.) x bar=2 µm.

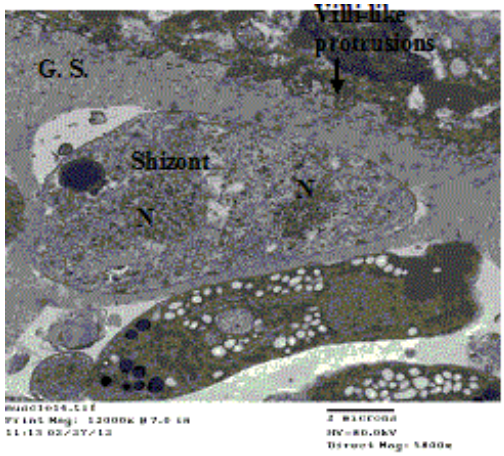

Figure 9: Transmission electron micrograph of cyst (Sarcosystis sp.) showing villi-like protrusions and schizont with more than nucleus (N) in parasitiphorous vacuole x bar=2 µm.

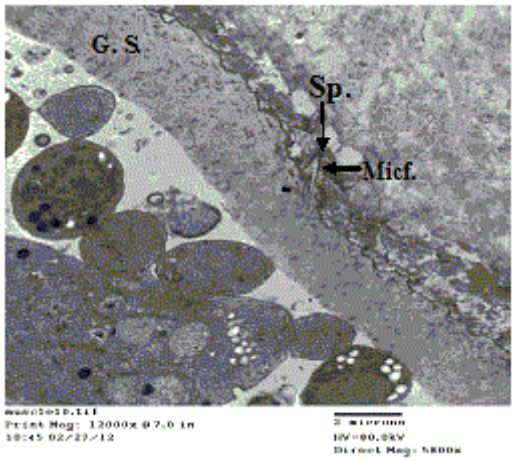

Figure 10: Transmission electron micrograph of cyst (Sarcosystis sp.) showing spine-like (sp.) protrusions and microfibrils (Micf.) appeared as they originated from ground substance (G.S.) x bar=2 µm.

The ground substances are located directly underneath the primary cyst wall and extend into the interior of the *Sarcosystis* forming numerous septa that divide the interior of the cyst into numerous chambers containing the cyst parasites. The ground substance consists mainly of fine dense homogenous granules and some fibrillar elements. The cyst parasites are usually differentiated into metrocytes, which are mostly located at the periphery of the cyst and directly underneath the ground substance, and the cyst merozoites, which are usually, fill the interior of the cyst Figure 11.

The metrocyts are globular to ovoid shaped. These stages were bordered by a clearly visible double membranous pellicle and showed inside remnants of the apical complex represented by a number of micronemes and dense bodies. Cell division resulted in the formation of two daughter cells figure 12. Large numbers of elongated cyst

merozoites, which were provided with all characteristics of Apicomplexa, were detected. Each cyst merozoite was characterized at the anterior end by a typical pellicle, conoid, rhoptries and micronemes. The micronemes appeared somewhat elongated in longitudinal sections but rounded in cross sections occupying the anterior third of the whole body Figure 13. besides that, large numbers of osmiophilic dense bodies, conoid and many amylopectin granules were found. Furthermore, there were two pores observed in the present study, one of them was at the anterior and the other was at the anterior third of the body Figures 14 and 15 respectively.

The sporozoites of *Sarcosystis* are elongated in shape and measured (14.11 × 3.5 pm) in size, with a subterminal nucleus and there is abundance of micronems, rhoptries, endoplasmic reticulum, dense granules and amylopectin granules but there is no crystalloid bodies or any retractile bodies as in Figure 16. The comparison between *Sarcosystis* sp. in the present study and Mandour et al. [35] shown in Table 2.

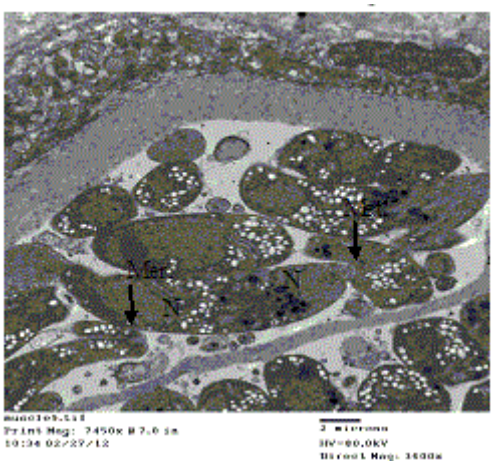

Figure 11: Transmission electron micrograph of cyst showing metrocyt (Met.) and merozoites (Mer.) x bar=2 µm.

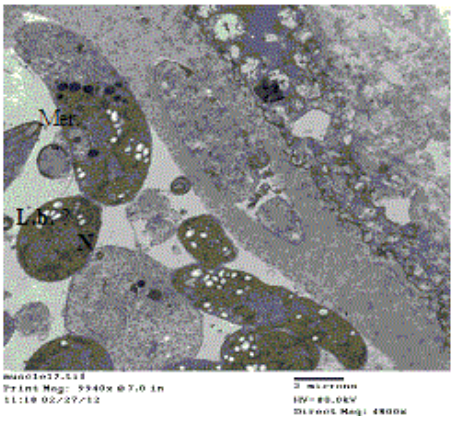

Figure 12: Transmission electron micrograph of cyst showing lipid body (L.b.) and cell division resulted in the formation of two daughter cells (D.C.). of merozoites (Mer.) x bar=2 µm.

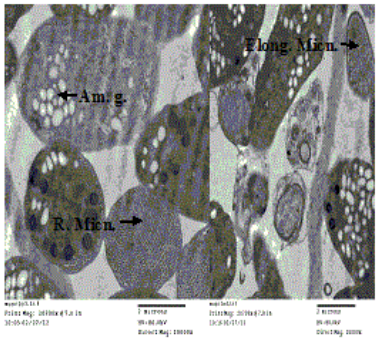

Figure 13: Transmission electron micrograph of cyst showing elongated and rounded micronemes (Elong, R. Micn.) and amylopectin granules (Am.g.) x bar=2 µm.

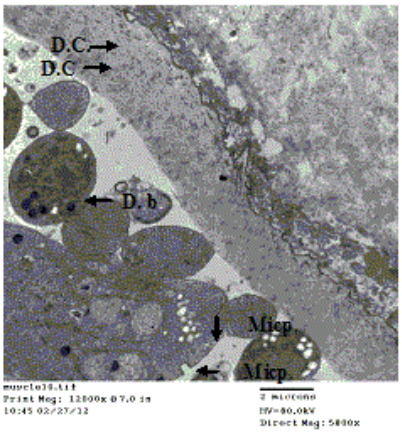

Figure 14: Transmission electron micrograph of cyst showing two micropores (Micp.) and dense bodies (D. b.) x bar=2 µm.

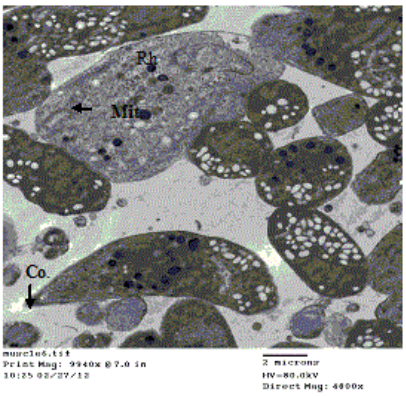

Figure 15: Transmission electron micrograph of cyst showing Rhoptry (Rh.), Conoid (co.) and Mitochondria (Mit.) x bar=2 µm.

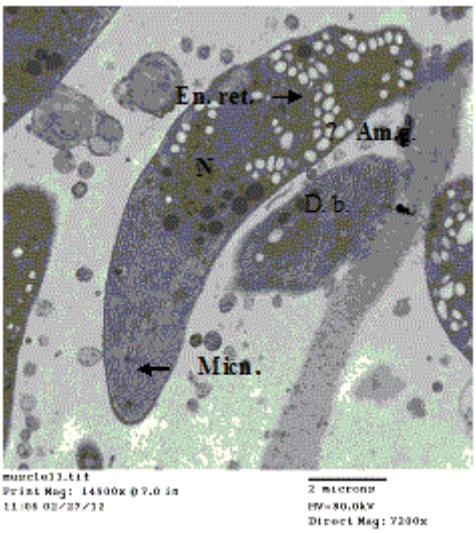

Figure 16: Transmission electron micrograph of Sporozoit of Sarcoststis showing Nucleus(N), micronemes (Micn.), dense bodies (D. b.), amylopectin granules (Am.g.) and endoplasmic reticulum (En.ret.) x bar=2 µm.

Discussion

Sarcosystis is worldwide in distribution. Parasites belonging to this genus have been reported from numerous mammals, especially sheep, cattle, buffaloes and pigs. Mandour et al. reported that, the infection rate with *Sarcosystis* sp. of camels at Qena Governorate was 41% [35]. In the present study, the infection with the present parasite in different muscles (esophageal, abdominal and tongue) to be the most common sites for *Sarcosystis* infection and the result of infection were (62%.) in esophagus, (52.2%) in abdominal muscles and (51.25%) in tongue. The rate of infection was recorded (100%) in sheep and cattle in the United States [36] and a high frequency of infection (91.6%) was also reported in goats in Sudan [37] and Argentina [38].

The ultrastructural characteristic of both cyst metrocytes and merozoites in the present work are similar to those described for many other *Sarcosystis* species. The merozoites in the present study resembled that the reported by Fatani et al. [20] and Mandour et al. [34] in shape where both of them were banana shaped with one end more pointed than the other. The nucleus located centrally or shifted towards the less pointed end, but their dimensions are different where in the present study it was longer and wider.

Mandour stated that, the structure of the wall of *Sarcosystis* cyst is the most important feature used in species identification [35]. Also David et al. stated that the structure of the *Sarcosystis* wall is the most important feature used in species identification. Light microscopic examination can be used to distinguish some species within an intermediate host, although examination with transmission electron microscopy (TEM) gave fine details for cyst wall of the parasite (spiny cyst wall, micro fibrils and ground substance) [18].

By comparing the present study with all the previous results was suggested that, this species in the present study is similar to the species reported by Mandour et al. [35,39] in Qena with some differences Table 2. So that, from the comparison between the two species in the last table the present species is *Sarcosystis* miescheri but Assuit I a new locality to its appearance for the first time in the governorate.

Sarcosystis sp.	Mandour, et al. [35]	The present work
Habitat	Esophageal muscles of camels at Qena.	Esophageal, Abdominal and Tongue muscles of camels at Assiut.
Cyst size	Length 229.0 µm – 2 mm. Width 77.76 - 137.85 µm.	179 µm - 2.5 mm. 70 - 112.5 µm.
Metrocytes	Length 4.1 – 7.3 µm Oval shaped. Width 3.5 – 4.8 µm.	4.5 – 7.5 µm Globular to ovoid 3.7 – 4.5 µm shaped
Merozoites	Length 21.56 -32.88µm. Banana shaped Width 7.76 - 17.76 µm.	20.95 - 32 µm. Banana shaped 7.5 - 14.2 µm.
Sporozoites	Not mentioned	(14.11 × 3.5 µm.) In size.

Table 2: Comparison between *Sarcosystis* sp. in the present work and Mandour et al. [35].

Conclusion

Results of the present study showed that a very high frequency of *Sarcosystis meisheri* Infections in camels slaughtered in Assiut, Egypt. Further investigations to determine in better detail the biology. Epidemiology, Life cycle, Ultrastructure and molecular differences of different species of *Sarcosystis meisheri* in Egyptian camels highly recommended.

A Conflict of Interest declaration

The authors declare no conflicts of interest.

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