

THE LIGHT MICROSCOPY AND ULTRASTRUCTURAL CHARACTERISTICS OF *SARCOCYSTIS FUSIFORMIS* INFECTING BUFFALOES IN IRAQ

By

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Abstract

Samples from fresh muscles of 2-12 years old buffalo (*Bubalus bubalis*) from Al-Shuala, Baghdad were examined for *Sarcocystis* infection. Macroscopic ovoid *Sarcocystis* embedded in the muscle tissues of the examined buffalo were detected, they measured 150-223 (209 \pm 7) μ m length and 35-130 (85 \pm 3) μ m width. Esophagus was the highly infected organ followed by diaphragm, tongue while the heart muscles were the lowest infected part. The cyst cavity was compartmentalized by septa derived from the ground substance located under the primary cyst wall. By TEM, the primary cyst wall bordered sarcocysts, 0.08-0.22 μ m in thickness, raised from the parasitophorous vacuolar membrane and surrounded by a secondary cyst wall of host origin. The primary cyst wall had irregular wall folds with numerous cauliflower projections of variable size and shape accompanied with a knob-like electron dense elevation. The interior of the cyst contained merozoites consisted of the structural elements of the Apicomplexa, polar rings, micronemes, conoid and rhoptries.

Keywords: *Bubalus bubalis*, *Sarcocystis fusiformis*, light and electron microscopy

Introduction

Sarcocystosis is a zoonotic and protozoan disease often prevalent in household animals like buffaloes, cattle, and pigs. *Sarcocystis* is an intracellular, cyst-forming coccidian parasite under the phylum Apicomplexa and family Sarcocystidae with two obligate hosts, including a definitive carnivorous host and an intermediate omnivorous or herbivorous host (Fayer *et al.*, 2015). Casualties of the pathogen in domestic cattle may lead to severe fatalities such as abortion, reduced milk yield, neurologic signs, and weight loss. The infection with macroscopic *Sarcocystis* leads to meat's low market profile along with condemnation of the infected hosts (Calero-Bernal *et al.*, 2016). The main source of *Sarcocystis* infection in humans is meat and meat-derivatives by ingestion of well-developed tissue cysts containing bradyzoites (Abu-Elwafa *et al.*, 2015). *Bubalus bubalis* (water buffalo) acts as the natural reservoir harbored four species of *Sarcocystis*: *S. fusiformis*, *S. buffalonis*, *S. levinei* & *S. sinensis* (Gjerde, 2016; Gjerde *et al.*, 2017). Many studies were done on *Sarcocystis* species infecting slaughtered animals in Iraq. Latif *et al.* (1999) recorded *Sarcocystis* spp. in edible animals, and added that the prevalence

percentage of macroscopic cysts were 4.1, 33.6, 0.2, 15.6 & 0, but the microscopic one were 97.0, 97.4, 97.8, 82.9 & 91.6 for sheep, goat, cattle, buffaloes & camels respectively. El-Dakhy *et al.* (2011) reported *S. fusiformis* & *S. levinei* in buffaloes. Whaeeb *et al.* (2016) in Baghdad studied the molecular structure of four *Sarcocystis* species from sheep esophagus and muscle. Zangana and Hussein (2017) in Duhok Province, Kurdistan region, Northern Iraq studied the prevalence of *S. ovis* and *S. capricanis* in sheep and goats tongue muscle. Scioscia *et al.* (2017) reported three *S. fusiformis*.

The present study aimed to describe *Sarcocystis fusiformis* isolated from the water buffaloes by the light and transmission electron microscopy.

Material and Methods

Macrocysts of *Sarcocystis* spp. were isolated from heavily infected esophagus of an adult male buffalo *Bubalus bubalis* Linnæus, 1758 (Mammalia: Bovidae) freshly slaughtered at Al-Shuala Abattoir, Baghdad over the year 2018. Tissue samples from the slaughterhouse were sent to the Parasitology Lab, Department of Biology, College of Science for Woman, preserved in ice-bags (Huong, 1997), and stored in refrigeration

tell needed. Macroscopically *Sarcocystis* was (3-4x1-2 mm) excised from esophagus, preserved in the desired fixative for light microscopy, and TEM examinations.

Histopathological studies: Buffered formal solution of 10% (pH 7.3) was used to immobilize small pieces of 3mm infected muscle samples for at least 12hrs. Phosphate buffer was used for washing for 2-3 times of 10 min. each, samples were dehydrated in ethanol 50%, 70%, 80%, 90%, 96% & 100%, cleared in xylol and embedded in paraffin at 62°C. Sections of 5µm thickness were cut by the rotary microtome (Ardalan *et al*, 2017). After wax removing, sections were hydrated in a descending series of ethanol and stained with hematoxylin and eosin (H&E). Sections were microscopically examined and photographed by attached digital camera (Canon).

TEM examination: Very small muscle pieces of the highly infected animals were immediately preserved in 3% glutaraldehyde fixative for at least 4hrs at 4°C. The muscles were rinsed overnight in 0.2M sodium cacodylate buffer at 4°C post-fixation and osmification performed in 1% cold osmium tetroxide in 0.2M sodium cacodylate at pH 7.2 for 2hrs. Buffer was done after dehydration in ethanol grades of 50%, 70%, 80%, 90%, 96% & 99.9% and clarified in propylene oxide for 20 minutes. The samples were put in equal volumes of propylene oxide and resin (Epon-Araldite mixture), rotated for at least one day and put into flat or BEEM capsule molds (positioned near molds tips). The resin was put overnight in an oven at 60°C (Mollenhauer, 1964). The semi-thin sections were stained with toluidine blue. Ultrathin sections were examined with a Philips (208S) TEM at 80-100 KV.

Results

The *Sarcocystis* infected buffalo; macroscopic cysts were embedded in the animals' muscle tissues (Figs. 1a). Cysts were ovoid with dimensions of 150-223 (209±7) µm length x 35-130 (85±3) µm width (Figs. 1b). Esophagus was the highly infected organ fo-

llowed by diaphragm, tongue and muscles, but the heart muscles were least infected.

Light microscopy: Histological sections (Figs. 1c) of infected muscle showed that cyst cavity was divided into many chamber-like compartments separated from each other by septa derived from the ground substance located under the primary cyst wall. In semithin sections via sarcocysts (Figs. 1d), cyst compartments were surrounded by primary cyst wall underneath was a ground substance. Two parasitic stages were within the cyst, merozoites that acquired a faint stain located directly under the ground substance and the merozoites, which filled most of the cysts interior area was darkly stained with toluidine blue.

In the present study, TEM examination of ultrathin sections showed that all cysts surrounded by a clear cyst wall of 0.08-0.22µm in thickness developed from parasitophorous vacuolar membrane (Figs. 2a-c). A well developed secondary cyst wall also presided. A crucial factor for species identification such as the structure of the primary cyst wall, had revealed that this wall had irregular folds with numerous cauliflower projections or protrusions of variable size and shape. Also, knob-like electron dense elevations were accompanied by projections. Within projections there were many fibrillar elements. Numerous vesicles, vacuoles and mitochondria of host cell were surrounding these projections. In L.S, protrusions measured 0.49-0.83 (0.67±0.02) µm, beneath primary cyst wall, a relatively thick homogenous ground substance measured 0.074-0.53µm (±0.29) thickness. Merozoites (Figs. 2d) of the sarcocysts showed the same ultrastructural merozoite patterns of all Apicomplexa members. They were banana shaped with blunt posterior and pointed anterior end, and measured 15.2-16.4 (14.9±4 µm) x 2.8- 6.1µm (5.5±2µm). Plasmalemma enclosed the whole cell, but was interrupted at the anterior and posterior polar rings and at micropore. The micropore (feeding organelle) was formed by the invagination of outer membrane of pellicle supp-

orted by two thickened structures raised by the interruption of the inner membranous layer. Merozoite apical complex (Fig. 2e) consisted of conoid at the anterior polar ring. It's a hollow cone-like or hump-structure. Rhoptries were paired club-shaped and dense structures with a gland like-appearance. Unlike their name implication, micronemes appeared as rice grains and were numerous filling anterior regions but, appeared round in cross sections and may be secretory in function.

Discussion

A wide range of mammals and avian harbor *Sarcocystis* was uncommon in ectothermic vertebrates (Dubey *et al*, 2017). The coccidian nature of *Sarcocystis* was elucidated (Fayer, 1972; Rommel *et al*, 1972). The infection profile in the present study indicated that these domestic animals are at a high risk of infection due to their surreal inter-relationship with other domestic animals (Collier *et al*, 1998). Prevalence of infection in domestic animals in different countries were 68% in pigs (Lukesová *et al*, 1986), 100% in sheep (Ford, 1987), 90% in cattle and horses 93 (Fukuyo *et al*, 2002), 93% in donkeys (El-Ganayni, 2003), 64% camels (Al-Quraishi *et al*, 2005), 84% in Egyptian caws and 28% in buffaloes Khalifa *et al*. (2008) to 25.5 % (Metwally *et al*, 2014), 77% & 79.4% goats (Morsy *et al*, 2011), 73.1% in Argentina lions (Moré *et al*, 2011), 25% in Egyptian horses (Sakran *et al*, 2013) 34.7% in swine (Grikienienė, 1994), in cats the incidence varied between 17.6% (Lukes ova *et al*, 1986), to 10% (Gillis *et al*, 2003) and 0.03% in dogs (Barutzki and Schaper 2011), in birds the incidence varied between 36.5% (Černá 1984) to 62% (Olias *et al*, 2011), to 8% in Egyptian swamp chicken (El-Morsey *et al*, 2013), 25% in Jackdaw birds (Prakas *et al*, 2013).

Generally, *Sarcocystis* varied in morphology as to species and age (JyothiSree *et al*, 2017). Commonest ones were filamentous, elongated, or globular (Dubey *et al*, 1989). The *Sarcocystis* was reported with different

forms according to species; either stumpy as in *S. zamani* (Mehlhorn *et al*, 1976), spindle-shaped as in *S. idahoensis* (Bledsoed, 1980), streak-like as in *S. crotali* (Entzeroth *et al*, 1985), slender as in *S. clethrionomyelaphis* (Matuschka, 1986), spherical as in *S. hoarensis* (Matuschka and Bannert, 1989), ovoid as in *S. moulei* (Abdel-Ghaffar *et al*, 1989), filiform as in *S. dubeyella* (Stolte *et al*, 1998), elliptical as in *S. felis* in cats (Elsheikha *et al*, 2006) or thick thread as in *S. corvusi* in jackdaw (Prakas *et al*, 2013). The morphological characters of primary cyst wall played a pivotal role in the genus *Sarcocystis* identification (Mehlhorn and Heydorn, 1978; Dubey *et al*, 2006). According to primary cyst wall, mature *Sarcocystis* was basically differentiated into thick-walled if the wall outer portions have prominent long projections or thin walled if the protrusions were short or disappear (Dar *et al*, 2017). Hence, the comparative parameter of species difference was restricted to the mature cysts (Abdel-Ghaffar *et al*, 1990; 2009; Modrý *et al*, 2000; Mehlhorn, 2008; Latif *et al*, 2015) as young cysts was somewhat similar.

In the present study, a unipolar membrane limit coccidian parasitophorous vacuole led to rise of recovered macroscopic *Sarcocystis* (Scholtyseck *et al*, 1974; Mehlhorn and Heydorn, 1978; Abdel-Ghaffar *et al*, 1989, Al-Quraishi *et al*, 2005). The cyst wall formation from the parasitophorous vacuole wall during cyst development was a typical feature of all *Sarcocystis* species. The origin of cyst wall was controversies. Some authors reported that it was of a host origin, others suggested that it was of a parasitic origin, but the third opinion claimed that the wall originated from both sources (Mehlhorn and Heydorn, 1978). Post-initial parasitophorous vacuole wall was formed and cyst formation began, the underlying layer of osmiophilic material strengthened to become the primary cyst wall (Mehlhorn, 2008; Dubey *et al*, 2016). The primary cyst wall structure widely varied among different developmental stages, and thus the cyst fine structure wall was

used with care, as species an identification criterion (Scholtyssek *et al*, 1974; Modrý *et al*, 2000; Dubey *et al*, 2006).

In some *Sarcocystis* species, the wall was thick and folded many times forming irregular wall folds with numerous cauliflower projections of variable size and shape, with knob-like electron dense elevations accompanied by projections. This agreed with Abdel-Ghaffar *et al*. (1989) in buffaloes. The primary wall and its complex structures a layer of fibril material of the host origin enclosing the parasitized host cell appeared as a secondary cyst wall, with a significant response from the host to parasite (Mehlhorn and Scholtyssek, 1973, Abdel-Ghaffar *et al*, 1989; Ahmadi *et al*, 2015). Under the primary cyst wall a relatively thin homogenous layer or ground substance extended outward via protrusions middle portion leaving a clear zone on both side inward via the cyst as septa, one of the characteristic features of genus *Sarcocystis* (Hu *et al*, 2017). The septa divided the cyst cavity into many chamber-like structures with two parasitic forms: the merocytes & merozoites. The globular merocytes occupied the cyst peripheral portion (chambers) just below the ground substance, with banana shaped merozoites concentrated in central region. This agreed with Matuschka and Bannert (1989) and Dubey *et al*. (2017). The present study coincided with *S. fusiformis* recorded in Iraqi buffaloes by Dakhil *et al*. (2017) focused on parasite macroscopic genetic identification.

Conclusion

The present study is the first documented light and ultrastructural study that confirmed the taxonomy of *Sarcocysts* isolated from the buffaloes in Baghdad as *Sarcocystis fusiformis*

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Explanation of figures

Fig. 1 (a, b): *Sarcocystis fusiformis* (arrows) embedded in a buffalo esophagus (a) and isolated (b). Macroscopic spindle shaped and whitish sarcocystis embedded in host muscles.

Fig. 1 (c, d): Photomicrograph showing: (c) a histological section through host muscles of esophagus infected with a parasite sarcocyst within parasitophorous vacuoles (PV) and stained with H&E. Cyst bordered with wall (CW) underlined by a layer of ground substance extended into the interior of the cyst as septa dividing it into compartments enclosing merozoites (ME), x 140. (d) Semi-thin section through a sarcocyst (Sc) stained with toluidine blue showing cyst compartments separated from each other by septa (SE), x 240

Fig. 2 (a-c): TEM of ultrathin section through sarcocysts showed ultrastructural characteristics of primary cyst wall underlined by a layer of thick ground substance (GS) extended into cyst as septa dividing it into compartments. Primary cyst wall with cauliflower-like protrusions (PT) of variable size, and shape with knob (KB) like structures surrounding each protrusion outer layer with internal microtubules (MT). HC abbreviated for host cell. x 13,000; x 22,000

Fig. 2 (d, e): TEM showed (d) different cross sections through merozoites (ME). Apical complex structural characteristics for motile infective stages as micronemes (MN), rhoptries (RH), and conoid (C). MP for a micropore. X 17,000. (e) TEM showed ultrastructural characteristics of the apical complex of merozoites. Anterior polar ring (APR) is the terminal part of merozoite, mechanical penetrating organ represented by two conoids (C), secretory organelles represented by micronemes (MN) and rhoptries (RH). X 25,000

