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Original Article

Prevalence and molecular identification of *Sarcocystis* spp. infecting water buffaloes (*Bubalus bubalis*) in Sharkia province, Egypt

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ABSTRACT

Sarcocystosis is regarded as one of the most important protozoal infections of buffaloes. This study was conducted to determine the prevalence of *Sarcocystis* spp. in water buffaloes (*Bubalus bubalis*) in slaughterhouses in Sharkia province, Egypt. In addition, effect of age and sex on the infection rates was reported. Therefore, *Sarcocystis* isolates were identified morphologically using macroscopic, microscopic and histopathological examinations, while molecular identification was carried out using amplification of 18S ribosomal subunit RNA (18S rRNA) by PCR and DNA sequencing. The current findings showed that the prevalence of *Sarcocystis* spp. in water buffaloes was 41.50%. Further, using both morphological and sequences analysis indicated that the isolates obtained from buffaloes were macroscopic *S. fusiformis*. This is the first morphological and genetic study of *S. fusiformis* in slaughtered buffaloes in Sharkia province which will provide useful information for monitoring and controlling of *Sarcocystis* infections.

Key words: Water buffaloes**,** *Sarcocystis fusiformis*, 18S rRNA, DNA sequencing, Sharkia, Egypt.

INTRODUCTION

Sarcocystis spp. is one of the most common protozoan foodborne parasites infecting both humans and many species of animals **(Amairia et al., 2016)**. While, *Sarcocystis* spp. are intracellular cyst forming parasites, it requires two obligatory hosts; the asexual stages develop in an herbivorous intermediate host and the sexual stages develop only in carnivorous definitive hosts **(Bucca et al., 2011; El-Dakhly et al., 2011)**. Hence, more than one *Sarcocystis* spp. can infect one host **(Mekibib et al., 2019)**. Therefore, water buffaloes (*Bubalus Bubalis*) are considered intermediate hosts

for four *Sarcocystis* species; *S. fusiformis* and *S. buffalonis* with cats as their definitive host and *S. levinei* to which dogs are the definitive host, and *S. dubeyi* whose the definitive host has not been identified yet **(Hilali et al., 2011)**. Moreover, **El-Refaii et al. (1995)** suggested that *S. fusiformis* of buffaloes and *S. cameli* of camels can be experimentally transmitted to lambs. On the other hand, some *Sarcocystis* spp. are associated with significant economic losses as they cause decreased weight gain, anemia, low feeding performance, muscle weakness, low milk production, abortion, condemnation of infected carcasses or offal in slaughterhouses and mortality of intermediate hosts in severe cases **(Hamidinejat et al., 2015; Ahmed et al., 2016)**. Sarcocystosis is also represented as a common zoonotic parasitic disease as humans can be may infected by consuming undercooked infected meat with some *Sarcocystis* spp. **(Metwally et al., 2014)**; such as *Sarcocystis hominis* and *S. suihominis* which cause human intestinal infections with digestive disorders as vomiting, diarrhea, nausea and abdominal pain **(Yu, 1991; Oryan et al., 2011)**.

In Egypt, few reports of sarcocystosis were recorded in buffaloes in different provinces as reported by **Abu-Elwafa et al. (2015a)** who detected *S. buffalonis* (8.72%) in Egyptian water buffaloes at Mansoura abattoirs, while **El-Dakhly et al. (2011)** found that overall prevalence of macroscopic *S. fusiformis* and microscopic *S. levinei* was 78.9% in Beni-Suef, Egypt. Furthermore, several global studies were carried out as **Jehle et al. (2009)** who reported 90% of water buffaloes infected with *Sarcocystis* spp. in Northern Vietnam.

Light and electron microscopy can be used to differentiate between different *Sarcocystis* spp. based on morphological features as *Sarcocystis* has unique main characters that can guide to identify species including size and cyst wall ultrastructure, however, those methods are of limiting value, time consuming and, require high number of samples **(More et al., 2011)**. Besides, those characters can be impaired by sarcocysts age, type of host cell and fixation methods **(Fayer, 2004)**. Therefore, molecular approaches seem to be an effective specific and sensitive tool for epidemiological analysis and detection of *Sarcocystis* spp. in the intermediate host **(El-Kady et al., 2018)**. In addition, the 18S rRNA gene with hypervariable regions anticipated a valuable information of identification and description of distinct species, also in the same genus **(Fischer and Odening, 1998)**. Although all

Sarcocystis spp. are host specific in livestock, however, sequences 18S rRNA gene of *Sarcocystis* derived from water buffaloes were nearly identical to *S. hominis* (0.1% difference), making the host specificity of certain *Sarcocystis* spp. of buffaloes controversial with reference to possible sources of human disease of that species **(Yang et al., 2001b)**. Accordingly, reports elucidated the epidemiological and molecular data of *Sarcocystis* spp. in water buffaloes in Sharkia province are lacking as the majority of studies focused on sarcocystosis in cattle. Also, **El-Bahy et al. (2019)** claimed that too little attention paid to molecular genetic studies of *S. fusiformis* in Egypt. Thus, the goal of the current study was to provide the more recent data on the prevalence of *Sarcocystis* spp. in water buffaloes slaughtered at abattoirs in Sharkia province, Egypt. In addition, we described the morphological and histopathological features of the examined specimens and performed molecular identification of *Sarcocystis* spp. using 18S rRNA genes sequencing analysis.

MATERIALS AND METHODS

Sample collection

The present work was conducted in Sharkia province slaughterhouses during the period between November, 2019 and October, 2020. A total number of 147 of slaughtered buffaloes were randomly examined from different abattoirs (Zagazig, Mina El-Kamah and Abou Hamad) in Sharkia province, Egypt. During normal routine postmortem meat inspection, oesophagus, tongue, heart and masseter muscles were examined. Tissue samples of those organs were sent the laboratory of Parasitology, Fac. Of Vet. Med., Zagazig Univ., Egypt for further macroscopic and microscopic investigations.

Macroscopic examination

Heart and oesophagus, tongue and masseter muscles were examined by the naked eye to detect macroscopic sarcocysts during normal post-mortem examinations at abattoirs **(Mohamed et al., 2020)**.

Microscopic examination

Microscopic examinations were performed through muscle squash method (impression technique); where 1 gm of muscular tissue was sliced into small pieces of about 5 mm thick and pressed forcibly between two glass slides to detect microscopic *Sarcocystis* cysts. Then, slides were fixed with methanol and stained with Giemsa stain to observe bradyzoites using light microscopy at 400x **(Hamidinejat et al., 2010)**.

Pepsin digestion method

Tissues were digested in acidic pepsin according to **Gareh et al. (2020)**. Muscular

tissue of 70 gm was homogenized and incubated with digestion solution of 1.5% HCL acid and 0.5% pepsin at 29 °C overnight. Then, digested liquid filtered, centrifuged at 1500 rpm for 10 min, pour off the supernatant. The sediment was stained with Giemsa stain and examined microscopically to detect bradyzoites.

Histopathological examination

Tissue specimens were fixed in 10% neutral buffered formalin for 48 hours then routinely processed for paraffin embedding and sectioned into 4-5 µm thick sections. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically **(Bancroft and Stevens, 1996)**.

DNA isolation

Genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Germany) with modifications manufacturer's instructions. 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 incubation, 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, and centrifuged. 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.

PCR amplification

18S ribosomal DNA genes were amplified according to protocol described by **Bahari et al. (2014)** and **Elmishmishy et al. (2018)** by using the primer pair Sar-F1 Forward (5'GCACTTGATGAATTCTGGCA3') and Sar-R1 Reverse (5'CACCACCCATAGAATCAAG3').

PCR reactions were performed in a total volume of 25 µl containing 1 µl of each primer, 12.5 µl Emerald Amp GTPCR master mix (Takara Bio Inc.), 6 µl template DNA and 4.5 µl nuclease free water. PCR cycles was as follows: 94 °C for 5 min as a hot start first step, followed by 35 cycles of 94 °C of 45 s, 1 min. at 55 °C, at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR amplicons were visualized on electrophoresis of 1.5% agarose gel.

Phylogenetic analysis

PCR amplicons were purified using QIAquick PCR extraction product kit (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for DNA sequence reaction with the aid of Applied Biosystems3130 genetic analyzer (HITACHI, Japan). BLAST was used to align and compare DNA sequences with GeneBank database of sequences

(Altschul et al., 1990). The obtained data sequences were submitted into GenBank databases under accession number MW324480.

Phylogenetic analyses were constructed using maximum likelihood and neighbour joining method by MEGA6 software **(Tamura et al., 2013)**.

Statistical analysis

Age and sex variables were analyzed with Chi-square ($\chi 2$) test using IBM SPSS Statistics for Windows software version 21. *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

Prevalence of *Sarcocystis* cysts in water buffaloes

The current study revealed that the overall prevalence of *Sarcocystis* spp. in naturally infected water buffaloes was 41.50%. Further, macroscopic sarcocysts were detected but no microscopic cysts were identified. Although oesophagus, tongue, heart and masseter muscles were examined during normal carcass inspection, the highest prevalence rates were recorded in oesophageal muscles 56 (91.80%) followed by tongue 5 (8.20%), while no cysts were detected in heart neither masseter muscles. The findings showed statistically significant difference (*P values ≤ 0.05*) between the infection rate among different age groups where the old animals of more than two years had a higher infection rate (58.73%) than younger ones (28.57%) (**Table 1**). Results of the present study showed no significant relation (*P values=0.636*) between the sex and the infection rate; although males had higher percentage (42.86%) than females (38.78%) (**Table 2**).

Morphological and histological identification

Macroscopically, the examined eosophageal specimens showed several elongated, fusiform, opaque whitish sarcocysts resembling rice grains of variable sizes embedded in the muscular layer near the serosal surface (**Fig. 1A**).

Microscopically, the sarcocysts were fusiform-shaped measuring 2-3.5 x 1-1.5 mm with a thin cyst wall (5-10 µm) (**Fig. 1B**). Eosinophilic thin villar protrusions were evident at some areas of the wall. The sarcocyst wall surrounded an amorphous eosinophilic ground substance among which metrocytes were observed. The ground substance extended eosinophilic septa dividing the sarcocyst into compartments full of banana shaped bradyzoites (**Fig. 1C**). The compartments were more crowded with bradyzoites at the periphery of the cyst and almost empty towards the center (**Fig. 1D**). The host muscular layer showed slight

intermuscular edema and muscular degeneration represented by hyalinization with loss of striations in some areas. No inflammatory infiltrations were observed.

Molecular analyses

PCR analysis revealed that macrocysts samples showed DNA fragments of 600 bp on agarose electrophoresis gels. Genotype of sequence of 18S rRNA gene was identified by BLAST analysis through comparing with *Sarcocystis* DNA sequences in the GenBank showed that the present macroscopic isolate sequences related to *S. fusiformis* reference sequences. The percent identity with previous Egyptian *S. fusiformis* strains published data of both accession numbers KR186121 and KR186123 were 99.8%. However, nucleotide homology reached 99.8% with Chinese strain (accession number AF176927). Whilst, identity percent of current isolate was 96% with previous identified Egyptian *S. buffalonis* isolate (accession number KU247901 and KU247903). Also, identity of our sequence with *S. hirsuta* reached 95.8% with a study submitted under accession number KT901160.

Phylogenetic analysis revealed that the identified sequence in the current study was closely related and clustered to previously reported data of *S. fusiformis* isolates as well as related somewhat to other clades with other *Sarcocystis* spp. as *S. buffalonis* and *S. hirsuta* (**Fig. 2**).

Table 1: Rate of infections among age groups

Age groups	No. examined animals	No. infected animals	%
< 2.5 years	84	24	28.57
≥ 2.5 years	63	37	58.73
Total	147	61	41.50

Table 2: Rate of infections related to sex.

Sex	No. examined animals	No. infected animals	%
Males	98	42	42.86
Females	49	19	38.78
Total	147	61	41.50

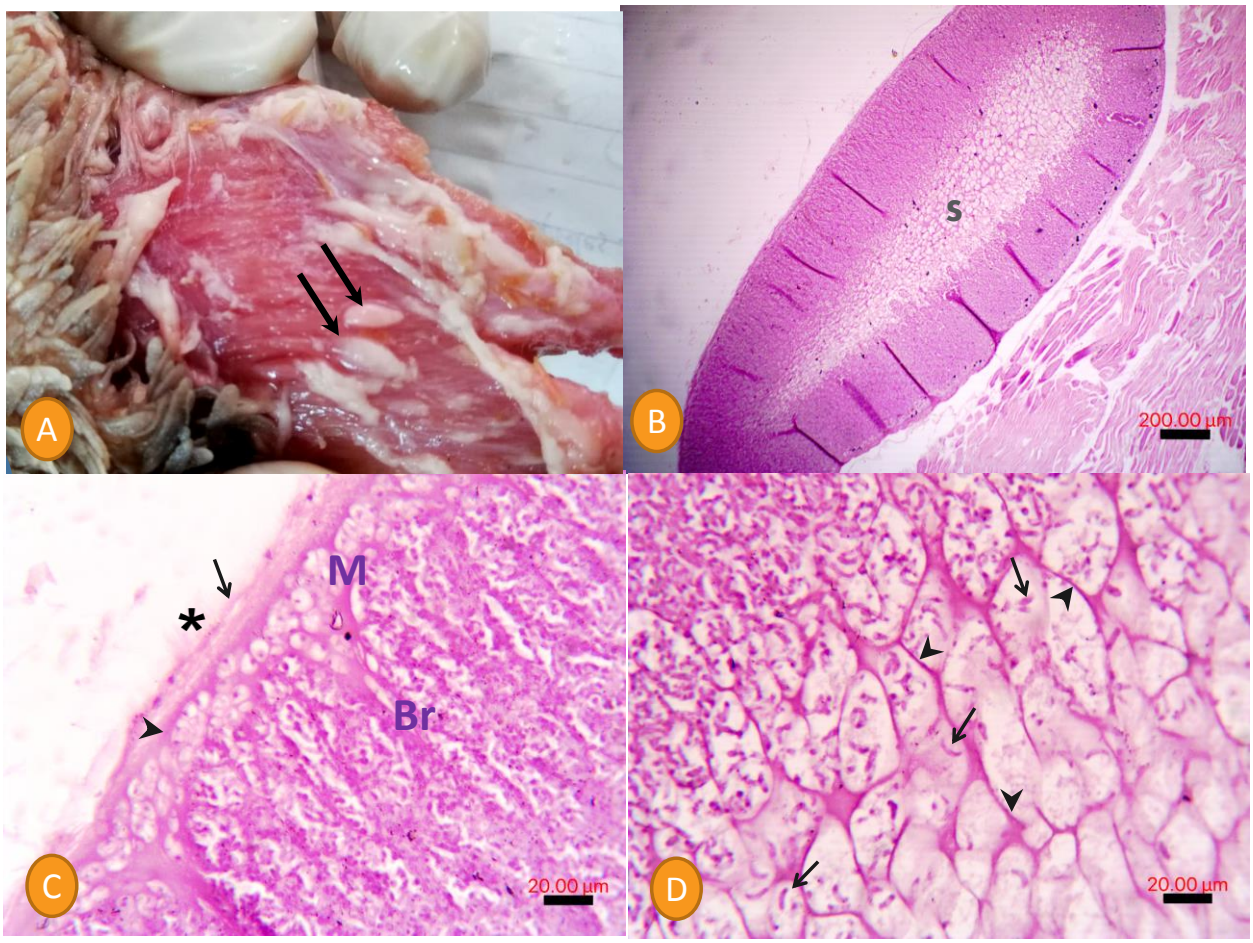

Fig. 1: Macroscopic *S. fusiformis* cysts in oesophagus of an infected buffalo

A) Macroscopic appearance of *S. fusiformis* in the eosophageal wall. The sarcocysts are elongated, fusiform, opaque whitish in color resembling rice grains and located near the serosal surface of the eosophagus (black arrows). **B-D)** Photomicrographs of a subserosal macroscopic cyst in the wall of the eosophagus. **B)** A longitudinal section of a sarcocyst (S) measuring 3 x 1 mm aligned along the longitudinal axis of the muscular layer which shows slight edema and degeneration. bar=200 µm **C)** The wall of the sarcocyst (arrow) is about 5-10 µm thick with villar protrusions evident at some areas of the wall (asterisk), enclosing a layer of eosinophilic ground substance (arrow head) containing metrocytes (M) and sending septa separating the sarcocyst into compartments containing bradyzoites (Br). bar=20 µm **D)** A higher magnification of the center of the sarcocyst showing the septal division (arrow heads) of the cyst into compartments containing banana shaped bradyzoites (arrows). bar=20 µm

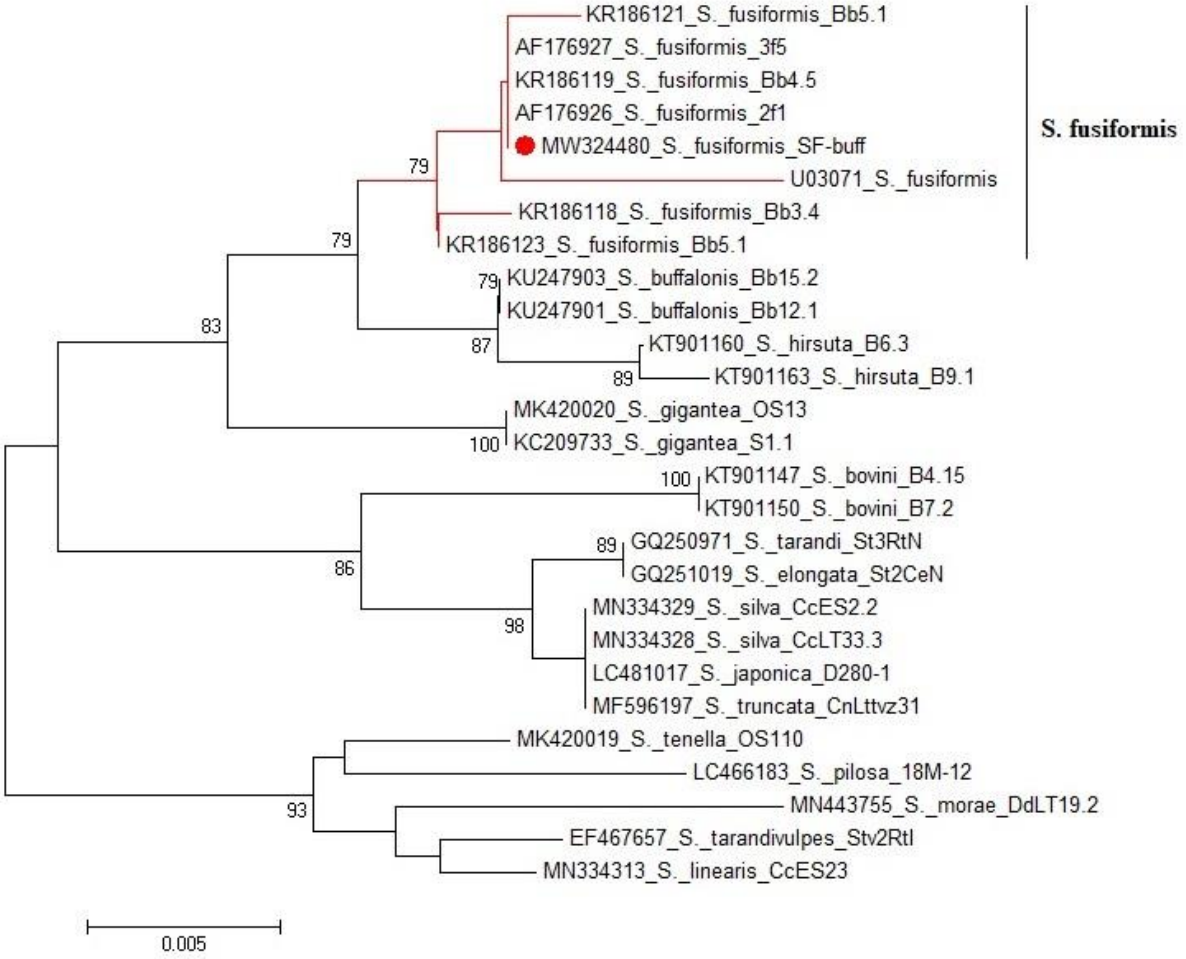

Fig. 2: Phylogenetic tree showing the relationship of the *S. fusiformis* sequences resulting from the present study (red circle), and other *Sarcocystis* spp., based on analysis of a partial sequence of the 18S rRNA gene and inferred by the maximum likelihood (ML) method.

DISCUSSION

Sarcocystosis is an intracellular protozoan disease caused by different *Sarcocystis* spp. which induce nervous disorders, abortion and significant economic losses with mortality of severe infected host animals **(Sun et al., 2021)**. The current study highlighted the morphology and prevalence of *Sarcocystis* spp. infecting water buffaloes as well as associated risk factors. To the authors' knowledge, this is the first molecular identification of *Sarcocystis* spp. isolated from tissues of slaughtered water buffaloes in Sharkia province using 18S rRNA genes sequencing and phylogenetic analysis. Although only one molecular identification of *Sarcocystis* spp. in water buffaloes using PCR-RFLP, was conducted by **Abd-ElRahman (2014)** in Sharkia province but this previous study didn't take account of DNA sequencing and phylogenetic approaches.

In the present study, examination of muscular tissues of water buffaloes showed that the prevalence of macroscopic *S. fusiformis* was 41.50%. These results were lower than those published in Egypt by **Ghaffar et al. (1978)**; **Abu-Elwafa et al. (2015b)**; **El-Bahy et al. (2019)** and **El Shanawany et al. (2019)** who found that the infection rate of water buffaloes with *S. fusiformis* was 100%, 58.72%, 85.96% and 74% respectively. However, the results observed in this investigation were far higher than those observed by **Khalifa et al. (2008); El-Dakhly et al. (2011)** and **Ahmed et al. (2016)** who reported 28%, 6.9% and 8.33% of water buffaloes infected with *S. fusiformis* respectively. *S. fusiformis* was recorded in water buffaloes of other countries; including Vietnam where **Huong (1999)** recorded a prevalence of 41%, Iraq where **Latif et al. (1999)** reported a prevalence of 15.6% and Andhra Pradesh India where the infection rate reached 22.62% **(JyothiSree et al., 2017)**.

We postulate that high prevalence of macroscopic *S. fusiformis* with no identified microscopic forms in our results might be explained as regular contact between abundance of infected cats (definitive hosts) with *S. fusiformis* and water buffaloes. Those infected cats can shed many *Sarcocystis fusiformis* sporocysts in their faces and contaminating environment, feed, water as well as pastures which represented a main source of infection for intermediate hosts as buffaloes to harbour macroscopic sarcocysts **(El-Dakhly et al., 2011)**. Further, our interpretation supported by **Oryan et al. (2010)** who detected only microscopic

Sarcocystis spp. with no *Sarcocystis fusiformis* in the examined buffaloes which explained by high abundance of dogs than cats in that study region which play an important role in transmission of such form of *Sarcocystis*. In addition, the inconsistency between the prevalence rates could be also attributed to other factors as viability of *Sarcocystis* sporocysts for long time under incompatible environmental conditions, suitable climatic conditions needed for survival of sporocysts as well as diagnostic methods in terms of sensitivity and specificity **(Anvari et al., 2020)**.

With regard to location of macroscopic cysts among different organs, *Sarcocystis* spp. mainly infect muscular tissues as the oesophagus, tongue, heart as well as other types of muscles **(Gareh et al., 2020)**. Our study indicated that the oesophagus was the most infected site followed by tongue which was consistent with **JyothiSree et al. (2017)** and **El-Bahy et al. (2019)**. Also, these results further accords with obtained data by **Huong (1999); Latif et al., (1999); Abu-Elwafa et al. (2015b)** and **Ardalan (2020)** who found a high prevalence rate in oesophagus. In the present study no *Sarcocystis* cysts were detected in heart muscles which corresponded to those observed in earlier studies by **Ahmed et al. (2016)**. Whereas for *S. levinei* and *S. dubeyi* the most infected muscular tissues were masseter muscles of water buffaloes **(Oryan et al., 2010)**. In accordance with the current investigation, previous studies demonstrated that oesophagus and tongue were the most common organs to be infected with *S. fusiformis*. Meanwhile, **El-Dakhly et al. (2011)** reported that distribution of *Sarocystis* cysts among affected organs of buffaloes didn't follow a particular pattern.

Concerning to the effect of age on the prevalence rate of *S. fusiformis*, our data revealed that older buffaloes of more than two years were more likely to be infected than younger ones. Furthermore, this study confirmed that old age was statistically associated with high *S. fuisformis* infection rate in water buffaloes. These results were in agreement with previous observations by **Huong (1999)** and **Ibrahim et al. (2018)** which showed high prevalence of sarcocystosis in old animals and **El-Bahy et al. (2019)** who reported that infection rate of *Sarcocystis fusiformis* was higher in animals older than 5 years (92.28%) than in animals 3 - 5 years old (29.32%). This correlation may be explained in part as old age animals were more presumably to prolonged exposure to sporocysts infections as well as macroscopic cysts required more time to be

visible in muscles **(Abu-Elwafa et al., 2015b; Ahmed et al., 2016)**.

The current study found that there was no statistical correlation between sex and the infection rate with *S. fusiformis* in water buffaloes but marginally higher prevalence was recorded in males (42.86%) than females (38.78%). These findings seemed to be consistent with other observations obtained by **Oryan et al., (2010)** and **Ghorbanpoor et al. (2007)**. This finding is contrary to previous studies which have suggested that the percentage of infected males was lower than the infected females **(Ahmed et al., 2016; Ibrahim et al., 2018; El Shanawany et al., 2019)**. Other possible explanations of those studies where female animals had higher infection rates than males can be the subjection of females to stress factors such as pregnancy and lactation which supersede the immune system **(Ibrahim et al., 2018)**.

Regarding to morphometric characters of the identified the macroscopic *S. fusiformis* isolated from esophageal muscles, our results have nearly similar pattern of findings reported by **El-Dakhly et al. (2011); JyothiSree et al., (2017)** and **Ibrahim et al. (2018)**. The current histopathological findings were in line with those previous studies done by **El-Dakhly et al. (2011)** who recorded thick cyst wall 2.6 - 14.5 µm; while, **El-Seify et al. (2014) and Abu-Elwafa et al. (2015b)** who reported a size of thin cyst wall of *S. fusiformis* measured 1-3 µm. Thus far based on macroscopical and histopathological features of previous studies affirmed our findings in terms of *S. fusiformis*. However, morphological and histopathological findings may be somewhat limited by maturation and age of *Sarcocystis* cyst as mature sarcocysts were either with thick wall of outer protrusions or thin cyst wall with short protrusions which the differentiation between species related to the mature cysts as young cysts was so far similar **(Böttner et al., 1987; Fayer, 2004; Morsy et al., 2018)**. Thus, those data needed to be interpreted carefully as it was difficult to identify and distinguish *Sarcocystis* spp. based on to morphological and histopathological findings **(Dubey et al., 2014; Hamidinejat et al., 2015)**. In consequence, molecular approaches were widely used to substantiate the obtained morphology data as well as data of gene sequencing was valuable to delineate if morphologically indiscernible sarcocysts that infect single or several intermediate hosts belongs to same or distinct species **(Kia et al., 2011; Gjerde, 2013)**.

In the present study, we utilized 18S rRNA gene to identify *Sarcocystis* spp. due to the 18S rRNA gene variable regions considered a relevant genetic target to discern between closely related *Sarcocystis* spp. **(Holmdahl et al., 1999; Yang et al., 2002)**. According to those gene sequence data assembled with morphological criteria analysis, we could infer that the tested isolates collected from water buffaloes belongs to *S. fusiformis*.

The phylogenetic analyses conducted in this study showed a close evolutionary relationship between current *S. fusiformis* isolate with Egyptian *S. fusiformis* isolate (accession number KR186121) **(Gjerde et al., 2015)**, Chinese isolate (accession number AF176927) **(Yang et al., 2001a)** which represented by 99.8 % sequence similarity with only one substitution nucleotide and 100% query coverage. However, **(El-Seify et al., 2014)** in Egypt; suggested high genetic variability of 18S rRNA gene within different isolates of the same *Sarcocystis* spp. regarding to geographic distribution. While our present Egyptian *S. fusiformis* differed from those previously recorded by **(El-Seify et al., 2014)** in Egypt and **(Holmdahl et al., 1994)** in Sweden by 3% and 1% respectively in terms of nucleotide sequences. Therefore; we assumed that high genetic variations within the same species were not related to geographical locations but with a small sample size, however, these findings might be questionable. Although the definitive host of *S. fusiformis*, *S. buffalonis* (parasites of water buffaloes) and *S. hirsuta* (Parasite of cattle) are feline origin, our phylogenetic tree analysis showed that the current *S. fusiformis* isolate was far relatedness to Egyptian *S. buffalonis* isolate (accession number KU247901) **(Gjerde et al., 2016)** and *Sarcocystis hirsuta* (accession number KT901160) in New Zealand **(Gjerde, 2016)**; whereas our study isolate demonstrated about 96% sequence identity with those two previously sequenced isolates. Hence, **(Hamidinejat et al., 2015)** and **(Morsy et al., 2018)** reported that the 18S rRNA gene sequence exhibit variable genotypic behaviors as well as high variable regions triggering assorted multiple copies of this gene which amplified from diverse merozoites in the *Sarcocystis* cysts. A further study with more focus on ultrastructural and molecular investigations at level of definitive and intermediate hosts is therefore needed to exemplify resemblance and differences of *Sarcocystis* spp. as well as host range and variations in host specificity in Egypt.

CONCLUSION

The results of the current study indicated macroscopic *S. fusiformis* was common in slaughtered water buffaloes in Sharkia province, Egypt employing structural and genetic data analysis. Those combined results provide an ongoing framework to understand the biology of *Sarcocystis* spp., transmission dynamics, and parasite identification which are necessary for controlling and prevention strategies.

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الملخص العربى

دراسات وبائية وجزيئية لأنواع الساركوسيست التى تصيب الجاموس فى محافظة الشرقية بمصر

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يعتبر داء الساركوسيستوزيس أحد أهم الأمراض الطفيلية التى تصيب الجاموس. حيث أجريت هذه الدراسة لتحديد معدلات إنتشار الأنواع المختلفة من طفيل الساركوسيستس في الجاموس في مجازر محافظة الشرقية ، مصر. بالإضافة إلى ذلك ، تم دراسة تأثير العمر والجنس على معدلات الإصابة.

تم التعرف على عزلات الساركوسيستس من خلال خصائصها المورفولوجية باستخدام الفحوصات الظاهرية والميكروسكوبية والفحص الباثولوجى للانسجة ، بينما تم إجراء التعريف الجزيئي باستخدام تفاعل البلمرة المتسلسل (PCR) أولا ثم تحديد التسلسل لجين (18S rRNA). حيث أظهرت النتائج الحالية أن معدل انتشار حويصلات الساركوسيست في الجاموس كانت ٤١,٥٠٪. علاوة على ذلك ، بإستخدام التحليل المورفولوجي والتحليل المتسلسل أشارت إلى أن عزلات الساركوسيستس التي تم الحصول عليها من الجاموس كانت للنوع ساركوسيستس فيوزيفورمز. وتعتبر هذه الدراسة الأولى المورفولوجية والوراثية لطفيل الساركوسيست فيوزيفورمز في الجاموس المذبوحة في مجازر محافظة الشرقية والتي ستوفر معلومات ذات أهمية كبيرة فى رصد والسيطرة على العدوى بطفيل الساركوسيست فى الجاموس.