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Morphological and molecular characterization of *Sarcocystis cameli* and *Sarcocystis ippeni* from the muscles of one-humped Camel (*Camelus dromedarius*) in New Valley governorate, Egypt**Soheir A. Rabie^{1*}, Reda M. El-S. Hassanine², Amal A. Hassan³, Obaida F. Abo Elhussien¹,
Mohammed B. M. EL-Mahdi¹**¹Department of Zoology, Faculty of Science, South Valley University, 83523 Qena, Egypt,²Department of Zoology, Faculty of Science, New valley University, EL-Kharga, 72511 Egypt,³Department of Zoology, Faculty of Science, Damnhour University, Damnhour, 22511 Egypt.**Abstract**

Sarcocystis spp. are cysts-forming coccidian parasites which infect several animals including camels as intermediate hosts. The present study was designed to study the *Sarcocystis* infection in camels (*Camelus dromedaries*) at morphological and molecular levels. Samples of the esophageal, heart and ocular muscle tissues were collected from infected camels in El-kharga, New Valley Governorate, Egypt. Samples were examined for *Sarcocystis* spp. infection using macroscopic evaluation, light microscopy (LM), transmission electron microscopy (TEM), and molecular analysis. LM examinations revealed 106 of 124 muscle samples (85.5%) were positive for *Sarcocystis*. Two species *Sarcocystis cameli* and *Sarcocystis ippeni* were recognized. In light microscopy, *Sarcocystis* were thin walled with barely visible projections. Using the TEM, the two structurally distinct *Sarcocystis* were recognized by unique villar protrusions (Vp). *Sarcocystis* of *S. cameli* had Vp of the type 9j. The *Sarcocystis* wall had upright slender Vp, up to 0.1-0.4 μm long and 0.05-0.1 μm wide. On each Vp, Rows of knob-like protrusions were observed to be interconnected. *Sarcocystis ippeni* had "type 32" *Sarcocystis* wall have been characterized by conical Vp with an electron dense knob. The Vp were approximately 175.27-266.76 nm long and 100.45-175.98 nm wide. In each *Sarcocystis*, the Vp had microtubules (Mt) that originated at midpoint of the ground substance (Gs) and continued up to the tip. Molecular data revealed the amplification of partial fragments of the 18S rRNA gene (~600 bp). The digestion analysis of obtained PCR products using Restriction fragment length polymorphism (RFLP) method utilizing (Mbo1) appeared 2 bands approximately 250 and 350 bp for each *Sarcocystis*. Molecular analysis demonstrated the ability of 18S rRNA gene for distinguishing *Sarcocystis* species in studied animals and to be used as molecular marker.

Keywords: *Camelus dromedaries*, EL-Kharga, Restriction fragment length polymorphism (RFLP), *Sarcocystis cameli*, *Sarcocystis ippeni*, 18S ribosomal gene.

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Introduction

Sarcocystis spp. are among the most important parasites affecting mammals and of considerable veterinary economic and public health importance. Camel as intermediate host becoming infected after ingestion of sporulated oocysts passed in faeces of the final host. The high prevalence of *Sarcocystis* infection has a significant economic impact on the production of domestic animals. They can cause anorexia, fever, anemia, muscle weakness, abortion and even death in intermediate hosts, as well as reducing meat quality and milk yield. Although more than 150 species of *Sarcocystis* were described in various animals, only a few data on camel *Sarcocystis* ultrastructure and their cyst wall details were published (Motamedi et al., 2011). The first study of *Sarcocystis* infection in camels (*Camelus dromedarius*) from Egypt reported in 1910 where *Sarcocystis* were less than 12 mm in length, 1 mm in width, and appeared to be white lines with thin or thick cyst walls and identified as *S. cameli* (Mason, 1910). Hilali and Mohamed (1980) observed that 63.6% of camels slaughtered in Egypt infected with microscopic *Sarcocystis cameli* cysts. The structure of thick- and thin-walled in *Sarcocystis* was confirmed in infected camels from Saudi Arabia (Fatani et al. 1996) and Somalia (Borrow et al. 1989). Abdel-Ghaffar et al. (2009) studied the prevalence of *Sarcocystis* infection in camels in Cairo, Egypt, microscopic *Sarcocystis* found in 116 of 180 samples, were $120\text{--}170 \times 50\text{--}100 \mu\text{m}$ in size and of one morphological type, also, in that study dogs that were fed infected camel meat excreted *Sarcocystis* sporocysts, the larger sporocyst in the dog assigned as a new species: *Sarcocystis camelocanis* with no description. In Sudan, Ishag et al. (2001 and 2006) studied the transmission of *Sarcocystis* between camels and dogs and they observed two species of *Sarcocystis*, thick- and thin –

walled, in camel fed sporocysts from dogs (Ishag et al. 2001) and two different sized sporocysts ($13.2\text{--}13.6 \times 6.5\text{--}9.5$ and $16.0 \times 9.9\text{--}11.5 \mu\text{m}$) in dogs that were fed camel meat (Ishag et al. 2006). They named the larger sporocyst in the dog that was fed camel meat as a new species, *Sarcocystis camelocanis*, but gave no description. Another new species from the camel was named, *Sarcocystis miescheri*, based on finding oocysts in faeces of dogs fed naturally infected camel meat (Mandour et al. 2011). Currently, there are five *Sarcocystis* species were identified in camels, namely *S. cameli*, *S. ippeni*, *S. camelicanis*, *S. camelocanis*, and *S. miescheri* (Dubey et al., 2015).

Molecular techniques have been widely used for the identification of *Sarcocystis* spp. in different animals. DNA sequences of 18S ribosomal gene, complemently with morphological investigations are useful for the molecular identification and characterization of *Sarcocystis* spp. from different hosts (Gjerde, 2013; Gjerde et al., 2015; Murata, et al., 2018).

This study aimed to examine the *Sarcocystis* infection that occurred in camels, New Valley Governorate (Egypt), studying the morphological and molecular characterization of *Sarcocystis cameli* and *Sarcocystis ippeni* from the muscles of One-Humped Camel (*Camelus dromedarius*).

Materials and methods

Ethical Approval:

Parasites collection from the examined animals was carried out according to the regulatory laws and ethical considerations regarding experimental ethics of animal use and collecting permits.

Sampling and Study Area:

A total of 124 muscle samples of the esophagus, heart and ocular muscles were randomly collected from freshly slaughtered camels (*Camelus dromedarius*) in the slaughterhouses of El-Kharga oasis, New Valley Governorate, Egypt. Tissue samples were preserved in ice bags according to Huong (1997) and transferred to Faculty of Science, New Valley University, Egypt for later examination. Specimens were stored under refrigeration prior to examination.

Macroscopic Examination:

All muscle samples were visually examined for the presence of *Sarcocystis* spp. according to the key of Valinezhad et al. (2008). Tissue samples (2– 8mm) were squashed between two glass slides and examined microscopically according to Hu et al., (2017).

Histological Examination:

Samples of infected muscles were fixed in 10 % buffered formalin for preparation of 5 µm paraffin sections. For histological examination, paraffin sections were stained with hematoxylin-eosin (H&E) stain., then photographs for each sample were taken.

Transmission Electron Microscopy:

For TEM, infected esophageal, heart and ocular muscle samples were fixed in 3% cold glutaraldehyde solution in sodium cacodylate buffer 0.1 M (pH 7.4) for 24 h, washed in sodium cacodylate buffer 0.1 M (pH 7.4), postfixed in 1 % Osmium tetroxide, dehydrated with ethyl alcohol, then embedded in resin (Faculty of Science, central laboratory). Obtained semi-thin sections were made to observe *Sarcocystis* by light Microscope to determine the infection. The Ultra-thin sections were examined under JEM 100 CXII electron microscope (Central Laboratory, South Valley University, Qena, Egypt).

Molecular data:

Infected oesophagus muscle tissues were cut off into small pieces and preserved in ethyl alcohol 95% at -20°C for total genomic DNA extraction. DNA was extracted using a DNA Extraction Kit (Qiagen, DNeasy® QIAamp DNA Mini, and Blood Mini Kit). DNA fragments of the 18S rRNA gene (~600 bp) from camel DNA samples (*Sarcosytis ippeni*, *Sarcosytis* sp. and *Sarcosytis cameli*) were amplified using primers Sar F 5' GCA CTT GAT GAA TTC TGG CA 3' and Sar R 5' CAC CAC CCA TAG AAT CAA G 3' (Wong and Pathmanathan, 1994). The cycling condition included 94°C for 5 minutes followed by 40 cycles of 94°C for 2 minutes, annealing at 55°C for 1 minute, and extension step 72°C for 90 seconds, followed by a final extension step at 72°C for 5 minutes. The final volume of PCR reaction was 25 ml including 3 ml of the sample DNA, 20 pmol of each primer, and 12.5 ml of PCR Master Mix and 7.5 ml distilled water. The PCR bands was separated 1% agarose gel/ethidium bromide, and visoilised on UV apparatus. The PCR products were subjected the digestion analysis by restriction fragment length polymorphism (RFLP) method using *Haemophilus influenza* Rf (Hinf) and *Moraxella bovis* (Mbo1) endonuclease enzymes. RFLP reaction was performed using 5 µL PCR product, 10 unit of each enzyme, and buffer reaction. The reaction is incubated 24 hours at 37°C.

Results

Prevalence and morphological structure:

The macroscopic evaluation revealed the infection with macroscopic *Sarcocystis*

in oesophagus muscles of camels. LM examination revealed the presence of thin-walled *Sarcocystis* sp. in 106 out of 124 camels (85.5 %); with high infection rate of 90.4 % in males (85 of 94) than females 70% (21 of 30). Result showed that the infection rate in camels above four years were more than in camels below four

years. In males, the infection recorded in 50 out of 52 (96.15%) from 4 years old and 35 of 42 (83.33%) below 4 years old. In females, the infection recorded in 14 of 17 (82.35%) above 4 years old, and in 7 of 13 (53.48%) below 4 years old (Table 1 and 2). Both macroscopical and microscopical *Sarcocystis* were detected by LM,

Sarcocystis were thin walled with barely visible projections on the cyst walls. In some *Sarcocystis*, conical projections could be seen on the *Sarcocystis* wall. In Toluidine blue-stained sections, the structure of the *Sarcocystis* wall was not clear. However, in one cyst, projection was visible (Fig. 1A and B).

Table 1: The total number of camels that were examined and the rate of infection with the present *Sarcocystis* sp. from both males and females:

Gender	No. examined	No. infected	No. non-infected	Prevalence %
Males	94	85	9	90.42
Females	30	21	9	70
Total	124	106	18	85.5

Table 2: Age-wise and sex-wise prevalence of *Sarcocystis* infection in camels:

Sex	Age group in years	No. examined	No. infected	Rate of infection %
Male	Below 4	42	35	83.33
	Above 4	52	50	96.15
	Total	94	85	90.42
Female	Below 4	13	7	53.84
	Above 4	17	14	82.35
	Total	30	21	70.00

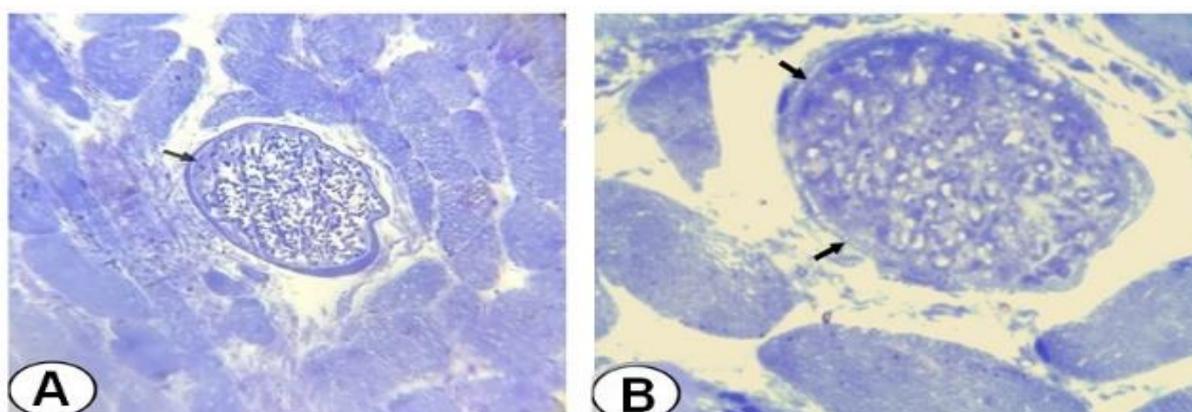


Fig. 1. Semithin sections of *Sarcocystis* from oesophagus of camels. (A): *Sarcocystis cameli* ($\times 100$); (B): *Sarcocystis ippeni* ($\times 400$), stained with Toluidine blue. Note projection of the cyst wall (arrow).

There are two structurally distinct *Sarcocystis* were recognized:

1- *Sarcocystis cameli*:

Sarcocystis appeared as macroscopic and microscopic cyst. In the fresh preparations, the cyst appeared as long cylindrical shaped cyst with spiral parts and as

elongated cyst with rounded end. The macroscopical cysts measured 1.2–3.4 mm long and 0.102 – 0.276 mm wide whereas, the cysts were measured 0.197–1.177 mm in long and 0.071–0.208 mm in wide microscopically (Fig. 2 A and B).

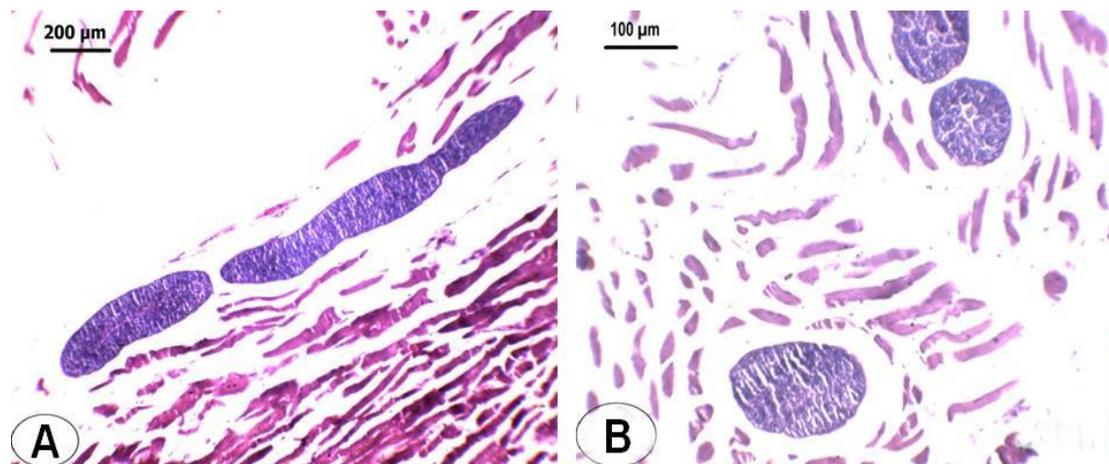


Fig. 2: Photomicrographs of longitudinal and transverse histological paraffin sections of esophagus muscles of camels infected with *Sarcocystis cameli*. stained with H&E stain. A ($\times 40$), B ($\times 100$)

Ultrastructure using TEM showed the *Sarcocystis* wall was smooth, $<1 \mu\text{m}$ thick, and without any visible protrusions. The *Sarcocystis* wall consisted of an outermost parasitophorous vacuolar membrane (Pvm) that was lined by an electron dense layer was $< 50 \text{ nm}$ thick (Fig. 3D). The Pvm had numerous villar protrusions (Vp), approximately $60 - 230 \text{ nm}$ apart from each other. The host myocyte was degenerated along the Vp to a varying degree, giving the impression that Vp were apart (Fig. 3A-D). The Vp were slender, $0.1 - 0.4 \mu\text{m}$ long and $0.05 - 0.1 \mu\text{m}$ wide; Several microtubules (Mt) were observed in the tip of the villus to the middle of ground substance (Gs) layer; the tubules were smooth, were without granules, but had fine cross-striations (Fig. 3B and D). Each villus had several rows of knob-like

projections (Pr) (Fig. 3D and E). Only cross-section showed a Vp, 9 projection $13- 40 \text{ nm}$ long, were visible at regular intervals, the projection seems to be interconnected (Fig. 3E). Electron dense layer (Edl) were observed on Vp tips. The Gs was $0.1 - 0.6 \mu\text{m}$ thick. The Gs continued into the interior of *Sarcocystis* as septa, and thus the Gs at the origin of septa appeared thicker than in other areas (Fig. 3F).

The Gs divided to 2 sections Gs1 and Gs2 (Fig. 4A). Bradyzoites (Br) were $0.57- 2.3 \mu\text{m}$ long and $0.3 - 0.8 \mu\text{m}$ wide long (Fig. 4A-D). However, most bradyzoites were poorly preserved, enormous numbers of micronemes (Mn), dense granules (Dg), mitochondrion (Mc) and nucleus (Nu) were visible (Fig. 4B-D).

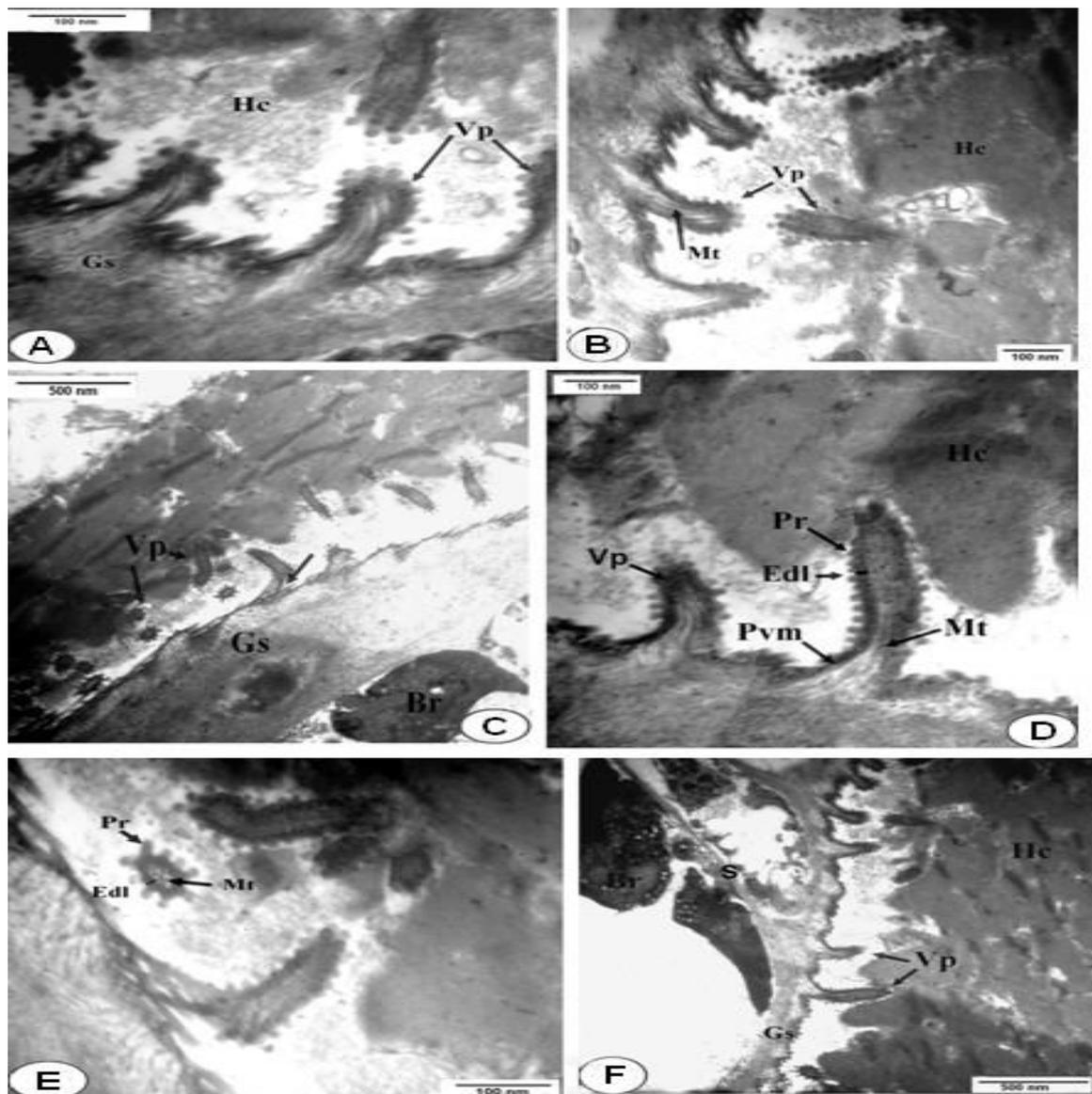


Fig. 3. TEM of *S. cameli Sarcocystis* wall showing: **(A)**: the Vp (villar protrusions) are interspersed with vacuolated (degenerated) Hc (host cell). **(B)**: Slender Vp with prominent Mt (microtubules) at the base of the Vp. **(C)**: Vp cut at an angle, note projections (arrow) from Vp . **(D)**: Pvm (parasitophorous vacuolar membrane) and Slender Vp with thick Edl (electron dense layer) and electron-lucent protrusions (Pr) along the villar length. **(E)**: Cross-section of Vp showing 9 pr (protrusions) at the periphery at regular intervals, and numerous internal Mt with electron lucent centers. **(F)**: Slender Vp and Gs (ground substance) which continued into the interior of *Sarcocystis* as septa (S). Abbreviations: TEM, transmission electron microscopy; Pvm, parasitophorous vacuolar membrane; Edl, electron dense layer; Vp, villar protrusions; Gs, ground substance; Pr,protrusions; Mt, microtubules; Hc, host cell; S, septa.

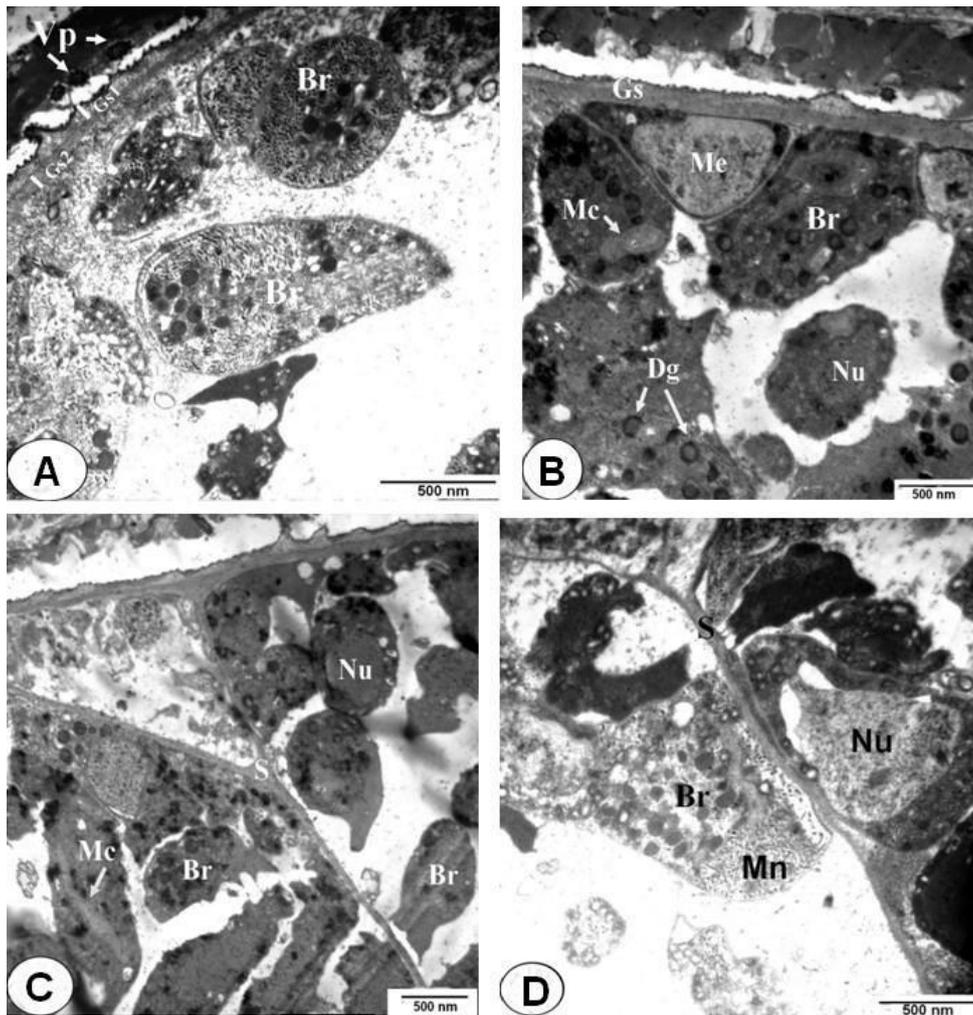


Fig. 4. TEM of *S. cameli* *Sarcocystis* Br showing **(A):** Br (bradyzoites) and Gs (ground substance) layers (Gs1, Gs2). **(B):** Me (metrocytes) and fully formed Br (bradyzoites), note Br with Nu (nucleus), Dg (dense granules) and Mc (mitochondrion). **(C):** Br (bradyzoites) and S (Septa), note Mc and Nu of Br. **(D):** fully formed Br with Nu (nucleus) and Mn (micronemes). Abbreviations: Me, metrocytes; Br, bradyzoites; Mn, micronemes; Dg, dense granules; Nu, nucleus; S, Septa; Gs, ground substance; Mc, mitochondrion.

Sarcocystis cameli has characteristic villar protrusions of “type 9j” (Dubey et al., 2016). The villar protrusions is one of the criterions of speciation of *Sarcocystis* species within a given host (Dubey et al., 2016). The structure of microscopic and macroscopic *Sarcocystis* of *S. cameli* is the same.

2- *Sarcocystis ippeni*:

The cyst appeared as microscopic spindle shaped in diaphragm, with light microscopy, *Sarcocystis* appeared as thin

cyst wall approximately 1 μ m, with small protrusions. *Sarcocystis* measure 230.36 - 309.42 \times 82.56 – 110.96 μ m in size (Fig. 5A and B).

In TEM, the *Sarcocystis* wall was < 1 μ m thick, and had villar protrusions (Vp) that were often conical in shape. The villar protrusions approximately 175.27 - 266.76 nm long, 100.45- 175.98 nm wide (Fig. 6A and B), some Vp were not conical and more finger-like and some were stubby Fig. (6A-D). Microtubules (Mt) in Vp

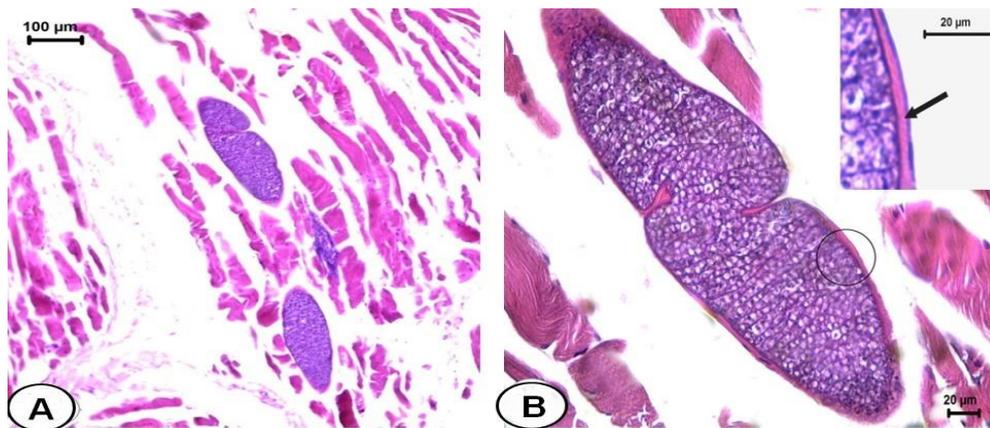


Fig. 5. photomicrographs of longitudinal histological paraffin sections of oesophagus muscles of camels infected with *Sarcocystis ippeni*. stained with H&E stain, note the protrusions in B (arrow). A (×100) and B (×400).

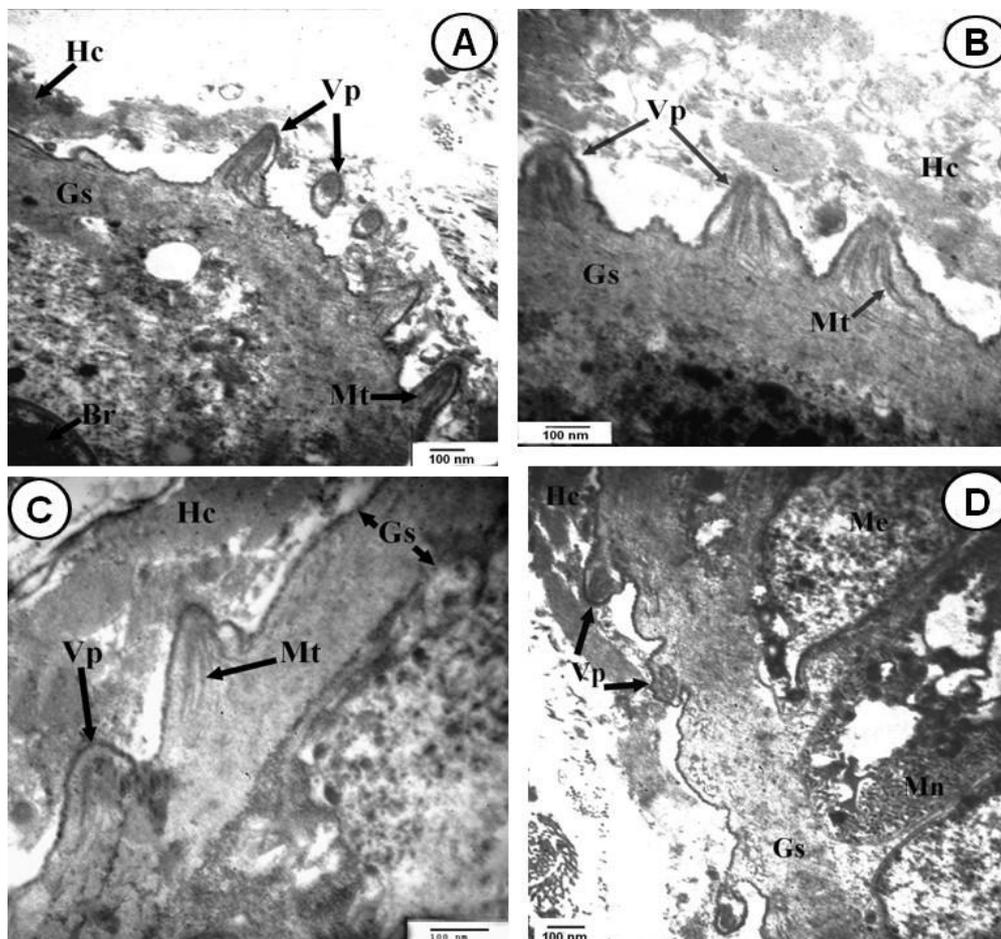


Fig. 6. TEM of *S. ippeni* *Sarcocystis* wall showing: **(A):** conical Vp (villar protrusions). Note criss-crossing Mt (microtubules) and knob-like thickening of the Vp. **(B):** conical Vp. Note the Vp are cut at different angles. **(C):** The Mt in Vp are more electron dense towards the villar tips. **(D):** Vp cut at different angles., The Gs (ground substance) layer is mostly electron lucent and not well demarcated. Abbreviations: Vp, villar protrusions; Gs, ground substance; Mt, microtubules; Hc, host cell; Me, merozoites; Br, bradyzoites; Mn, micronemes.

originate at mid point of ground substance (Gs) and continue up to tip, criss-crossed, smooth and without granules or dense areas (Fig. 6B and C). The total thickness of *Sarcocystis* wall (from the base of ground substance to Vp tip) was 329.34 – 478.47 nm. Bradyzoites (Br) 572.63 – 1325.73 × 435.25 – 585.99 nm in size.

Bradyzoites had double - membraned plasmalemma consisting of an outer membrane (Om) and an inner membrane (Im) (Fig. 7B). They contained numerous

micronemes (Mn), subterminal nucleus (Nu) and numerous dense granules (Dg) (Fig. 7C and D). The ground substance (Gs) was 184.14 – 359.45nm thickness. Within the same *Sarcocystis*, metrocytes were nucleated and contained very few organelles (Fig. 7A and B). *Sarcocystis ippeni* has unique “type 32” Vp with electron dense knobs and microtubules from villar tips to the middle of the Gs layer

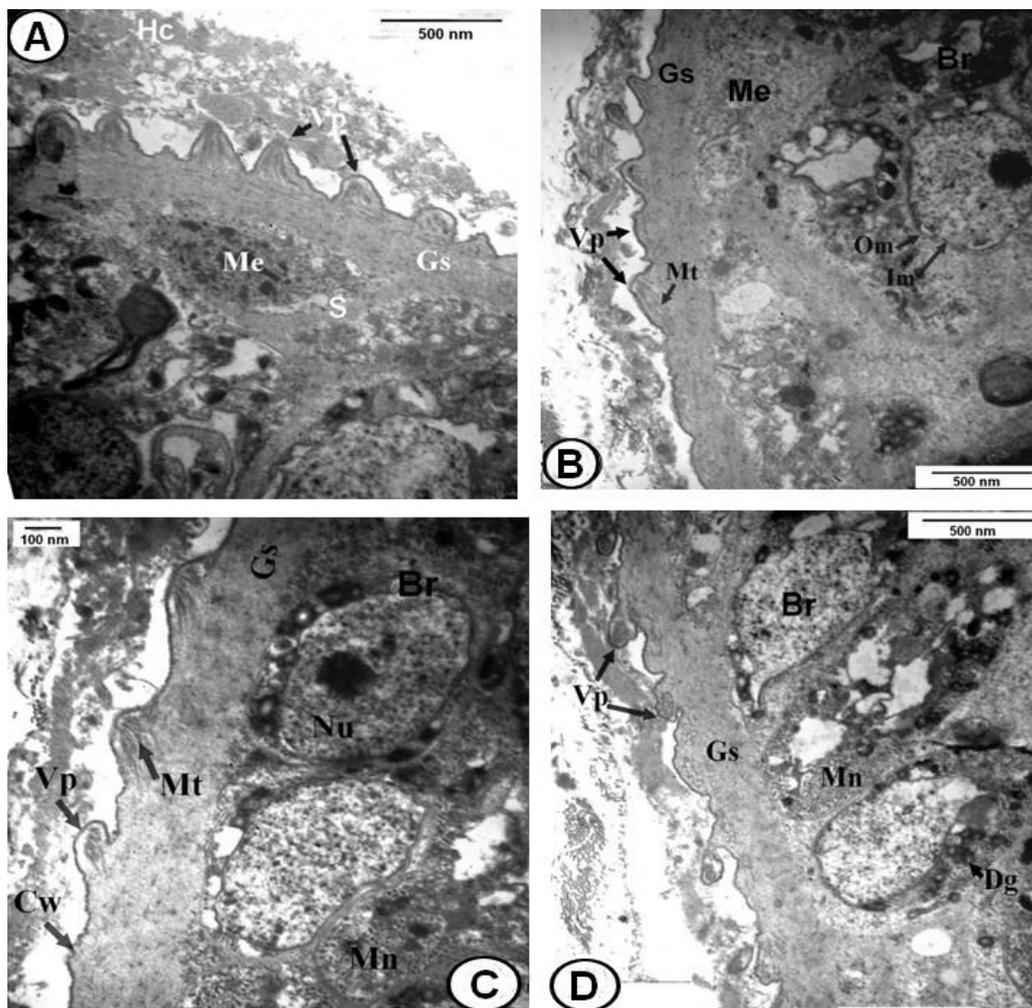


Fig. 7. TEM of *S. ippeni Sarcocystis* showing: **(A):** Me (metrocytes) below indistinct Gs (ground substance) layer. Note S (septa). **(B):** Br (bradyzoites) with plasmalemma Om (outer plasmalemma membrane) and Im (inner membrane), note Mt below Gs layer. **(C):** Br with Nu (nucleus) and Mn (micronemes), note Cw (cyst wall) and Vp. **(D):** Br with Mn and Dg (dense granules) below the Gs layer. Abbreviations: Gs, ground substance; Mt, microtubules; S, septa; Cw, cyst wall; Me, metrocytes; Br, bradyzoites; Mn, micronemes; Dg, dense granules; Nu; nucleus; Om, outer plasmalemma membrane; Im, inner membrane.

Molecular identification

The oesophageal muscle samples previously examined using light microscope, were selected for processing molecular analysis. In the present study, we used PCR-RFLP molecular technique, Specific primers for *Sarcocystis* species to amplify 18s ribosomal gene from selected camels' samples (*Sarcosytis ippeni*, *Sarcosytis* sp. and *Sarcosytis cameli*). DNA fragments of about 600 base pair were amplified as shown in (Fig. 8). Samples

were subjected to the restriction digestion analysis by RFLP and using 2 restricted enzymes (*Hinf*1 and *Mbo*1 enzymes). As shown in Fig. 9, the *Hinf*1 enzyme has no cutting effects on the PCR yields. The *Mbo*1 restricted enzyme was digested the PCR product of *Sarcocystis ippeni* and *Sarcocystis cameli* into 2 bands approximately 250 and 350 bp. For sample 2 (*Sarcocystis* sp.), no cutting effect was observed from both enzymes as shown. In (Fig. 9).

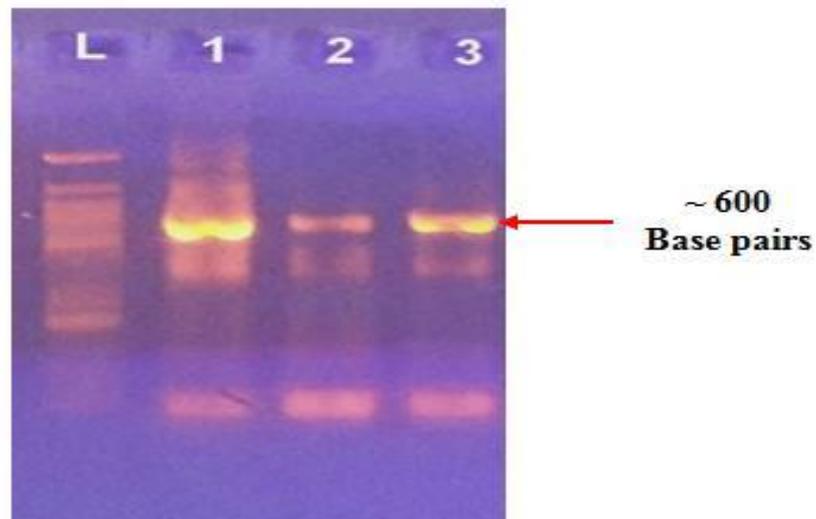


Fig. 8. The Electrophoresis of the PCR Product of ribosomal gene of *Sarcocystis* in Camel samples showing a band with 600 bp. Samples: 1= *Sarcocystis ippeni*; 2= *Sarcocystis* sp. and 3= *Sarcocystis cameli* ; L= DNA ladder (50-1350 bae pair)

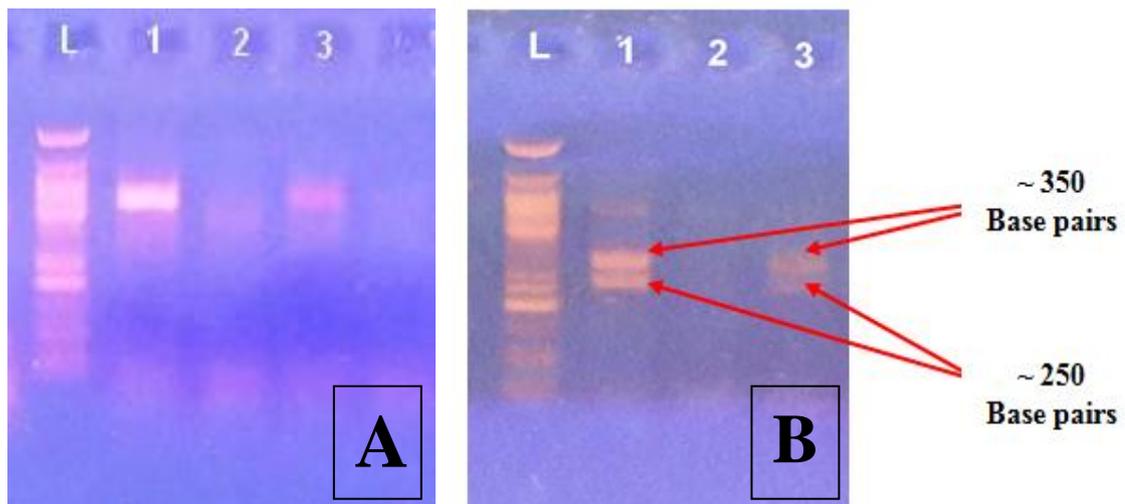


Fig. 9. Restriction digestion analysis by RFLP and using 2 restricted enzymes *Hinf* (A) and *Mbo*1 (B) enzymes. Samples: 1= *Sarcocystis ippeni*; 2= *Sarcocystis* sp and 3= *Sarcocystis cameli* ; L= DNA ladder (50-1350 base pair). In sample 1, there is an undigested DNA obsreveded.

Discussion

Morphological and ultra-examination studies:

Sarcocystis spp. are among the most common parasites in domestic ruminants, and some of them 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., 2015).

Prevalence data on *Sarcocystis* species in camels have been reported in various countries, including Sudan (Hussein & Warrag, 1985), Jordan (Al-Ani and Amr, 2017), Afghanistan (Kirmse & Mohanbabu, 1986), Morocco (Kirmse 1986) and Egypt (Abdel Ghafar et al. 1979), Somalia (Borrow et al. 1989), Saudi Arabia (Fatani et al. 1996), Southern Ethiopia (Woldemeskel & Gumi 2001), Iraq (Latif et al. 1999), and Mongolia (Fukuyo et al. 2002).

In the present study, the prevalence of *Sarcocystis* infection in slaughtered camels was 85.5%. The observed prevalence is higher than that reported in Egypt, Southern Ethiopia, and the Yazd Province (Iran), where the reported prevalence rates were 42.3 – 60.0%, 45.45%, and 51.5%, respectively (Mandour et al. 2011, Abdel Ghafar et al. 1979, Woldemeskel & Gumi 2001, Hamidinejat et al. 2013). Moreover, previous reports on *Sarcocystis* spp. in camels from Saudi Arabia, Afghanistan, Jordan, and Morocco reported prevalence rates of 56.7%, 47.3–66.3%, 6.6% and 60%, respectively (Hussein 1991, Kirmse & Mohanbabu 1986, Al-Ani and Amr, 2017, Kirmse 1986). In contrast, higher infection rates of 100% and 91.6% were reported in Mongolia and Iraq, respectively (Fukuyo et al. 2002, Latif et al. 1999). Furthermore, in a previous study in Egypt, a prevalence of 81% and 75% was reported (El-Etreby 1970, Gareh et al. 2020), whereas a prevalence of 83.6% was

reported in the eastern Provinces of Iran (Valinezhad et al. 2008).

The variation between our present results and those previously mentioned 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, differences in the systems used for camel keeping could influence the infection rate (Valinezhad et al. 2008, Dubey et al. 2015).

Sarcocystis spp. infect muscular tissue of the heart, tongue, oesophagus, and diaphragm. However, *Sarcocystis* spp. cysts have been reported in several other types of muscle tissue (Bucca et al. 2011, Ono & Ohsumi 1999). In the present study, *Sarcocystis* was observed in oesophageal, heart and ocular muscles, particularly in the oesophagus, with a prevalence rate of 85.5% which is according to several previous reports either in the same species or different species (Valinezhad et al. 2008, Wahba et al. 2014, Hamidinejat et al. 2013, Woldemeskel & Gumi 2001, Ahmed et al. 2016). Meanwhile, some studies found the diaphragm of camels to be the most commonly affected site (Fukuyo et al. 2002, Al-Ani and Amr, 2017), whereas another study identified the heart as the most commonly infected organ (Shekarforoush et al. 2006).

The sex of the animal found to be a significant variable associated with infection. Males being at higher risk of infection than females. Prevalence rates of 90.4% and 70% were reported in male and female camels, respectively (Table 1). Our results are consistent with several previous studies in Egypt (Mandour et al., 2011), southern Ethiopia (Woldemeskel & Gumi 2001), and Iran (Valinezhad et al. 2008). 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).

Age was another significant risk factor associated with infection. Older camels appeared to be at greater risk of infection than younger ones; animals aged 4 years or older were infected to a significantly larger extent (92.8%) than younger animals (76.4%). Similar findings were reported in previous studies in the Menofia Governorate (Egypt) (El-Bahy et al. 2019), Yazd Province (Iran) (Hamidinejat et al. 2013), and in Riyadh city (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; moreover, 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).

In the previous study, the two *Sarcocystis* species under study were detected in *Camelus dromedarius*, as *Sarcocystis cameli* and *Sarcocystis ippeni*. Mason (1910) was first to report *Sarcocystis* in camel with no description and gave its name as *S. cameli*. This name was largely ignored until Dubey et al. (1989) arbitrarily assigned one *Sarcocystis* species to be named *S. cameli*. Abdel-Ghaffar et al. (1979) had reported a unique structure of this parasite, but they did not name it. Odening (1997) proposed a new name, *S. ippeni*, for the parasite that was described by Entzeroth et al. (1981). Abdel-Ghaffar et al. (2009) ignored all previously assigned names and called the parasite as *S.*

camelicanis in continuing with the earlier philosophy of Heydorn et al. (1975).

The previous description of these two species *S. cameli* and *S. ippeni* and their taxonomical position summarized as follow:

1- *Sarcocystis cameli* (Mason, 1910)

Diagnosis: *Sarcocystis* microscopic and macroscopic, appear thin walled by LM. By TEM, *Sarcocystis* wall has unique Vp, type 9j (Dubey et al. 2015), these are slender, up to , 0.1 – 0.4 µm long and 0.05 – 0.1 µm wide, with knob-like protrusions, microtubules in Vp are smooth, originate at midpoint of the Gs and continue up to the tip. The Gs was 0.1 – 0.6 µm thick. Bradyzoites were approximately 0.57- 2.3 µm long and 0.3 – 0.8 µm wide. Dog is the definitive host.

2- *Sarcocystis ippeni* (Odening, 1997)

Diagnosis: *Sarcocystis* microscopic, appearing thin walled by LM. By TEM, *Sarcocystis* wall has unique conical Vp with an electron dense knob. The Vp approximately 175.27 - 366.76 nm long, 100.45- 175.98 nm wide, microtubules in Vp originate at midpoint of Gs and continue up to tip, criss-crossed, smooth and without granules or dense areas. The total thickness of the *Sarcocystis* wall (from the base of Gs to Vp tip) was 329.34 – 478.47 nm. Bradyzoites 572.63 – 1325.73 × 435.25 – 585.99 nm in size.

For molecular identification of *Sarcocystis* species, oesophagus samples from camels were analyzed to determinate species of the parasite by PCR-RFLP method. This help to distinguish the risk of human Sarcocystosis due to consumption of contaminated meat that is consider a public health problem. Currently, identification of *Sarcocystis* species in animals and humans is conducted using transmission electron microscopy to examine cyst' wall structure (Agholi et al. 2016). However, this method has some limitations due to the extended

epidemiology study and detection of little morphology variation in species (Gjerde 2013). This led researchers to look for an alternative means which can give more references clues for *Sarcocystis* identifications. Molecular approaches have been widely used and applied for a more accurate analysis. Several studies reported using the molecular approach for identification of *Sarcocystis* species in different hosts, where the DNA sequences of 18S ribosomal gene inside to morphological investigations consider helpful and useful for identification/characterization of *Sarcocystis* spp from different hosts (Gjerde, 2013; Gjerde et al., 2015; Murata, et al., 2018).

Data here showed the partial DNA fragment of 600 bp of 18S rRNA gene, which when digested using Hinf (A) and MboI (B) restriction enzymes, produced two fragments (~250, ~350) which is in accordance to Motamedi et al. (2011) who characterized *Sarcocystis* isolated from camels by PCR-RFLP method using MboI enzyme and other enzymes (XbaI, EcoRI, AvaI). This reflected the reliability of the PCR-RFLP technique for distinguishing of *Sarcocystis* spp. In other study, Rahdar and Kardooni, (2017) used the Hinf, MboI enzymes to digest the 18S ribosomal RNA gene amplicon of 600 bp from sheep which used for distinguishing *Sarcocystis* species. Furthermore, Omer et al. (2017) extracted DNA from purified bradyzoites revealing high molecular weight DNA when using 18S rRNA gene specific primers. Therefore, RFLP resolved by agarose gel electrophoresis demonstrated to be an economic and rapid method of discriminating between *Sarcocystis* species from different hosts.

In this study, the 18S rRNA gene was used for distinguishing *Sarcocystis* species in slaughtered camels. Data showed that all *Sarcocystis* isolates in camel samples

belonged to *S. cameli* and *S. ippeni*. The results of the PCR of samples presented a 600-nucleotide bp fragment. The PCR-RFLP analysis revealed the detection of two similar size cutting bands in both *S. cameli* and *S. ippeni*. This work demonstrated that ultrastructural study, PCR amplification of 18S rRNA and RFLP-based analysis are useful and helpful to distinguish camel species of *Sarcocystis* from different hosts.

In conclusion, this study is the first to demonstrate the presence of *Sarcocystis cameli* and *Sarcocystis ippeni* in the oesophagus, heart and ocular muscles of slaughtered One-humid camel for human consumption in New Valley Governorate. The study presented the isolated *Sarcocystis* species from camels belonged to *Sarcocystis cameli* and *Sarcocystis ippeni*. This may could have an important role for transmission human Sarcocystosis due to human consumption. The molecular analysis demonstrated that the PCR amplification of 18S rRNA and RFLP-based analysis are useful and helpful to distinguish camel species of *Sarcocystis* from slaughtered camels.

Conflict of interests

The authors report no conflicts of interest associated with this manuscript.

References

- Abdel Ghafar F, Entzeroth R, Chobotar B and Scholtyssek E (1979). Ultrastructural studies of *Sarcocystis* sp. From the camel (*Camelus dromedarius*) in Egypt. Tropical Medical Parasitology, 30: 434-438.
- Abdel-Ghaffar F, Mehlhorn H, Bashtar A, Al-Rasheid K, Sakran T and El-Fayoumi H (2009). Life cycle of *Sarcocystis camelicanis* infecting the camel (*Camelus dromedarius*) and the dog (*Canis familiaris*), light and

- electron microscopic study. *Parasitology Research*, 106: 189-195.
- Agholi M, Shahabadi SN, Motazedian MH and Hatam GR (2016). Prevalence of Enteric Protozoan Oocysts with Special Reference to *Sarcocystis cruzi* among Fecal Samples of Diarrheic Immunodeficient Patients in Iran. *Korean Journal of Parasitology*, 54(3): 339-344.
- Ahmed AM, Elshraway NT and Youssef A I (2016). Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egyptian Veterinary World, 9: 1461–1465.
- Al-Ani FK and Amr Z (2017). *Sarcocystis* spp Prevalence in Camel Meat in Jordan. *Dairy Veterinary Science Journal*, 4: 1–3.
- Borrow HA., Mohammed HA and Di Sacco B (1989). *Sarcocystis* in Somali camel. *Parassitologia*, 31(2-3): 133-136.
- Bucca M, Brianti E, Giuffrida A, Ziino G, Cicciani S, Panebianco A (2011). Prevalence and distribution of *Sarcocystis* spp. cysts in several muscles of cattle slaughtered in Sicily, Southern Italy. *Food Control*, 22(1): 105-108.
- Dubey J, Hilali M, van Wilpe, E, Calero-Bernal R, Verma SK and Abbas I (2015). A review of Sarcocystisosis in camels and redescription of *Sarcocystis cameli* and *Sarcocystis ippeni* Sarcocysts from the one-humped camel (*Camelus dromedarius*). *Parasitology*, 142.
- Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R (2016). *Sarcocystisosis of Animals and Humans*, 2nd Edn. CRC Press, Boca Raton, FL, USA.
- Dubey JP, Speer CA and Fayer R (1989). *Sarcocystisosis in animals and man*. CRC Press. Boca Raton, Florida, 215.
- El-Bahy N, El-Bagory AER, AbouLaila M, Elkhatam A and Mady HM (2019). Prevalence of *Sarcocystis fusiformis* and Hydatid cyst among Different Ruminants at Menofia Governorate, Egypt. *Journal of Current Veterinary Research*, 1(1): 1-10.
- El-Etreby M (1970). Myocardial sarcosporidiosis in the camel. *Pathology Veterinary*, 7: 7–11.
- Entzeroth R, Abdel Ghaffar F, Chobotar B and Scholtyseck E (1981). Fine structural study of *Sarcocystis* sp. from Egyptian camels (*Camelus dromedarius*). *Acta Veterinaria Hungarica*, 29(3): 335-339.
- Fatani A, Hilali M, Al-Atiya S and Al-Shami S (1996). Prevalence of *Sarcocystis* in camels (*Camelus dromedarius*) from Al-Ahsa, Saudi Arabia. *Veterinary Parasitology*, 62: 241-245.
- Fukuyo M, Battsetseg G, Byambaa B (2002). Prevalence of *Sarcocystis* infection in meat-producing animals in Mongolia. *Southeast Asian Journal of Tropical Medical Public Health*, 33: 490–495.
- Gareh A, Soliman M, Amira A, Saleh AA, Fatma A, El-Gohary FA, El-Sherbiny H, Mohamed RM, Elmahallawy E (2020). Epidemiological and Histopathological Investigation of *Sarcocystis* spp. in Slaughtered Dromedary Camels (*Camelus dromedarius*). *Egyptian Veterinary Sciences*, 7: 162.
- Gjerde B (2013). Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase

- subunit I gene. *International Journal of Parasitology*, 43(7): 579–591.
- Gjerde B, Hilali M and Mawgood SA (2015). Molecular characterisation of three regions of the nuclear ribosomal DNA unit and the mitochondrial *cox1* gene of *Sarcocystis fusiformis* from water buffaloes (*Bubalus bubalis*) in Egypt. *Parasitology Research*, 114: 3401-3413.
- Hamidinejat H, Hekmatimoghaddam S, Jafari H, Sazmand A, Haddad Molayan P, Derakhshan L and Mirabdollahi S (2013). Prevalence and distribution patterns of *Sarcocystis* in camels (*Camelus dromedarius*) in Yazd province, Iran. *Journal Parasitology Diseases*, 37: 163–165.
- Heydorn AO, Gestrich R, Mehlhorn H and Rommel M (1975). Proposal for a new nomenclature of the Sarcosporidia. *Zeitschrift für Parasitenkunde (Now Parasitology Research)* 48: 73–82.
- Hilali M and Mohamed A (1980). The dog (*Canis familiaris*) as the final host of *Sarcocystis cameli* (Mason, 1910). *Tropmenmed.*, 31: 213-214.
- Hu JJ, Huang S, Wen T, Esch GW, Liang Y and Li HL (2017). *Sarcocystis* spp. in domestic sheep in Kunming City, China: prevalence, morphology, and molecular characteristics. *Parasite*. 24, 30DOI: 10.1051/parasite/2017025.
- Huong LTT, Dubey JP, Nikkila T, Uggla A (1997). *Sarcocystis buffalonis* n. sp. (Protozoa: Sarcocystidae) from the water buffalo (*Bubalus bubalis*) in Vietnam. *Journal of Parasitology*, 83: 471–474.
- Hussein HS and Warrag M (1985). Prevalence of *Sarcocystis* in food animals in the Sudan. *Tropical Animal Health and Production*, 17: 100–101.
- Hussein NM, Hassan AA, Abd-Ella OH (2018). Morphological, Ultrastructural, and Molecular Characterization of *Sarcocystis tenella* from Sheep in Qena Governorate, Upper Egypt. *Egyptian Academy Journal of Biological Sciences*, 10 (1): 11-19.
- Hussein SH (1991). The prevalence of *Sarcocystis* infection in Saudi Arabian Najdi sheep and camels. *Biology Science*, 1: 43–56.
- Ishag MY, El Amin EA, Osman AY (2001). Camel experimentally infected with *Sarcocystis*. *Sudan Journal of Veterinary Research*, 17: 27–33.
- Ishag MY, Majid AM, Magzoub AM (2006). Isolation of a new *Sarcocystis* species from Sudanese camels (*Camelus dromedarius*). *International Journal of Tropical Medicine* 1: 167–169.
- Kirmse P (1986). Sarcosporidiosis in equines of Morocco. *British Veterinary Journal*, 142: 70–72.
- Kirmse P, Mohanbabu B (1986). *Sarcocystis* sp. in the one-humped camel (*Camelus dromedarius*) from Afghanistan. *British Veterinary Journal*, 142: 73–74.
- Latif BM, Al-Delemi JK, Mohammed BS, Al-Bayati SM, Al-Amiry AM (1999). Prevalence of *Sarcocystis* spp. in meat-producing animals in Iraqi. *Veterinary Parasitology*, 84: 85–90.
- Mandour AM, Rabie SA, Mohammed NI and Hussein NM (2011). On the presence of *Sarcocystis miescheri* sp. nov. in camels of Qena Governorate. *Egyptian Academic Journal of Biological Sciences* 3: 1–7.

- Mason FE (1910). *Sarcocystis* in the camel in Egyptian Journal of Comparative Pathology, 23: 168-176.
- Motamedi GR, Dalimi A, Nouri A, Aghaeipour K (2011). Ultrastructure and molecular characterization of *Sarcocystis* isolated from camel (*Camelus dromedarius*) in Iran. Parasitology Research, 108: 949-954.
- Murata R, Suzuki J, Hyuga A, Shinkai T and Sadamasu K (2018). Molecular identification and characterization of *Sarcocystis* spp. in horsemeat and beef marketed in Japan. Parasite, 25.
- Odening K (1997). The Sarcocystis Infection: Interactions Between Wildlife, Domestic Animals, and Zoo Animals "Die Sarcocystis-Infektion: Wechselbeziehungen zwischen freilebenden Wildtieren, Haustieren und Zootieren. Zoologische Garten". Zoological garden 67: 317–340.
- Omer SA, Alzuraiq AA, Mohammed OB (2017). Prevalence and molecular detection of *Sarcocystis* spp. Infection in the dromedary camel (*Camelus dromedarius*) in Riyadh city, Saudi Arabia. Biomedical Research, 28: 4962–4965.
- Ono M. and Ohsumi T. (1999). Prevalence of *Sarcocystis* spp. cysts in Japanese and imported beef (Loin: *Musculus longissimus*). Parasitology International Journal, 48: 91–94.
- Rahdar M, Karadooni T (2017). Molecular Identification of *Sarcocystis* spp. in Sheep and Cattle by PCR- RFLP from Southwest of Iran, Jundishapur Journal of Microbiology.
- Romero S, Carletti T, Decker Franco C, More G, Schnittger L, Florin-Christensen M (2017). Seropositivity to *Sarcocystis* infection of llamas correlates with breeding practices. Veterinary Parasitology: Regional Studies and Reports, 10: 65–70.
- Shekarforoush SS, Shakerian A, Hasanpoor MM (2006). Prevalence of *Sarcocystis* in slaughtered one-humped camels (*Camelus dromedarius*) in Iran. Tropical Animal Health and Production, 38: 301–303.
- Valinezhad A, Oryan A, Ahmadi N (2008). *Sarcocystis* and its complications in camels (*Camelus dromedarius*) of eastern provinces of Iran. Korean Journal of Parasitology, 46(4): 229–234.
- Wahba A, Ayoub M, Soliman K (2014). Light and ultrastructure of *Sarcocystis* spp. of camels and associated pathological changes. Animal Health Research Journal, 2: 143–158.
- Woldemeskel M and Gumi B (2001). Prevalence of *Sarcocystis* in one-humped camel (*Camelus dromedarius*) from southern Ethiopia. Journal of Veterinary Medicine, B, Infectious Diseases and Veterinary Public Health, 48: 223–226.
- Wong KT and Pathmanathan R (1994). Ultrastructure of the human skeletal muscle *Sarcocystis*. Journal of Parasitology, 80(2): 327–330.