Animal Health Research Journal Vol. 7, No. 4, November 2019 pp. 903-914

ISSN: 2356-7767

Incidence and identification of *Sarcocystis* species and its patholgical effects in water buffaloes (*Bubalus bubalis*)

Neveen, S. Satour[*]; Samy, S. Mohammed[**] and Abd-Alla, S. Hamed[***]
[*]Parasitology Department, Animal Health Research Institute, Alexandria Lab.
[**]Parasitology Department, Animal Health Research Institute, Zagazig Lab.
[***]Parasitology Department, Animal Health Research Institute, Zagazig Lab.
Animal Health Research Institute (AHRI),
Agriculture Research Center (ARC), Egypt.

Received in 04/10/2019
Accepted in 22/10/2019

Abstract
Sarcocystis species have the ability to infect wide range of animals, birds and reptiles. The current study was established to determine the prevalence of *Sarcocystis* spp., morphological, pathological changes and its molecular identification in Egyptian water buffaloes in Alexandria governorate. Samples were collected from esophagus and tongue muscles of 117 slaughtered buffaloes. The result revealed that the percentage of infection was 25.6% with macroscopical examination and 29.1 % with microscopical examination. The samples were macroscopically, microscopically and histopathologically examined. Macroscopic and microscopic cysts were detected. They were crushed, stained with Giemsa stain and morphologically described. Histopathological examination revealed degenerative changes, oedema in between infected muscle fibers and coagulative necrosis with some inflammatory cells infiltration. Also, *Sarcocystis* spp. was molecularly identified using 18S ribosomal subunit DNA through PCR and DNA sequencing. Sequencing and genotyping revealed one isolate had 100% identity to *Sarcocystis cruzi*.

Keywords: *Sarcocystis species, water buffaloes, PCR, 18S ribosomal subunit DNA.*

Introduction

Sarcocystis species (Apicomplexa: *Sarcocystidae*) are coccidian parasites of warm blooded and poikilothermic animals, including humans. They have two-host, prey-predator life cycle pattern, with herbivores as intermediate hosts and carnivores as definitive hosts **(Dubey *et al.* 2015).**

Cattle and water buffaloes are the intermediate hosts of some important species of *Sarcocystis*, that is, may harbor macro or micro *Sarcocystis* on their muscles. Intermediate hosts become infected with the parasite via ingesting sporocysts or sometimes sporulated oocysts existed in the food or water **(Dubey and Lindsay 2006).**

The parasites are most prevalent in livestock animals all around the world which the infection rate in cattle reported up to 100 percent in many countries **(Dubey *et al.* 1989)**. Cattle are mainly infected with *Sarcocystis cruzi*, *Sarcocystis hominis* and *Sarcocystis hirsute* **(Dubey and Lindsay 2006)** and water buffaloes are usually infected with *Sarcocystis fusiformis*, *Sarcocystis levinei*, *Sarcocystis dubeyi* and *Sarcocystis buffalonis* **(Dubey *et al.* 1989)**. Some *Sarcocystis* species induce weight loss, general weakness, fever, anorexia, abortion and death in domestic animals but, macrocyst inducing *Sarcocystis* species are often considered as economic loss producers in slaughterhouses **(Dubey *et al.* 1989)**.

Sarcocystosis is a zoonotic and parasitic disease commonly seen in domestic animals such as buffaloes, cattle, and pigs. Among these, *Sarcocystis hominis* has a significant impact on public health. Meats and meat products are the main source of infection in human beings, who become infected when ingesting well-developed tissue cysts containing bradyzoites **(Juyal and Bhatia 1989).**

In fact corresponds to *Sarcocystis cruzi*, occu-

pying an intermediate host range that is larger than previously understood. A report employing genetic and ultrastructural methods to investigate the parasites of cattle and water buffalo in Vietnam concluded that certain parasites are shared by water buffalo and cattle **(Jehle *et al*. 2009).**

Dubey *et al*. (1989) recorded that all *Sarcocystis* species found in livestock show high specificity at the level of intermediate hosts. For example, those species infecting cattle including *S. cruzi* are not supposed to occur in water buffaloes and vice versa. In support of this, **Jain and Shah (1985)** performed the first cross-transmission studies of *S. cruzi* from cattle and were unable to infect water buffaloes. On the other hand, **Wang *et al*. (1992) and Xiao *et al*. (1993)** reported cross transmission of *Sarcocystis* species between water buffalo and cattle and demonstrated the infection of water buffalo with *S. cruzi.* **Badawy *et al*., (2012)** mentioned that there were many economic impacts of *Sarcocystosis*. The pathogenic species affect cattle may lead to abortion, reduced milk yield, neurologic signs, and loss of weight.

The present work is an attempt to study the prevalence, morphological identification, pathological changes and molecular characterization of different *Sarcocystis* species infecting slaughtered buffaloes in Alexandria governorate.

Materials and Methods

Sample collection: A total number of 117 water buffaloes (*Bubalus bubalis*) aged 2-3 years and over 5 years were surveyed for the presence of *Sarcocystis* during the period from February to August 2019 from Alamria and Abies abattoirs in Alexandria governorate. Samples were sent to the laboratory of Parasitology Department of Animal Health Research Institute; Alexandria. Tissue samples were collected from esophagus and tongue of each freshly slaughtered animal, preserved in clean labeled bags and transported in ice box to the Parasitology Laboratory for further investigation**.** Specimens were kept refrigerated prior to examination.

Examination of macrocysts by naked eyes: Each tongue and esophagus muscle sample was cut into small pieces and examined by naked eyes for the presence of macrocysts **(Lam *et al*. 1999).**

Examination of microcysts by compression technique: For detection of microscopic *Sarcocystis* cysts, small pieces of fresh muscle were compressed between two slides and examined microscopically with 100x magnification **(Latif *et al*. 1999).**

Morphology of bradyzoites and metrocytes: Some of macrocysts were crushed on clean microscopic slides with a few drops of saline, left to dry, fixed with methyl alcohol and stained with Giemsa stain. Microcysts were separated and picked out from between muscle fibers by means of needles under the microscope, then crushed on microscopic slides and stained also with Giemsa stain. Morphology of bradyzoites and metrocytes were described microscopically under oil immersion lens (x1000).

Histopathological studies: Muscular samples (tongue and esophagus) collected from slaughter animals were preserved in 10 % neutral buffered formalin solution till send to laboratory. Positive samples from macroscopic and microscopic examinations were dehydrated, cleared and embedded in paraffin wax, then they were sectioned to 4-5 micron thickness and stained with Hematoxylin and Eosin stain (H&E) and examined microscopically **(Survarna *et al.*, 2013)**.

Molecular identification using 18S rRNA: Small pieces of infected tongue and esophagus muscle with microscopic cysts were incubated in ethanol (70%) for DNA analysis. After removing the ethanol, genomic DNA was extracted.

DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at $56^{O}C$ overnight. After incu-

bation, 200 µl of AL buffer was added to the lysate, incubated for 10 min. at 72^{O}C, then 200 µl of 100% ethanol was added to the lysate. The lysate was then transferred to silica column, centrifugated. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit **(Bahari *et al*., 2014).**

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in table (1).

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpiolt 100 bp ladder (Qiagen, Gmbh, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Primary denaturation	Amplification (35 cycles)			Final extension	Amplified segment (bp)	Reference
			Secondary denaturation	Annealing	Extension			
***Sarcocyst* 18S rRNA**	GCACTT-GATGAATTCTGG CA CACCACCCA-TAGAATCAAG	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	600	**Bahari *et al*., (2014)**

Sequencing and phylogenetic analysis: PCR products were purified using QIAquick PCR product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool). **Altschul *et al*. (1990)** was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module of Lasergene DNA Star version 12.1 **(Thompson *et al*., 1994)** and phylogenetic analyses was done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 **(Tamura *et al*., 2013).**

Results

Prevalence of macroscopic and microscopic *Sarcocystis* cysts: Two muscle samples (tongue and esophagus) from 117 water buffaloes were examined macroscopically and microscopically. The animal considered to be positive, if *Sarcocystis* cysts were found in one or both of the examined muscle samples (tongue and esophagus). Macroscopic cysts were detected in 25.6% (30/117) of examined animals, while microscopic cysts were detected in 29.1% (34/117) of them (table2).

Table (2). Prevalence of macroscopic and microscopic *Sarcocystis* cysts in water buffaloes:-

No. of examined animals	No. of +ve animals infected with macroscopic cysts	%	No. of +ve animals infected with microscopic cysts	%
117	30	25.6	34	29.1

Table (3) showed that the prevalence of macroscopic and microscopic *Sarcocystis* cysts according to age were 16.4% (9/55) and 21.8 % (12/55) respectively in samples from buffaloes aged between 2-3 years old and 33.9 % (21/62) and 35.5% (22/62) respectively in samples from buffaloes over 5 years old. The prevalence of macroscopic and microscopic *Sarcocystis* cysts was higher in females 35.1% (20/57) and 33.3% (19/57) than in males 16.7% (10/60) and 25% (15/60) respectively.

Table (3). Prevalence of macroscopic and microscopic *Sarcocystis* cysts in water buffaloes according to age and sex:-

		No. of examined animals	Macroscopic examination		Microscopic examination	
			No. of animals	%	No. of animals	%
Age	**2-3 years**	55	9	16.4	12	21.8
	Over 5 years	62	21	33.9	22	35.5
Sex	**Female**	57	20	35.1	19	33.3
	Male	60	10	16.7	15	25

Morphology of macrocysts: The macrocysts appeared opaque white in color, spindle shaped or rice seeds shaped and located sometimes under the serosal sheet of the esophageal muscles (Fig.1) and deeply situated within tongue muscles (Fig.2). Macroscopic cysts ranged in size from 2.5-3 × 1-1.2 mm.

Morphology of microcysts: With compression technique, *Sarcocystis* cysts appeared elongated with one rounded end and other more or less pointed one (210-236 x 42-48 µm) running in between esophageal muscle fibers and parallel with them (Fig.3). Stumped shaped cysts also seen in in between esophageal muscle fibers (142-170 x 54-60 µm) (Fig.4). Cylindrical shaped cysts (420-486 x 31-38 µm) appeared in between muscle fibers of tongue (Fig.5). The microcysts had thin and smooth wall and divided internally with trabeculae into several compartments.

Morphology of bradyzoites and metrocytes: Bradyzoites and metrocytes from macrocysts of esophageal muscle fibers that stained with Giemsa stain, appeared blue in colour with light or dark pink nucleus and dark blue amylopectin granules (Fig. 6 and 7). Bradyzoites were banana-shaped and their width appeared either thin (8.8-10 x 1.1-1.4 µm) or wide (9.0-9.6 x 2.2-2.4 µm) (Fig. 8). Metrocytes were stumped and less curved in shape (4.8-5.6 x 2-2.4 µm) (Fig. 7). Figures 9 and 10 showed that bradyzoites and metrocytes from microcysts of tongue of water buffaloes appeared lightly stained with Giemsa stain and with ill-defined cell walls. Bradyzoites were banana shaped (8.6-9.2 x 2.0-3.1 µm) and some of them appeared with one end more broader than other. Metrocytes appeared stumped in shape (5.4-5.8 x 2-2.2 µm). Some of bradyzoites and metrocytes appeared with ruptured walls.

Histopathological examination: Present investigation showed some pathological changes in esophageal and tongue muscles of water buffaloes infected with *Sarcocystis* cysts. In longitudinal sections, the esophageal muscle showed spindle shaped microscopic cyst with thin, smooth wall, dividing septa and inflammatory cells infiltration around microscopic cyst (Fig.11), *Sarcocystis* cyst appeared filled with bradyzoites. There were loss striation of muscles fibers and inter muscular oedema with few inflammatory cells infiltration (fig.12).

In cross section, the tongue muscles of buffalo, revealed macroscopic cyst with thick cyst wall and oedema around the cyst (Fig.13). Microscopic cyst appeared with thin wall, measured 0.2-0.5 µm, loss of striation and coagulative necrosis of some muscles fibers around the cyst (Fig.14). Some microscopic cysts appeared without separating septa, filled with bradyzoites and oedema in between some degenerated muscle fibers (Fig.15). The esophageal muscle showed macroscopic cyst with thick wall, measured 2-5 µm, bradyzoites tended to be overcrowded at the periphery of the cyst and decrease in number towards the center and with separating septa in addition to intermuscular oedema around the cyst (Fig.16). Fig. (17) showed degeneration and coagulative necrosis of infected muscle fibers with inflammatory cells infiltration.

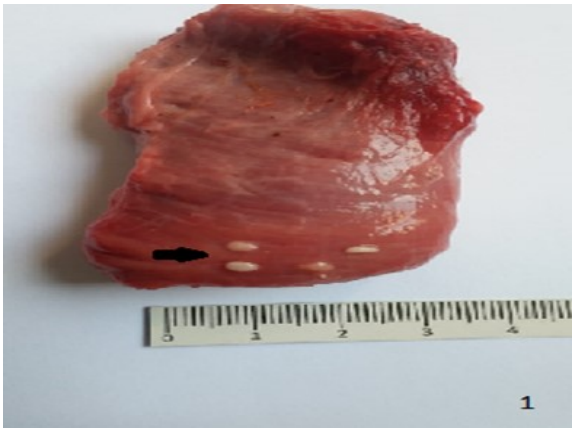

Fig. (1): Macroscopic *Sarcocystis* cysts in tongue muscle of water buffalo.

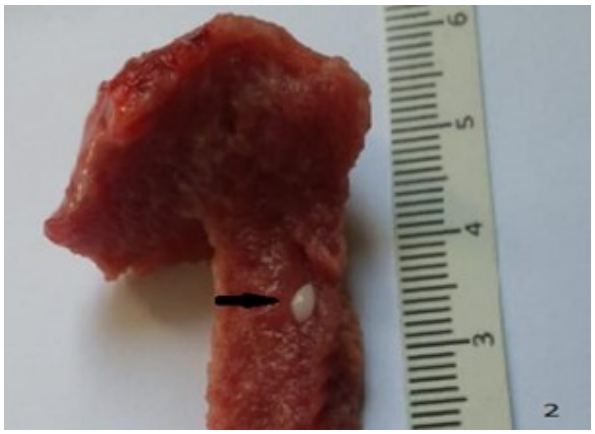

Fig. (2): Macroscopic *Sarcocystis* cysts in esophageal muscle of water buffalo.

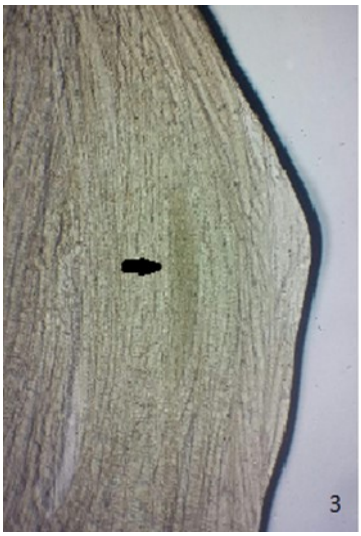

Fig. (3): Elongated microcyst of *Sarcocystis* spp. from esophageal muscle of water buffalo appeared with one rounded end and the other end more or less pointed (x100).

Fig. (4): Stumped shaped microcyst in esophageal muscle of water buffalo (x100).

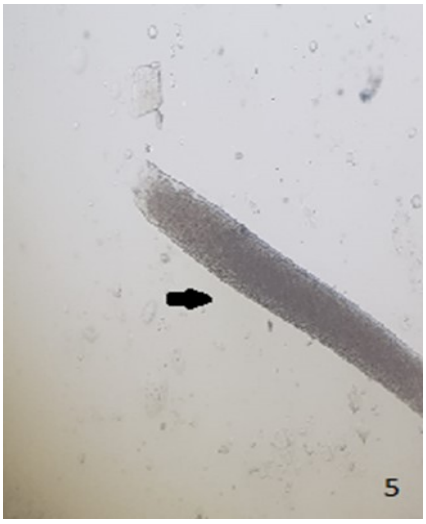

Fig. (5): Cylindrical shaped cyst from tongue muscle of water buffalo (x100).

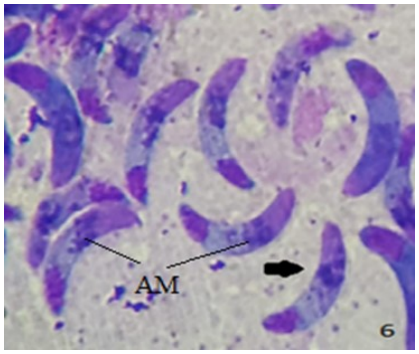

Fig. (6): Banana shaped bradyzoites stained with Giemsa stain from macrocyst of *Sarcocystis* spp. from esophagus of water buffalo (x1000).

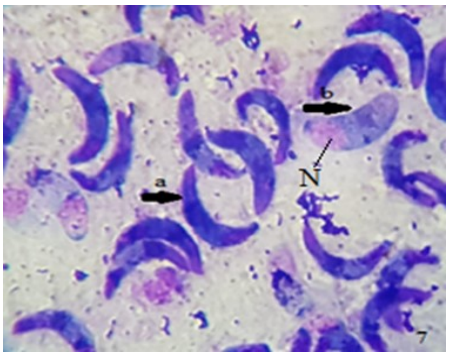

Fig. (7): Banana shaped bradyzoites (a) and stumped shaped metrocytes (b) stained with Giemsa stain from macrocyst of *Sarcocystis* spp. from esophagus water buffalo (x1000).
N: Nucleus. AM: Amylopectin granules.

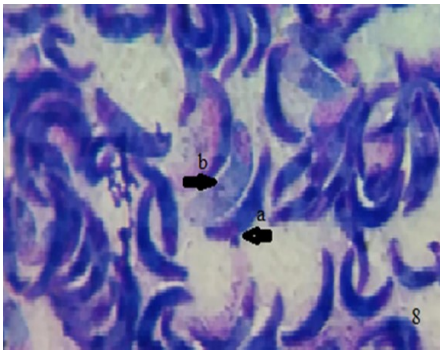

Fig. (8): Thin (a) and wide (b) Banana shaped bradyzoites stained with Giemsa stain from macrocyst of *Sarcocystis* spp. from esophagus of water buffalo (x1000).

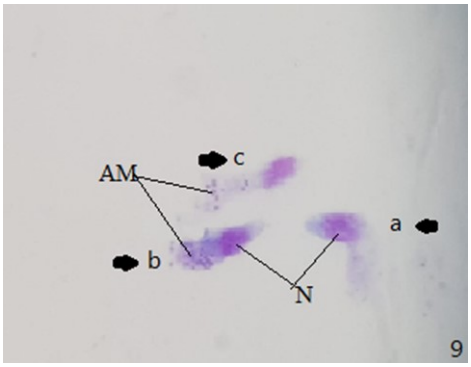

Fig. (9): Bradyzoites with ill-defined cell wall stained with Giemsa stain from of *Sarcocystis* spp. from tongue of water microcyst buffaloes (x1000):
a) Banana shaped bradyzoite.
b) Bradyzoite with one end more broader than other.
c) Bradyzoite with ruptured wall.

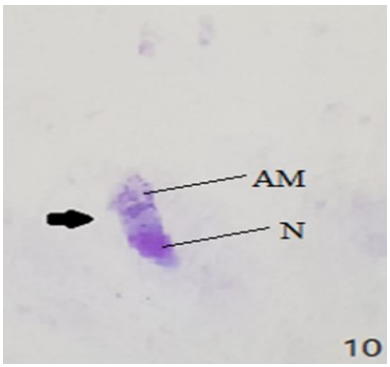

Fig. (10): Stumped shaped metrocyte with ill-defined and ruptured cell wall stained with Giemsa stain from microcyst of *Sarcocystis* spp. from tongue of water buffaloes (x1000).
N: Nucleus.
AM: Amylopectin granules.

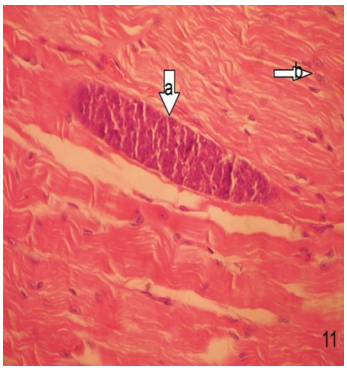

Fig. (11): Longitudinal section of esophageal muscle of buffalo showing spindle shaped microscopic *Sarcocystis* cyst with thin, smooth wall, dividing septa (a) and inflammatory cells infiltration (b). (H&E100)

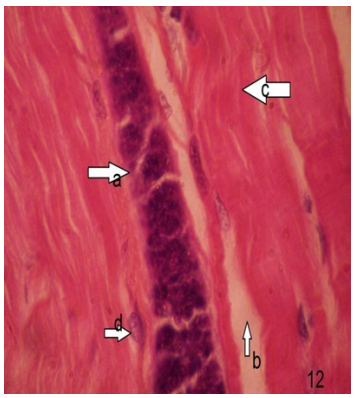

Fig. (12): Longitudinal section of esophageal muscle of buffalo showing *Sarcocystis* cyst filled with bradyzoites (a), inter muscular oedema (b) and loss striation of muscle fibers around microscopic cyst (c) with few infilammatory cells infiltration (d). (H&E1000)

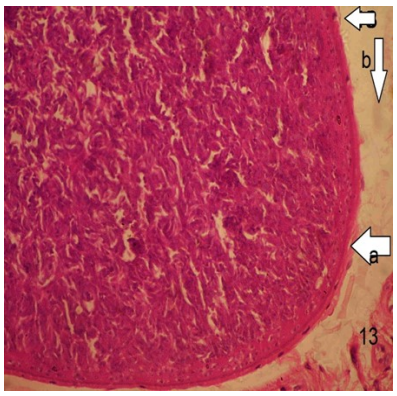

Fig. (13): Cross section of tongue muscle of buffalo showing macroscopic cyst with thick wall (a) and oedema around the cyst (b). (H&E1000)

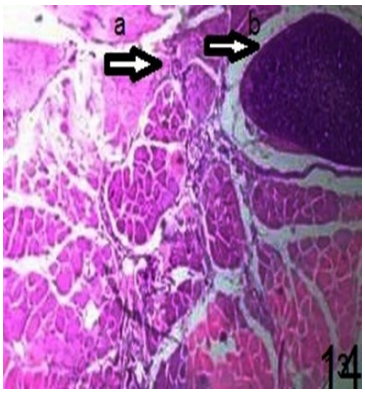

Fig. (14): Cross section of tongue muscle of buffalo showing loss striation and coagulative necrosis of some muscle fibers (a) around thin walled microscopic cyst (b). (H&E100)

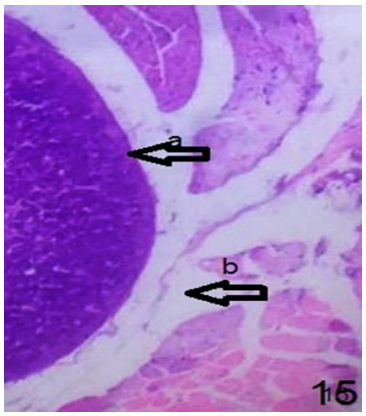

Fig. (15): Cross section of tongue muscle of buffalo showing microscopic cyst appeared without separating septa, filled with bradyzoites (a) and oedema in between some degenerated muscle fibers (b). (H&E400)

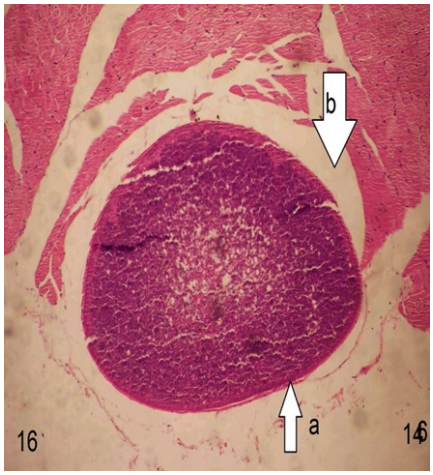

Fig. (16): Cross section of esophageal muscle of buffalo showing macroscopic cyst with thick wall, overcrowding with bradyzoites behind the wall with separating septa (a) and inter muscular oedema around the macroscopic cyst (b). (H&E 400)

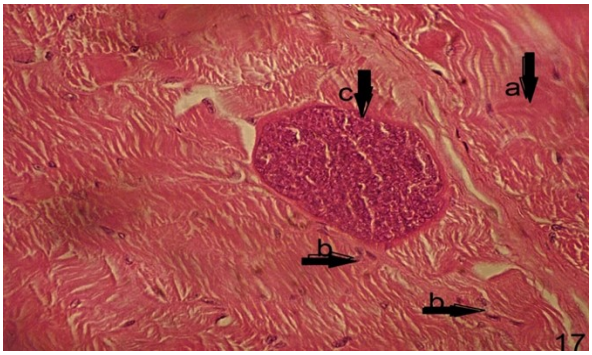

Fig. (17): Esophageal muscle of buffalo showing microscopic *Sarcocystis* cysts with degeneration and coagulative necrosis (a) of infected muscle fibers with inflammatory cells infiltration (b). (H&E200)

Sequencing and genotyping of isolates: The *18S rRNA* gene of the *Sarcocystis* species was amplified and yielded the expected PCR product size (600 bp) from microscopic cyst samples (Fig. 17), then published (GenBank accession number MN396435). As shown in sequence distance figure, the sequenced strains showed 100% identity to *S. cruzi* strains confirming the clustering of the study strain with *S. cruzi.* The closest identities to other strains were as follow; 98.9% (*S. capracanis, S. tenella, S. taeniata* and *S. morae*), 98.5% (*S. fusiformis*), 98.4 % (*S. buffalonis*) (Fig.18). Phylogenetic tree cleared the clustering of the collected *sarcocystis cruzi* with *sarcocystis cruzi* strains (Fig. 19).

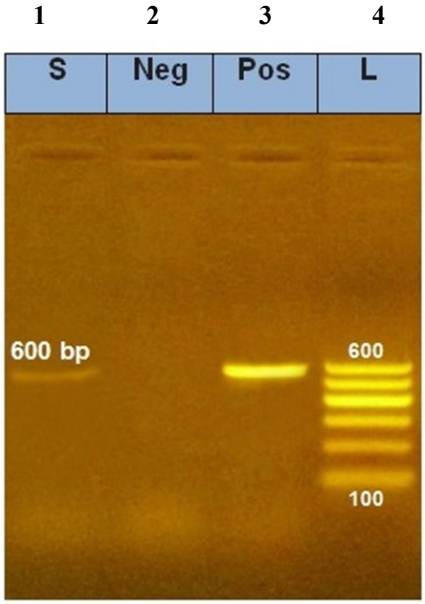

Fig. (17): PCR results for the *18S rRNA* gene of the *Sarcocystis* species showing positive amplification of 600 bp.L [Gelpilot100 bp. ladder (Qiagen, 100-600 bp.)].

Lane 1: Sample Lane 2: Negative control
Lane 3: Positive control Lane 4: 100-600 bp. ladder

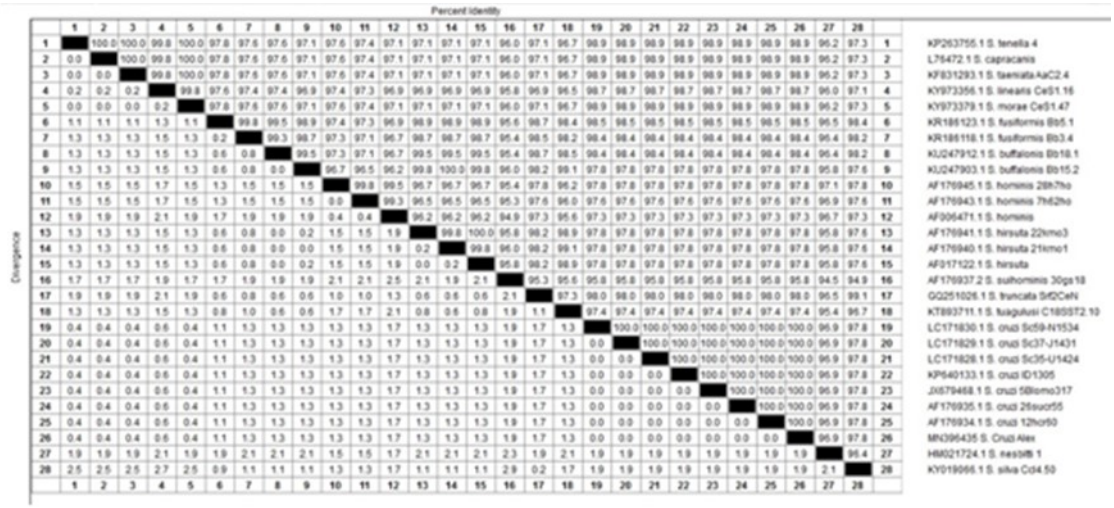

Fig. (18): Sequence distance of the *18S rRNA* gene of the tested *Sarcocystis* strain (generated by lasergene software) showing identity range of 100% with *Sarcocystis cruzi* strains.

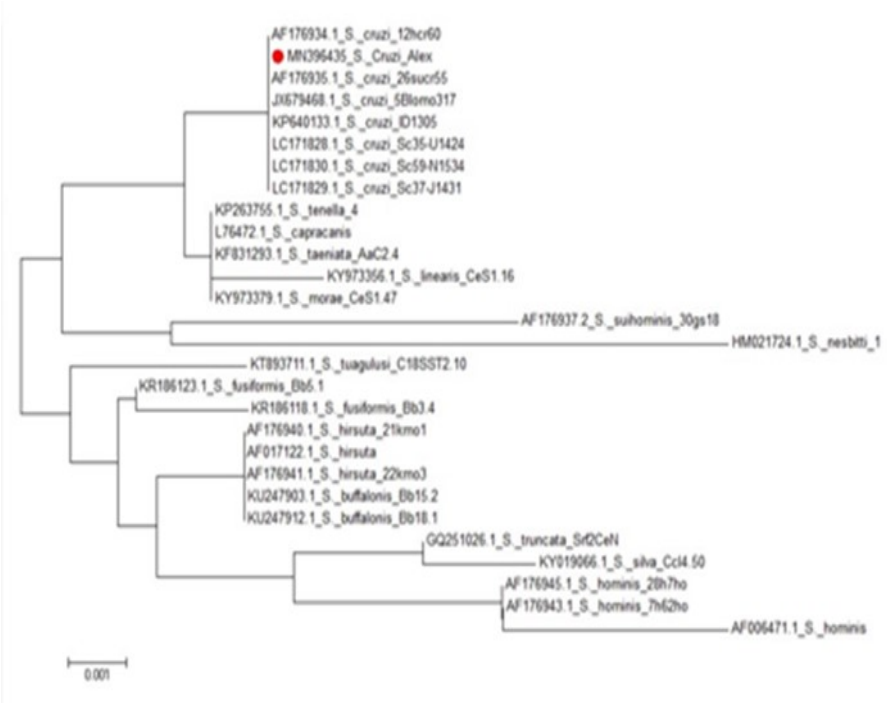

Fig. (19): Phylogenetic relatedness of the *18S rRNA* gene. Maximum-likelihood un rooted tree indicated clustering of the tested strain with *Sarcocystis cruzi* strains apart from other *Sarcocystis* types.

Discussion

In the present study, both morphological and molecular examinations were utilized to identify *Sarcocystis* infection in water buffaloes in some localities in Alexandria governorate.

The prevalence of macroscopic *Sarcocystis* cysts (25.6%) was nearly similar to that recorded by **Metwally *et al*., (2014)** in Egypt (25.5%), **Mousa *et al*., (2016)** in Egypt (23.6%) and **Jyothisree *et al*., (2017)** in Andra Pradesh (22.62%). Higher prevalence was recorded by **El-Shanawany *et al*., (2019)** in Egypt (74%). On the other hand, lower prevalence rate was recorded by **Ahmed *et al*., (2016)** in Egypt (8.33%), **Dar *et al*. (2017)** in Punjab (0.6%) and **Dakhil *et al*. (2017)** in Iraq (2.8%). In the present study the prevalence of microscopic *Sarcocystis* cysts was 29.1%.

Nearly similar infection rate was reported by **Metwally *et al*., (2014)** in Egypt (27.7 %). Higher infection rate was recorded by **Arafa *et al*., (2003)** in Egypt (34.6%), **Latif *et al*., (2013)** in Malaysia (66.7%), **Dar *et al*., (2017)** in Punjab (95.5%), **Jyothisree *et al*., (2017)** in Andra Pradesh (43.79%) and **Murata *et al*., (2018)** in Japan (49.5%). On the other hand, slightly lower prevalence rate was reported by **Mousa *et al*., (2016)** in Egypt (20%)**.** These results indicated that buffaloes were frequently exposed to the infection with *Sarcocystis* species due to close contact with dogs and other wild animals which act as definitive hosts.

The present investigation revealed higher prevalence rate of macroscopic *Sarcocystis* cysts in aged animals (33.9%) than in younger ones (16.4%). The result was nearly agreed with the previous studies obtained by **El-Seify *et al*. (2014**) who reported higher prevalence of macroscopic *Sarcocystis* cysts in old -aged Egyptian water buffaloes (68.2%) than younger ones (17.2%). **Jyothisree *et al*. (2017)** recorded higher prevalence in animals more than 5 years (39.51%) than in those aged 18 and 36 months (13.4%). **Ibrahim *et al*. (2018)** found higher incidence in old buffaloes (48.6%) than in young ones (41.2%). **El-Shanawany *et al*. (2019)** found that the infection rate was 20% in 2- 4 years group and 82.3% in 5-8 years group. The present result indicated higher incidence of microscopic *Sarcocystis* cysts in aged animals (35.5%) than in younger ones (21.8%). **Jyothisree *et al*. (2017)** revealed slight variation in prevalence rate in aged animals (45.83%) and younger animals (42.69%). **Ibrahim *et al*. (2018)** found higher incidence in old buffaloes (63.2%) than in young buffaloes (53.8%). The higher incidence in old animals than in young ones may be due to in old ages there are longer and repeated exposure periods to the infectious sporocysts, also due to lower immunity in old ages.

In the present study, the prevalence of macroscopic *Sarcocystis* cysts was higher in females (35.1%) than in males (16.7%). This was agreed with the earlier report by **Ibrahim *et al*. (2018)** who reported higher incidence in females (37%) than in males (25.8%). **El-Shanawany *et al*. (2019)** found that the infection rate was 90.7% in females and 20% in males. The prevalence of microscopic *Sarcocystis* cysts in the present work was higher in females (33.33%) than in males (25%). The result was agreed with the previous studies obtained by **Arafa *et al*. (2003)** who found higher incidence in females (57.14%) than in males (15%). **Ibrahim *et al*. (2018)** found that the infection rate was 49.6% in females and 36.2% in males. The low percentage of the infected males may be attributed to the animal management system in Egypt, as most of the males were kept only for fattening system and being slaughtered around 2 years old while females were kept for longer time for milk production.

Morphometry and micrometric analysis was compared with the descriptions of previous reports and strongly supports that they are *S. fusiformis* and *S. cruzi*.

In the present work, the macrocysts from esophageal and tongue muscles appeared opaque white in color, spindle shaped or rice seeds shaped and ranged in size from 2.5-3x1-1.2 mm. **Metwally *et al*. (2014)** found that macroscopic cysts were spindle in shape and consisted of opaque bodies, milky white in color, ranged in size from 1.27 to 22.0 × 0.5 to 0.8 mm. **El-Shanawany *et al*. (2019)** said that the obtained cysts were of *S. fusiformis* and characterized by milky white opaque color with fusiform shape measured 0.5-3.5 × 0.3-0.8 cm. In the present study, the microcysts from esophageal and tongue muscles appeared elongated, stumped and cylindrical shaped cysts. They had thin and smooth wall and divided internally with trabeculae into several compartments. **Wahba *et al*. (2014)** found that microcysts of camel appeared elongated, cylindrical and spindle in shape and had thin, smooth cyst wall and divided internally by trabeculae into several compartments. **Ibrahim *et al*. (2018)** found microscopic stumped shaped *Sarcocystis* cyst in esophagus of buffaloes.

Bradyzoites from macrocysts were banana-shaped and their width appeared either thin (8.8-10 x 1.1-1.4 µm) or wide (9.0-9.6 x 2.2-2.4 µm). This may be different types of bradyzoites or different developmental stages. Metrocytes were stumped and less curved in shape (4.8-5.6 x 2-2.4 µm). **Metwally *et al*. (2014)** found banana-shaped bradyzoites and ranged in size from 8.4 to 15.6 × 2.5 to 5.4 µm. In case of microcysts, bradyzoites were banana shaped (8.6-9.2 x 2.0-3.1 µm) and some of them ap-

peared with one end more broader than other end. Metrocytes appeared stumped in shape (5.4-5.8 x 2-2.2 µm). Some of bradyzoites and metrocytes appeared with ruptured walls and thus may be due to its walls are ill-defined and fragile.

By histopathological section, some pathological changes were observed only with heavy infection, represented by oedema in between muscles, with loss of their striation and degeneration of some infected muscle fibers in addition to inflammatory cells infiltration and coagulative necrosis. After ingestion of sporocysts and subsequent migration of sporozoites through body vessels, acute lesions such as oedema, and necrosis developed may be attributed to rupture of the cysts at irregular intervals with resultant release of antigens into the circulation, thus forming antigen and antibody complexes that in turn lesions occurred **(McLeod *et al.*, 1980)**. Our investigation were supported by the findings of **Fatma *et al*., (2008)** who reported that some pathological changes as muscular degeneration of the esophagus were only associated with heavy infection with microscopic cysts (*S. cruzi*) infection in cattle. **Wahba *et al.* (2014)** mentioned that tunica muscularis of esophageal camel revealed Zenker's necrosis with oedema between muscle fibres as well as hyperplastic proliferation in tunica mucosa may be attributed to necrotizing vasculitis which decreases blood supply to muscles and epithelium. **Hussein *et al*. (2017)** when examined imported frozen beef found that the infected muscles fiber with microscopic *Sarcocystis* showed loss of striation, fragmentation and intermuscular oedema. **Abdel Aziz *et al*., (2017)** observed degeneration and necrosis of muscle fibers with infiltration of mononuclear cells in connective tissue of tongue muscles in buffaloes.

A correlation between pathological changes and the degree of infection prove that the pathological reactions could be detected with heavily infected cases by microscopic cysts (*S. cruzi*). This is agreed with both **Dubey *et al.*, (1989)** and **Collier *et al.*, (1998)** who reported that *S. cruzi* is more pathogenic for cattle than *S. hirsute* and *S. hominis*.

The obtained results revealed few pathological changes observed in infection with *Sarcocystis cruzi*,. In consistency with this results **Meistro *et al*., (2015)** mentioned that no pathological changes were observed in muscular fibres or surround interstitium. Few pathological changes accompanied the infection with *Sarcocystis* species in the present study may be attributed to that the infection was not severe enough to produce severe pathological changes.

A molecular approach is widely utilized for the identification of *Sarcocystis* species variation, as transmission electron microscopy is of limited value in the detection of slight morphology variations **(Gjerde, 2013)**. In the present study, 18S rRNA gene was used for the differentiation of *Sarcocystis* species in water buffaloes using genus-specific primers as in previous studies of **DalimiAsl *et al*., (2008)** and **El-Kady *et al*., (2018)**.

The present study recorded that *Sarcocystis* isolates in the water buffaloes was belonged to *S. cruzi* (accession number MN396435). However, previous studies reported that water buffalo used as an intermediate host to *S. cruzi* and infection is not bounded to cattle only **(Li *et al*., 2002; Jehle *et al*., 2009; Xiang *et al*., 2011; Metwally *et al*., 2014; Gjerde *et al*., 2016 and Mousa *et al*., 2016)**. Water buffaloes are usually infected with *Sarcocystis fusiformis*, *Sarcocystis dubeyi*, *Sarcocystis levinei* and *Sarcocystis buffalonis* **(Dubey *et al*., 1989; Jehle *et al*., 2009 and Abu-Elwafa *et al*., 2015)**.

In conclusion, the present study reported two *Sarcocystis* spp. (*S. fusiformis* and *S. cruzi*) infecting buffaloes in Egypt. Our findings prove the hypothesis that *S. cruzi* is able to use animals such as water buffaloes as intermediate hosts. Also, this study confirmed the isolated samples of *S. cruzi* were genetically identical and 18S rRNA gene used as genetic marker to identify *S. cruzi*.

References

Abdel Aziz, A.R.; Ali, F.A. and Salman, D. (2017). Microscopic and IFAT Based Incidence of *Sarcocystis* in Naturally Infected Buffaloes (*Bubalus bubalis*) at Sohag, Egypt**.** PSM Veterinary Research; 2(2): 22-28

Abu-Elwafa, S.A.; Al-Araby, M.A. and Abbas, I.E.A. (2015). *Sarcocystis fusiformis* (Railliet, 1897) infecting water buffaloes (Bubalus bubalis) in Dakahlia Province, Egypt. I.J.A.R., 3: 116-120.

Ahmed, A.M.; Elshraway, N.T. and Youssef, A.I. (2016). Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egypt. Veterinary World, 9(12): 1461-1465.

Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W. and Lipmanl, D.J. (1990). Basic Local Alignment Search Tool. J. Mol. Biol., 215: 403-410.

Arafa, M.I.; Monib, M.M.; Dyab, A.K. and Abdel-Ghaffar, S.KH. (2003). Studies on ocular *Sarcocystis* in buffaloes in Assuit Governorate. Ass. Univ. Bull. Environ. Res., 6 (1): 27-35.

Badawy, A.I.; Abouzaid, N.Z. and Ahmed, H.A. (2012). *Sarcocystis hominis* and other *Sarcocystis* species infecting cattle in Sharkia province, Egypt. J. Am. Sci., 8(8): 271-275.

Bahari, P.; Salehi, M.; Seyedabadi, M. and Mohammadi, A. (2014). Molecular Identification of Macroscopic and Microscopic Cysts of *Sarcocystis* in Sheep in North Khorasan Province, Iran. Int. J. Mol. Cell Med., 3(1): 51-56.

Dakhil1, H.G.; Abdallah, B.H. and Abdallah, F.A. (2017). Molecular identification of *Sarcocystis fusiformis* and *S. moulei* infecting water buffaloes (*Bubalus bubalis*) in southern Iraq. World Journal of Pharmaceutical Research, 6(3): 215-229.

DalimiAsl, A.H.; Mutamedi, G. and Paykari, H. (2008). Detection of *Sarcocystis* spp. of slaughtered sheep in GazvinZiaran slaughter house by molecular assay. J. Modarres Med. Sci., 11: 65-72.

Dar, K.H.; Bhat, M.M. and Shafi, M. (2017). Prevalence and organwise distribution of Sarcocystiosis in buffaloes of Mohali, Punjab. J. Parasit. Dis., 41(2): 318–321.

Dubey, J.P.; Speer, C.A. and Epling, G.P. (1982). Sarcocystosis in newborn calves fed *Sarcocystis cruzi* sporocysts from coyotes. Am. J. Vet. Res.; 43: 2147-2164.

Dubey, J.P.; Speer, C.A. and Fayer, R. (1989). Sarcocystosis of animals and man. Boca Raton: C.A.C. Press Inc., 105-145.

Dubey, J.P. and Lindsay, D.S. (2006). Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. Vet. Clin. Food Anim., 22: 645 -671.

Dubey, J.P.; Calero-Bernal, R.; Rosenthal, B.M.; Speer, C.A. and Fayer, R. (2015a). Sarcocystosis of animals and humans. CRC press, Boca Raton, Florida.1-481.

El-kady, A.M.; Nermean M. Hussein, M.N. and Hassan, A.A. (2018). First molecular characterization of *Sarcocystis* spp. in cattle in Qena Governorate, Upper Egypt. J. Parasit. Dis., 42(1): 114–121.

El-Seify, M.; El-Morsey, A.; Hilali, M.; Zayed, A.; El-Dakhly, K.; Haridy, M.; Sakai, H. and Yana, T. (2014). Molecular characterization of *Sarcocystis fusiformis* and *Sarcocystis buffalonis* infecting water buffaloes (*Bubalus bubalis*) from Egypt. AJAVS9 (2): 95-104.

El Shanawany, E.; Nassar, S.A. and Ata, E.B. (2019). Detection of humoral and cellular immune responses in buffaloes naturally infected with Sarcocystosis with risk factor assessment. Acta Veterinaria-Beograd, 69 (3): 275-289.

Fatma, G. Sayed; Maha, S.I. Shaheen; Mohsen, I. Arafa and Hoda, M. Koraa (2008). *Sarcocystis* infection in cattle at Assiut abattoir: Ass. Univ. Bull. Environ. Res.,11: 47-57.

Gjerde, B. (2013). Phylogenetic relationships among *Sarcocystis* species in cervids, cattle and sheep inferred from the mitochondrial cytochrome c oxidase subunit I gene. Int. J. Parasitol., 43(7): 579-591.

Gjerde, B.; Hilali, M. and Abbas, I.E. (2016). Molecular differentiation of *Sarcocystis Buffalonis* and *Sarcocystis levinei* in water buffaloes (*Bubalus bubalis*) from *Sarcocystis hirsute* and *Sarcocystis cruzi* in cattle (*Bostaurus*). Parasitol. Res., 115: 2459-2471.

Hussein, D.E.; Abu-Akkada, S.S.; Bessat, M.S.; Aggour, M.G. and Otify, Y.Z. (2017). Molecular identification of *Sarcocystis* species in imported frozen beef in Egypt. Alexandria Journal of Veterinary Sciences. 53 (2): 72-82.

Ibrahim, H.M.; El Sabagh, R.; Wahba, A.A. and Abd El Rahman, E.A. (2018). The Incidence of *Sarcocystis* in Slaughtered Food Animals. Benha Vet. Med., 35: 106-122.

Jain, P.C. and Shah, H.L. (1985). Cross-transmission studies of *Sarcocystis cruzi* of the cattle to the buffalo calves. Indian J. Anim. Sci., 55: 27–28.

Jehle, C.; Dinkel, A.; Sander, A.; Morent, M.; Romig, T.; Luc, P.V.; De, T.V.; Thai, V.V. and Mackenstedt, U. (2009). Diagnosis of *Sarcocystis* spp. Vet. Parasitol., 166: 314-320.

Juyal, P.D. and Bhatia, B.B. (1989). Sarcocystosis: an emerging zoonosis. Indian Vet. Med. J., 13: 66-69.

Jyothisree, Ch.; Venu, R.; Samatha, V.; Malakondaiah, P. and Rayulu, V.C. (2017). Prevalence and microscopic studies of *Sarcocystis* infection in naturally infected water buffaloes (*Bubalus bubalis*) of Andhra Pradesh. J. Parasit. Dis., 41(2): 476-482.

Lam, K.K.; Watkins, K.L. and Chan, C.W. (1999). First report of equine protozoa lmyeloencephalitis in Hong Kong. Equine Vet. Educ., 11: 54-56.

Latif, B.; Al-Delemi, J.K.; Mohammed, B.S.; Al-Bayati, S.M. and Al-Amiry, A.M. (1999). Prevalence of *Sarcocystis* spp. in meat producing animals in Iraq. Vet Parasitol., 84: 85-90.

Latif, B.; Vellayan, S.; Heo, C.C.; Kannan-Kutty, M.; Omar, E.; Abdullah, S. and Tappe, D. (2013). High prevalence of muscular sarcocystosis in cattle and water buffaloes from Selangor, Malaysia. Tropical Biomedicine, 30(4): 699-705.

Li, Q.Q.; Yang, Z.Q.; Zuo, Y.X.; Attwood, S.W.; Chen, X.W. and Zhang, Y.P. (2002). A PCR-based RFLP analysis of *Sarcocystis* cruzi (Protozoa: Sarcocystidae) in Yunnan Province, PR China, reveals the water buffalo (*Bubalus bubalis*) as a natural intermediate host. J. Parasitol., 88: 1259-1261.

McLeod, R.; Hirabayashi, R.N.; Rothman, W. and Renington, J.S. (1980). Necrotizing vasculitis and *Sarcocystis*: A cause and effect relationship. South Med. J., 73: 1380-3.

Meistro, S.; Peletto, S., Pezzolato, M.;Katia Varello, K.; Botta, M.; Richelmi, G.; Biglia, C.; Baioni, E.; Modesto, P.; Acutis, P. and Bozzetta, E. (2015). *Sarcocystis* spp. Prevalence in Bovine Minced Meat: A Histological and Molecular Study. Ital. J. Food Saf., 28: 4(2): 4626.

Metwally, A.M.; AbdEllah, M.R.; AL-Hosary, A.A. and Omar, M.A. (2014). Microscopical and serological studies on *Sarcocystis* infection with first report of *S. cruzi* in buffaloes (*Bubalus bubalis*) in Assiut, Egypt. J. Parasit, Dis. 38(4): 378-382.

Mousa, M.M.; Hiekal, F.A.; El-Hoshy, S.M.; Radwan, M.M. and Abd El Naby, W.H. (2016). Characterization of *Sarcocystis* Species Based on Traditional and Molecular Methods in imported Frozen Buffalo Meat in Egypt. Alexandria Journal of Veterinary Sciences, 51(1): 155-161.

Murata, R.; Suzuki, J.; Hyuga, A.; Shinkai, T. and Sadamasu, K. (2018). Molecular identification and characterization of *Sarcocystis* spp. in horse meat and beef marketed in Japan. Parasite., 25: 27.

Survarna, K.; Lyton, C. and Bancroft, J. (2013). Bancroft's theory and practice of histological Techniques. 7th Ed. Churchil Livingston Elsevier. Cell Dev. Biol., 5:1

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30: 2725-2729.

Thompson, J.D.; Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22): 4673-4680.

Wahba, A.A.; Ayoub, M.B. and Soliman, K.M. (2014). Light and ultrastructure of *Sarcocystis* spp. of camels and associated pathological changes. Anim . Hlth. Res. J., 2(4): 143-158.

Wang, M.Q.; Lin, Q.W.; Liu, H.H., Xiao, N.B.; Zhang, G.C. and Gong, F.Z. (1992). Comparative ultrastructural studies on the cysts of *Sarcocystis cruzi* in cattle and buffalo after cross-infections. Acta Veterinaria ET Zootechnica Sinica., 23: 347-353.

Xiao, N.B.; Zeng, N.D.; Zhang, C.G.; Wang, M.; Li, Y. and Gong, F.Z. (1993). Development of *Sarcocystis cruzi* in buffalo (*Bubalus bubalis*) and cattle (*Bos Taurus*) Acta Veterinariaet Zootechnica Sinica., 24: 185-192.

Xiang, Z.; He, Y.; Zhao, H.; Rosenthal, B.M.; Dunams, D.B.; Li, X.; Zuo, G.; Feng, Y.; Cui, L. and Yang, Z. (2011). *Sarcocystis cruzi*: comparative studies confirm natural infections of buffaloes. Exp. Parasitol., 127: 460-6.