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RESEARCH ARTICLE

Sarcocystis fusiformis (Railliet, 1897) infecting water buffaloes (Bubalus bubalis) in Dakahlia Province, Egypt

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Abstract

A great problem was raised about the imported Indian buffalo meats which compensate the decrease in the meat industry in Egypt. This is due to the infection of the Indian meat with *Sarcocystis* species specially the prevalent macroscopic *S. fusiformis.* So, we planned this work in order to update the prevalence and distribution patterns of *S. fusiformis* infecting the Egyptian water buffaloes, as well as studying their ultrastructure. Tissue specimens from esophagus, heart, tongue, diaphragm and throat muscles were recovered from 550 water buffaloes slaughtered at Mansoura abattoir in Dakahlia Province, Egypt, at the period between July 2009 and June 2010. *S. fusiformis* cysts were recovered from 58.72% of the examined animals. Aged buffaloes were more infected than young buffaloes. The prevalence of infection was 100% in the esophagus, while no infection was detected in the heart. Ultrastructurally, the cyst wall was thin (1-3µm) exhibiting highly branched and anastomosed cauliflower-like villar protrusions.

INTRODUCTION

Sarcocystis spp. are cyst-forming intracellular protozoan parasites with an obligate two host life cycle between predators as final hosts and their prey animals as intermediate hosts. *Sarcocystis spp.* are highly prevalent in livestock animals and are considered to be very host specific. Most *Sarcocystis species* have been named based on their intermediate host occurrence and their sarcocyst structure. The morphology of *Sarcocystis spp*. has received more attention macroscopically, microscopically and ultramicroscopy.

Dubey et al. (1989 a), and Dubey and Odening (2001) recognized 35 types of sarcocysts based on the structure of the sarcocyst walls. Water buffaloes (*Bubalus bubalis*) harbour four *Sarcocystis spp.* (Huong 1999). Two macroscopic *(S. fusiformis* and *S. buffalonis)* with cats as definitive hosts and two microscopic (*S. levinei* with dogs as definitive hosts and *S. dubeyi* with unknown definitive host but thought to be zoonotic). The infection of water buffaloes with macroscopic *Sarcocystis* cyst renders the meat unmarketable leading to downgrading and condemnation of the carcass.

S. fusiformis infecting water buffaloes (Bubalus bubalis) was studied in different countries and showed high incidences like, 83.3% in Turkey (Retzlaff and Weise, 1969), 94% in China (Xiao et al., 1988) and 88% in Vietnam (Huong et al., 1995). In Egypt, reports about *S. fusiformis* prevalence were variable from high (100% Ghaffar et al., 1978) to low (6.9% El-Dakhly et al., 2011). Many authors studied the ultrastructure of *S. fusiformis* in water buffaloes (Ghaffar et al., 1978; Kan and Dissanaike, 1978; Dubey et al., 1989 b; Claveria and Cruz, 1999; Khalifa et al., 2008 and Jehle et al., 2010).

In Egypt, due to the increased demands on protein foods in consistence with the sharp decrease in meat industry, the government refuge to the importation of water buffalo meat from India with possible introduction of *S. fusiformis* cysts which attract our interest to update the knowledge about their prevalence and distribution patterns. In addition

to that, studying the ultrastructure of *S. fusiformis* cysts in tissues of slaughtered buffaloes in Dakahlia Province, Egypt

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Material and methods

Study area, animals and specimens:

Tissue specimens from esophagus, heart, tongue, diaphragm and throat muscles were recovered from 550 water buffaloes slaughtered at Mansoura abattoir at Dakahlia Province, Egypt, at the period between July 2009 and June 2010. Animals under investigation were assigned into two age groups, the first one was over five years of age (n=400), while the other group was 2-3 years old (n=150). Fresh samples were obtained, and then transported in an ice box to the Parasitology laboratory, Faculty of Veterinary Medicine, Mansoura Univeristy.

Examination of the collected specimens:

Detection of *S. fusiformis* cysts was done by visual inspection of the muscular tissues. The revealed cysts were dissected out and measured by transparent plastic ruler. Samples were then cut off into about 1 cm^3 thick specimens, fixed by neutral buffered formalin 10% and processed for histopathological technique (Bancroft and Stevens, 1996) through dehydration in graded ethanol, embedded in Paraffin wax, sectioned at 5µm in thickness, stained by hematoxylin and eosin and examined under ordinary light microscope. Photos were taken by Digital Camera (AGFA 12 mega pixel). For ultrastructural studies, specimens were fixed in 2.5% cold gluteraldehyde and processed for TEM according (Dubey et al., 1989a). Sarcocysts were located in 1 µm-thick resin sections stained with aqueous toluidine blue. Ultra-thin sections were obtained from the sarcocysts at a thickness of 60-80 A^o by means of diamond knife, collected on copper grids, stained by uranyl acetate and then lead citrate, and Examined by TEM at Faculty of Science, Ain Shams University.

Results

a- Prevalence and distribution of *S. fusiformis* cysts:

Examination of buffalo's muscular tissues revealed a relatively high prevalence of *S. fusiformis* cysts 58.72% (323 out of 550 examined animals). The investigated slaughtered buffaloes were assigned into two age groups. The prevalence of *S. fusiformis* infection was higher in older animals 67.5 % (270 out of 323 infected animal) than in younger ones 35. 33 % (53). Concerning distribution patterns of sarcocysts in different tissues, It is found that esophagus (100%) was infected with *S. fusiformis* cysts in the all examined positive animals, while no infection was reported in the heart. Variable prevalences were noted in throat muscles 56.35% (182 out of 323 infected animal), tongue 40.25% (130) and finally the diaphragm 14.86% (48).

b- Morphological description of *S. fusiformis* cysts:

1-: Gross appearance: Sarcocysts of *S. fusiformis* appeared as macroscopic (4-35 X1-8 mm) broad spindle or fusiform shaped, may be round, milky-white opaque cysts resembling large rice grains or cucumber seeds and located superficially along the longitudinal axis of muscle fibers. Sometimes, embedded deeply in the tissues.

2- Histological examination: Examination of the histological (stained by H&E) and semithin sections (stained by toluidine blue) revealed that the cyst had thin cyst wall (1-3 µm in thickness) projecting highly branched villar protrusions toward the parasitophorous vacuolar membrane which is nucleated and formed from the intact muscle fibers layer. Metrocytes are found on the periphery of the sarcocyst and bradyzoites toward the center. Moreover the center of older sarcocysts is empty and devoid of bradyzoites or metrocytes**.**

3- Ultrastructure Examination: Sarcocysts wall exhibited highly denderitic and branched cauliflower-like villar protrusions, and the primary cyst wall has irregularly spaced invaginations (30-60 nm). Due to the branching, anastomosing status, and the discontinuity of the villar protrusions, the length of them is so difficult to be measured. Numerous course electron-dense granules are scattered between the microfilaments within the villar protrusions. Ground substance layer is found to be located under the PCW with a thickness of 1.5-3.3 µm and contains few and fine electron dense granules. Ground substance is deeply invaginated into the sarcocyst to form prominent septa (1-2.3 µm thick).

Metrocytes of *S. fusiformis* were globular or oval shaped and pale colored, their size ranged from 5-8.5 X 4-6 µm. The most characteristic feature of the metrocytes is the deeply invaginated double membranous pellicle (PE). They might contain small lipid droplet (L), large amylopectin granules (Ag), rough endoplasmic reticulum (RET), and a large nucleus (1.5 X 2.5 µm) with small pale and large dense granules. Also, metrocytes contain rhoptries (Rh) and micrinemes (Mn).

Bradyzoites are banana or crescent shaped cells with the anterior end more pointed than the posterior one and measured 10.5-17 X 2-3 µm. The whole zoite is surrounded by a double membranous pellicle (PE). Apical complex structure is found in the anterior region of the zoite and composed of, conoid (C) which measured nearly 400 nm

long, 300 nm wide at the base and 150 nm wide at the apex. Also, 8 rhoptries and about 300-400 elongated micronemes are found. The middle zone of the zoite is filled with large amount of different sized (150-600 nm) amylopectin granules (Ag) which might extended to the anterior zone. Posteriorly, a large nucleus with corrugated edges (1.5 X 2.5 µm) is located subterminally and contained different types of electron granules, small pale and large dense granules. Near to the nucleus, Mitochondria is situated.

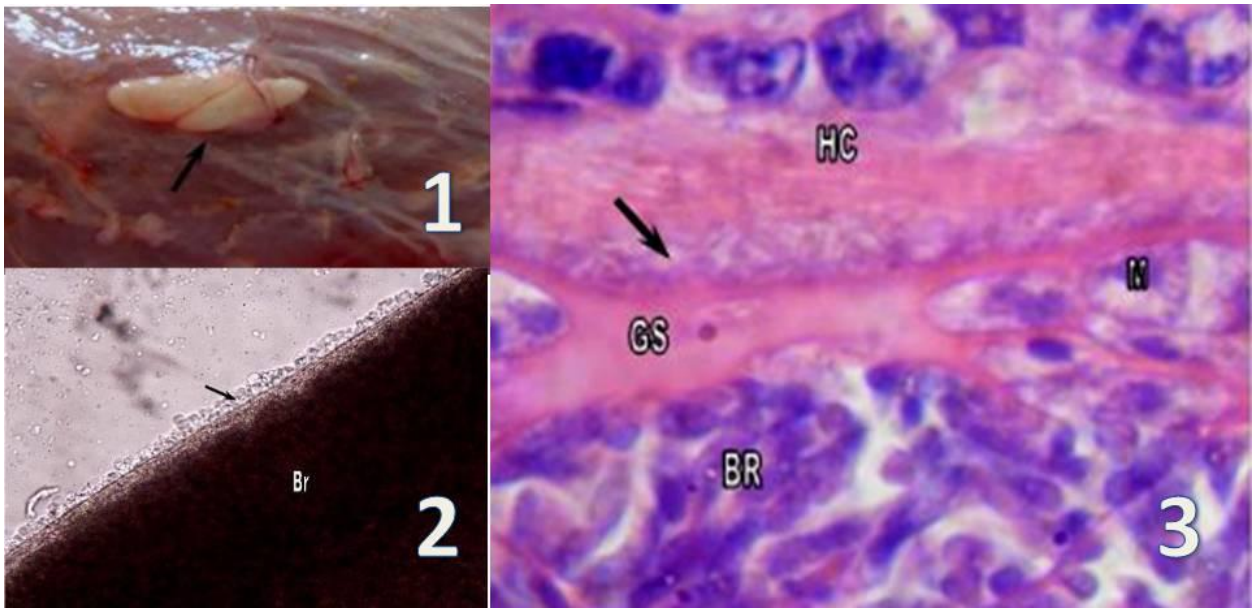


Fig. (1): Gross picture of Large spindle shaped *S. fusiformis* sarcocyst (arrow) in the esophagus.
Fig. (2): Fresh preparation of *S. fusiformis* sarcocyst. Note the highly branched villar protrusions (arrow). Br: bradyzoites. Stereomicroscope X 4.
Fig. (3): Histological section of *S. fusiformis* sarcocyst, H&E staining. Note the branched villar protrusions (arrow), bradyzoites (BR), metrocytes (M), ground substance (GS) and the host cell (HC). X100.
Fig. (4): Semithin section of *S. fusiformis*. Sarcocyst, Toluidine blue staining. Note the highly branched villar protrusions (VP), bradyzoites (BR), metrocytes (MET), ground substance (GS) and the host cell (HC). X 100.

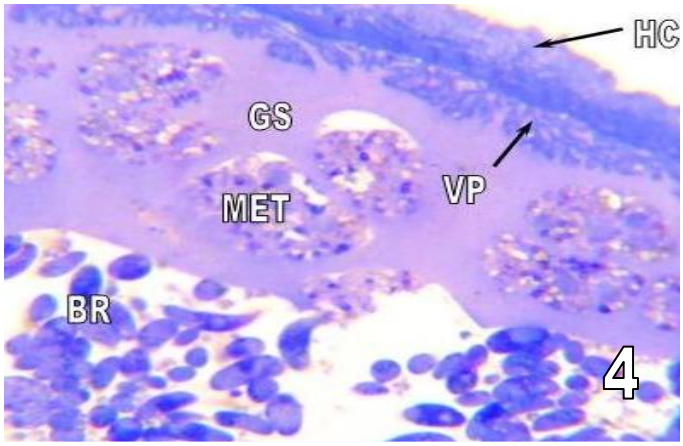
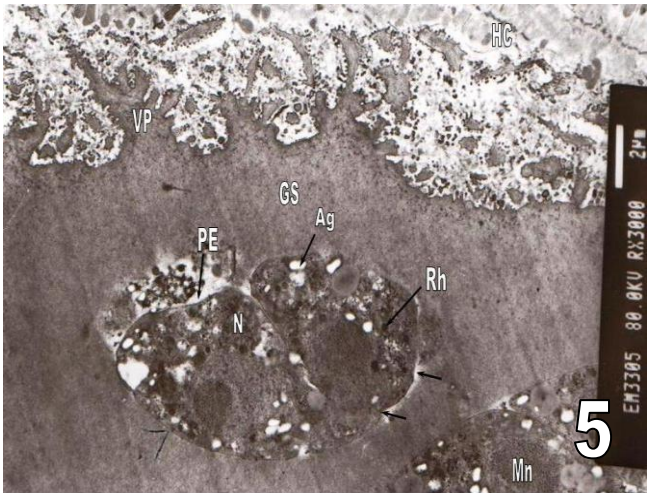
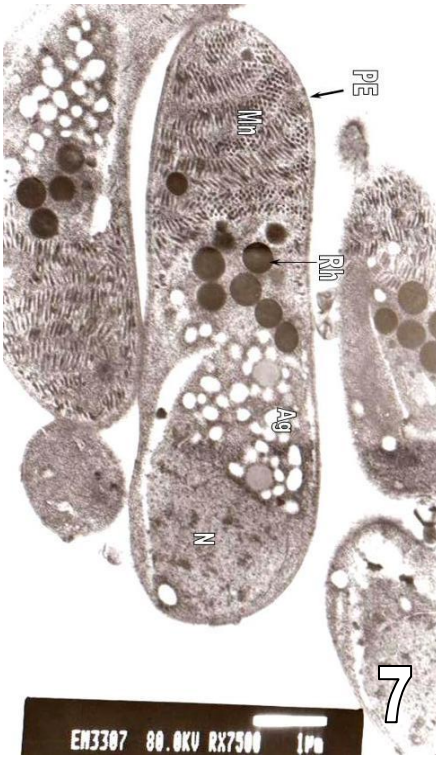


Fig. (5): TEM picture showing *S. fusiformis* cyst wall and the metrocytes showing the highly branched cauliflower-like villar protrusions (VP), and the pale coloured metrocytes bounded by a double membrane pellicle and contain nucleus (N), amylopectin granules (Ag), rhoptries (Rh) and micronemes (Mn). (GS) ground substance and (HC).the host cell. Scale bar = 2 µm.





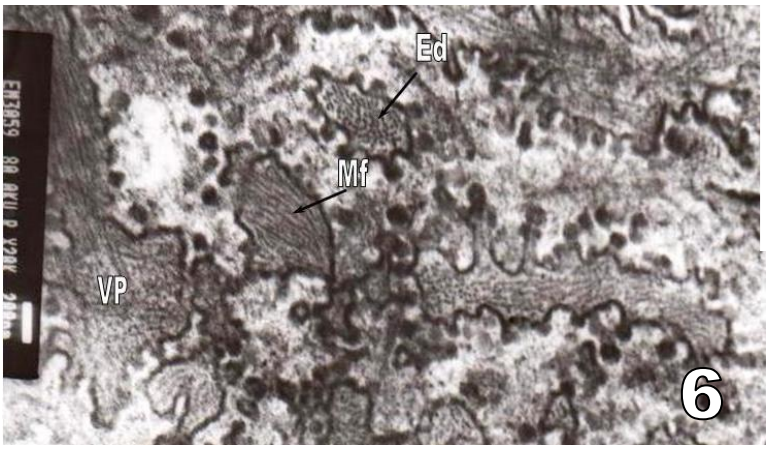


Fig. (6): TEM picture showing *S. fusiformis* villar protrusions (VP). Note the highly branched cauliflower –like VP occluded with microfilaments (Mf) and electron dense granules (Ed). Scale bar = 200 nm.
Fig. (7): TEM pictures showing *S. fusiformis* bradyzoite. Note the double membrane pellicle (PE), micronemes (Mn) and rhoptries (Rh) in the anterior third, amylopectin granules (Ag) in the midddle and the posteriorly located nucleus (N). Scale bar = 1 µm.

Disscusion

The recorded high incidence of *S. fusiformis* (58.72%) in this work indicates the widespread existence of their sporocysts in the environment, which attributed to the abundance of definitive host (cat) El-Dakhly et al. (2011), and the ability of sporocyst to survive in unsuitable harsh environmental conditions (Savini et al., 1996). Previous investigations recorded high incidences of *S. fusiformis* in water buffaloes like 83.3% in Turkey (Retzlaff and Weise, 1969), 100% in Egypt (Ghaffar et al., 1978), 94% in China (Xiao et al., 1988) and 88% in Vietnam (Huong et al., 1995). Other authors mentioned lower incidence as Camisasca et al., 1996 (33% in Italy), Latif et al., 1999 (15.6% in Iraq), Khalifa et al., 2008 (28% in Egypt) and El-Dakhly et al., 2011 (6.9%). Moreover, Oryan et al. (2010) found no *S. fusiformis* infection in the Iranian water buffaloes.

ur results revealed that the older age of the animal, the high prevalence of infection. This finding which is most likely due to a longer exposure periods of aged animals to the sporocysts infection and the cysts needed long time to appear macroscopically, is coincided with Huong (1999), El-Dakhly et al. (2011) and Dubey et al. (1989a).

In the present study, esophagus, heart, tongue, diaphragm and throat muscles were examined, as recommended by previous investigations to be the predilection sites of *Sarcocystis* infection in the water buffaloes (Huong, 1999) and Khalifa et al., 2008). Evidently, distribution of sarcocysts does not follow a specific pattern in most of the affected organs in buffalo (El-Dakhly et al., 2011).The obtained results showed that esophagus was the mostly affected

tissue, which agreed with that reported by Latif et al. (1999), Huong et al. (1995), Dundar and Ozer (1993) and Abbas (2008).

Concerning the morphological features of the revealed *Sarcocystis* species, the spindle shaped cysts (4-35 X 1-8 mm) in the present study was similar to that reported by Ghaffar et al., 1978 (7-30 X 3-7 mm), Dubey et al., 1989b (up to 32mm) and Huong, 1999 (3-38 mm), but smaller than that noted by Kan ad Dissanaike, 1978 (1-2.5 X 0.5-5 cm), and larger than Claveria and Cruz, 1999 (1-18 X 1-7 mm) and El-Dakhly et al., 2011 (1.2-19 X 0.7-7 mm).

Light microscopic description of *S. fusiformis* cyst revealed a thin cyst wall (1-3 µm) with branched villar protrusions. These results were in agreement with Ghaffar et al. (1978), Claveria and Cruz (1999) and Huong, 1999 (1-2 µm), while disagreed with El-Dakhly et al., 2011 (2.6-14.5 µm thick cyst wall).

Furthermore, the cyst wall in this study was typically type 23 (according to Dubey et al., 1989a) exhibiting highly branched cauliflower-like villar protrusions. This is harmonized with that noted by Kan and Dissanaike (1978), Dubey et al. (1989 b), Wang et al. (1991), Claveria and Cruz (1999), Arafa et al. (2003), Khalifa et al. (2008) and Jhele et al (2009).

We couldn't measure the length of the villar protrusions in this work due to their branched and anastomosed nature, this is agreed with Huong (1999) but disagreed with Kan and Dissanaike (1978) who found that the length of the villar protrusions was 5.4 µm. The ground substance layer in the present investigation was 1.5-3.3 µm thick and contain few and fine electron dense granules. These results were coincided with that noted by Kan and Dissanaike, 1978 (2.5 µm), Tongson and Molina, 1979 (1.4-3.75 µm), Huong, 1999 (1.5-3.7 µm), and Khalifa et al., 2008. Moreover, the morphological features of the metrocytes or bradyzoites is not a vital point for differentiation between *Sarcocystis* species (Huong, 1999 and Dubey et al., 1989a).

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