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# Prevalence, Histopathology and Molecular Characterization of *Sarcocystis* species Infecting Buffaloes in Menoufia Governorate, Egypt

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## ABSTRACT

The present study was done to assess the infection rate, histopathological and molecular characterization of *Sarcocystis* species infecting buffaloes in Menoufia governorate, Egypt. Different muscles of 3513 slaughtered buffaloes in three abattoirs from Menoufia governorate were inspected by naked eye. Histopathological examination was applied on infected muscles with cysts. The DNA from each individual sarcocysts was extracted, amplified and sequenced. The results indicated that the total infection rate of sarcocystosis in examined buffaloes was (12.87 %). The prevalence in females was higher (20.23%) than males was (10.42%). The most affected muscles with *Sarcocystis* spp. cysts were esophagus. There was slight variation in the prevalence of sarcocystosis between the three abattoirs. The occurrence of sarcocystosis was slightly higher in winter and spring seasons than Autumn and Summer seasons. The amplified DNA was yielded the expected band size. The sequence of *Sarcocystis* cyst in this study was aligned with similar sequences of Sarcocystis spp. which was deposited in gene bank. The sequence of 18S rRNA was highly identical (94-99%) to sequences of *Sarcocystis fusiformis* from Egypt and other countries. The phylogenetic tree revealed that *Sarcocystis* in the current study was founded in the same genetic clade with the gene bank sequences of *Sarcocystis fusiformis*. In conclusion, the infection rate of sarcocystosis was low in current study compared with previous studies may be due to the enhanced or proper disposal of carnivores and human feces.

**Keywords:** *Sarcocystis* spp., Buffaloes. Prevalence, histopathology, molecular characterization, Egypt.

## INTRODUCTION

*Sarcocystis* species were a coccidian protozoan which caused cyst in tissues, its life cycle had two hosts, including herbivorous (intermediate host) and carnivorous (definitive host) (Soulsby, 1982). Sarcocystis spp. were host specific and very common in animals (Dubey et al., 1989). Several species could infect the same host (Dubey et al., 1996; and Bhatia, 2000). Sarcocystosis disease was parasitic and zoonotic which was frequently

common in cattle and buffaloes. *S. hominis* was one of the parasitic diseases that was significantly affects public health. Human infections were commonly caused by meats and meat products and infect people when they consume tissue cysts contain bradyzoites (Juyal and Bhatia 1989). Buffaloes were identified as an intermediate host *S. dubeyi*, dogs were a definitive host for *S. levinei* and cats were definitive hosts for *S. fusiformis* and *S. buffalonis* (Hilali et al., 2011). Bovine eosinophilic myositis (BEM) was an inflammatory response which caused degenerated muscle fibers and condemnation of meat could be resulted from *Sarcocystis* species. Concerned to macroscopic cysts of *S. hirsute*, it caused financial losses during the process of meat detection (Dubey and Rosenthal 2023). Concerned to histopathological examination there was fibrosis which caused the white color, degradation of tissue and the concentration of eosinophil granulocytes was produced the green color of the infected muscles (Dubey and Rosenthal 2023). *Sarcocystis* could be examined in muscle through macroscopic identification of sarcocysts or through the histological study (Urqhurt et al., 1996). Customary meat inspection ways in abattoirs were not accurate for determination of the parasite prevalence due to there was small sarcocysts. So, it was strongly recommending the need to use microscopical identification in the serological (ELISA) and postmortem for identification of the disease in Egypt (Metwally et al., 2014). Meat may be detected by cutting meat itself in hydrochloric acid, pepsin and then concentrated the content for presence of bradyzoites (Fayer, 2004). Detection of sporocysts in dogs faces or cats could be helpful in the diagnosis (Urqhurt et al., 1996). The application of control measures of sarcocystosis on animals' food, hygiene, farm pets and control of uncooked meat all help limitation of the infection (Urqhurt et al., 1996; and Fayer, 2004). PCR had been applied for identification of the Sarcocysts. *S. cruzi* and *S. hominis* from cattle depending on 18S rRNA gene (Fischer and Odening 1998). Using 18S ribosomal RNA on s*arcocystis* from buffalo gave nearly identical sequence to *S. hominis* and this was mean that multiple species that affect ruminant were served as an intermediate host and also a potential source for human infection by this parasite (Yang et al., 2001). This study was focused on the infection rate, histopathology and molecular study of sarcocystis spp. in slaughtered buffaloes in Menoufia province, Egypt.

## MATERIAL AND METHODS

### *1. Sample collection and study area*

This work was brought through the year of 2022 from January to December in Menoufia governorate, Egypt, to conclude the prevalence of Sarcocysts in buffaloes at 3 abattoirs (Ashmoun, El-Shohada and Quisna) in Menoufia, Egypt.

A total of 3513 buffaloes (875 females and 2638 males) were examined macroscopically by naked eye for the immediacy of the parasite. The examined buffaloes were divided into two groups (1st group from 2 to 3 years and 2nd group over 5 years).

The esophagus, pharyngeal, diaphragm, neck, heart, masseter, tongue, thigh and other muscles were investigated grossly for presence of macroscopic sarcocysts.

For microscopic finding of the sarcocystis cysts, specimens of infected tissue were putted and compressed between two slides then were examined under microscope (Mowafy, 1993).

### *2. Histopathological examination*

Infected muscle samples were fixed in formalin (10%). After three days, the muscular tissues were dehydrated, embedded in paraffin. and sectioned to (3µm) for staining by hematoxylin and eosin stain (H&E) (Bancroft and Gamble 2002).

***3. Sample preparation and DNA extraction***

The recovered sarcocysts were washed at first several times with Phosphate Buffer Saline. DNA was pried from each individual sarcocysts from infected buffaloes by using Qiagen DNeasy Tissue and Blood kit® according to manufacturer guidance.

***4. PCR analysis***

Partial gene sequence of 18S rRNA of *Sarcocystis fusiformis* was amplified by using the forward primer (5`-CGCCCTTTTAGTGAGGGTGT3`) and reverse primer (5` TACGAATGCCCCCAACTGTC 3`) (El-Seify et al., 2014). The PCR reactions were concluded in 25 µl volume which was contained 12 µl of Emerald Amp® GT PCR Master Mix [Takara Biotechnology], 1 µl of the DNA genome, 10 µl of sterile distilled water (DW) and 1 µl from each primer. The annealing was done at 55 °C for 40 sec. and extension was at 72 °C for 45 sec. An initial denaturation step at 94 °C for 5 min and 35 cycles of final denaturation at 94 °C for 45 sec. and a final extension step at 72 °C for 10 min. Products were screened by electrophoresis in 1.5 % agarose gels.

***5. DNA Sequencing, Sequence Alignment and phylogenic Analysis***

The expected band size was at (600 bp), DNA was purified by using extraction kit gel and sequenced. The18S rRNA sequences were blasted with the NCBI BLAST methods and the sequence was aligned in GenBank. The phylogenetic tree was premediated by the neighbor-joining. The 18S rRNA gene sequence of the parasite from Menoufia, Egypt was used to create a tree with other sequences from the GenBank.

## RESULTS

***1. Infection rate of Sarcocystis in buffaloes:***

A total number of 3513 buffalo carcasses (875 females and 2638 males) were all examined grossly through three abattoirs from Menoufia governorate, Egypt throughout one year to detect the prevalence of *Sarcocystis* species.

The overall prevalence with *Sarcocystis* spp. in buffaloes was 12.87% (452 out of 3513). The prevalence in males was 10.42 % (275 out of 2638), while the prevalence in females was 20.23 % (177 out of 875) (Table 1).

Result recorded in Table (2) revealed the prevalence of sarcocystosis (three abattoirs) in Menoufia province. Prevalence of *Sarcocystis* spp. was 10.9%, 14.38 % and 11.38 % in Ashmoun, El shohada and Quesna abattoirs, respectively. Females showed higher infection rates than males in different three abattoirs.

The distribution of the *Sarcocystis* species in slaughtered male buffaloes was 145 (52.72%) in esophagus and pharyngeal muscles, and 130 (47.27%) in the tongue, while in females were 135 (48.74%) in esophagus and pharyngeal muscles 142 (51.26%) in the tongue (Table 3).

Regarding to seasonal prevalence in Table (4), the females in different seasons recorded higher infection rate with sarcocystosis than males. The prevalence of sarcocystosis was higher in winter (14.82 %) and spring (14.27%) than Autumn (10.67%) and summer (10.27 %), respectively.

**Table 1.** Overall prevalence of *Sarcocystis* species cysts in buffaloes carcasses at 3 abattoirs in Menoufia governorate

| sex | No. examined | No. infected | percent of infection |
|---|---|---|---|
| Male | 2638 | 275 | 10.42 % |
| Female | 875 | 177 | 20.23 % |
| Total | 3513 | 452 | 12.87 % |

**Table 2.** The infection rate of *Sarcocystis* spp. cysts in examined buffaloes carcasses of 3 abattoirs in Menoufia governorate.

| **Abattoir** | **Examined carcasses** | | | **Infected carcasses** | | | | **Total** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | | Females | | Infected | non infected | % |
| | | | | N | % | N | % | | | |
| **Ashmoun** | 637 | 116 | 753 | 55 | 8.63 | 21 | 18.1 | 76 | 677 | 10.09 % |
| **El-shohada** | 811 | 579 | 1390 | 120 | 14.79 | 100 | 17.27 | 220 | 1170 | 14.38 % |
| **Quesna** | 1100 | 270 | 1370 | 100 | 9.09 | 56 | 20.74 | 156 | 1214 | 11.38 % |
| **Total** | 2538 | 975 | 3513 | 275 | 10.42 | 177 | 19.48 | 452 | 3061 | 12.87 % |

**Table 3.** The distribution of *Sarcocystis* spp. cysts in different muscles of infected buffaloes carcasses.

| **Infected organ** | **Male** | **Female** | **Total** |
|---|---|---|---|
| Esophagus and Pharyngeal muscle | 145 (52.72%) | 135 (48.74% ) | 280 |
| Tongue | 130 (47.27%) | 142 (51.26%) | 272 |
| Total | 275 | 277 | 552 |

**Table 4.** Seasonal infection rate of *Sarcocystis* spp. cysts in buffaloes carcasses at 3 abattoirs in Menoufia governorate.

| Season | Genus | | | | Total | | | Percent of infection |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | | | | |
| | Infected | Non infected | Infected | Non infected | Infected | Non infected | total | |
| **Autumn** | 45 | 454 | 26 | 140 | 71 | 594 | 665 | 10.67% |
| **Winter** | 85 | 620 | 57 | 196 | 142 | 816 | 958 | 14.82% |
| **Spring** | 95 | 764 | 65 | 197 | 160 | 961 | 1121 | 14.27% |
| **Summer** | 50 | 524 | 29 | 166 | 79 | 690 | 769 | 10.27% |
| **Total** | 275 | 2362 | 177 | 699 | 452 | 3061 | 3513 | 12.87% |

***2. Morphological and histopathological examination of Sarcocystis spp. in buffaloes:***

The macroscopic *Sarcocystis* cyst appeared grossly as a spindle shape cyst, white colored and distributed under the serosal membrane between the muscle bundles parallel to the longitudinal axis of the muscle fiber, their measurements were ranged from 0.3-2.5 x 0.2-0.7 cm. The most infected muscles were esophagus and tongue muscle (Fig.1).

Microscopically, the cyst had a thick prominent wall (Fig.2). Numerous thin septa were attached from the wall of cyst which was separated the cyst into irregular several chambers filled with oval, elongated and other large organisms, which were found as bradyzoites. The bradyzoites were crowded peripherally, while in the central part of the sarcocysts, there was a small area where the septa inhibited very few bradyzoites, moreover there was no inflammatory response around the sarcocysts was associated.

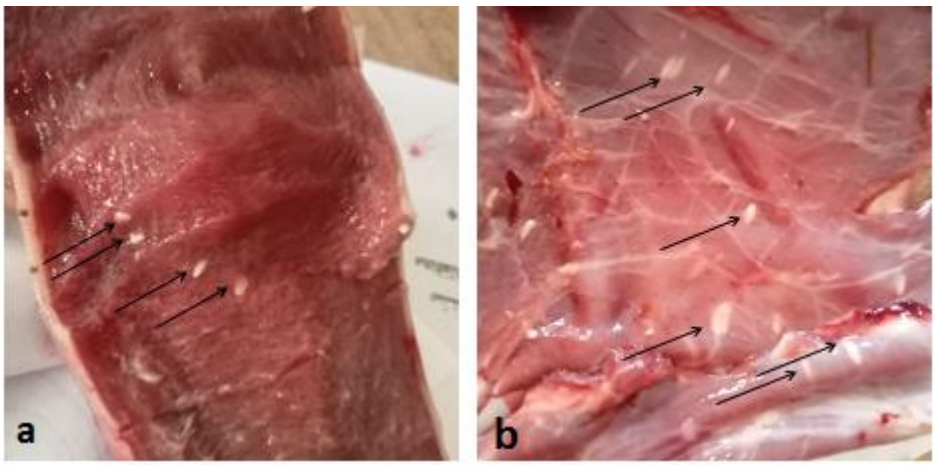


**Fig. 1.** Photograph of several macroscopic *Sarcocystis* spp. cysts in Tongue (a) and esophagus (b) of infected buffalo carcass

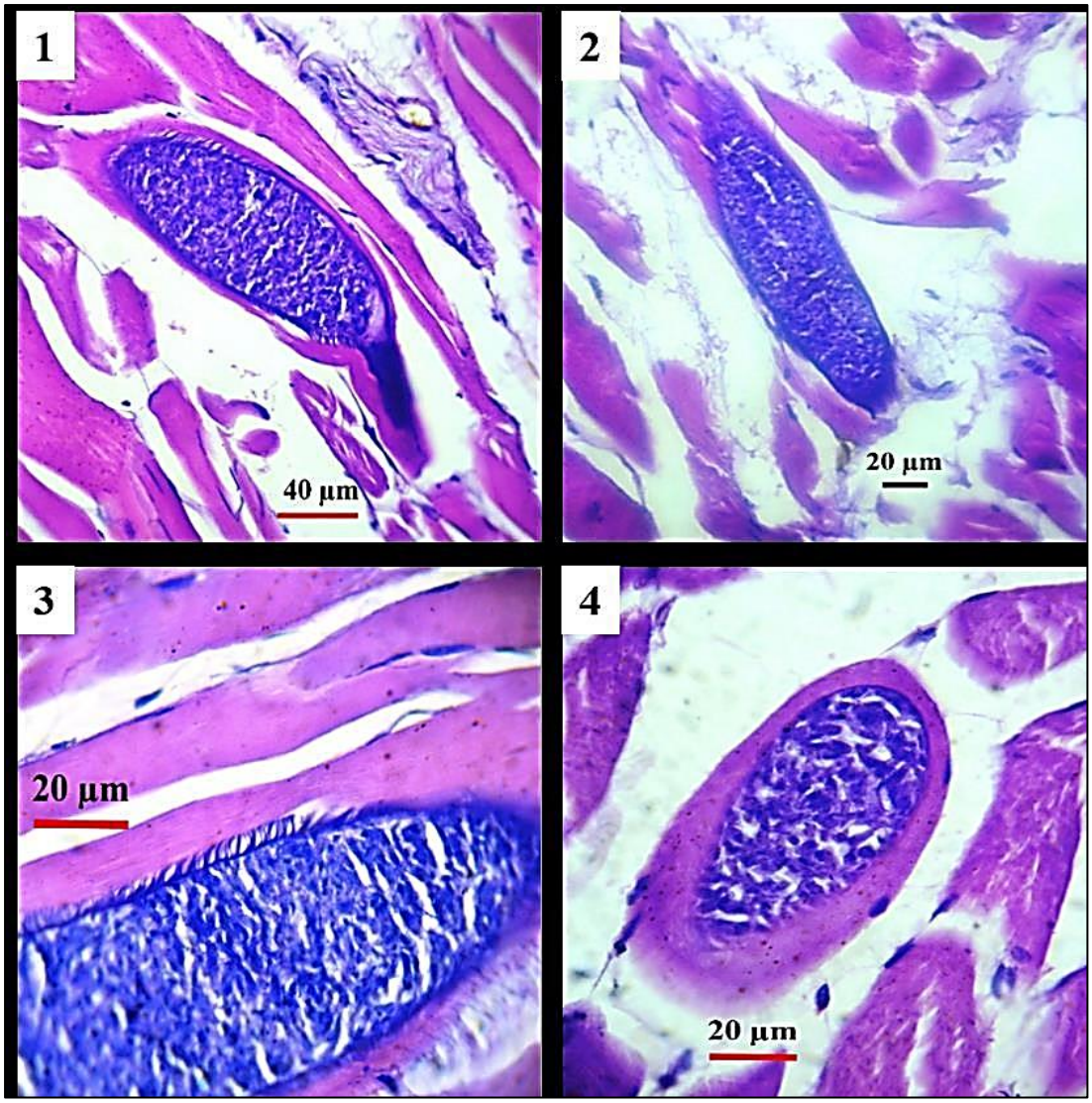


**Fig. 2.** Micrograph of *Sarcocystis* species cyst in muscle of infected buffalo (H & E stained)

***3. Molecular characterization of Sarcocystis species***

The DNA which extracted was then amplified from each macroscopic cyst by using forward and reverse primers of targeted 18S ribosomal RNA (18S rRNA). The amplified DNA was yielded band at 600 bp as in (Fig. 3).

Sequences of Sarcocystis cyst in current study was aligned with the sequences of similar Sarcocystis species. which deposited in gene bank. The sequence of 18S rRNA was highly identical (about 94-99%) with sequences of *Sarcocystis fusiformis* from Egypt and other countries. The phylogenetic tree revealed that *Sarcocystis* in the current study was founded in the same genetic clade with the *Sarcocystis fusiformis* in gene bank (Fig.4).

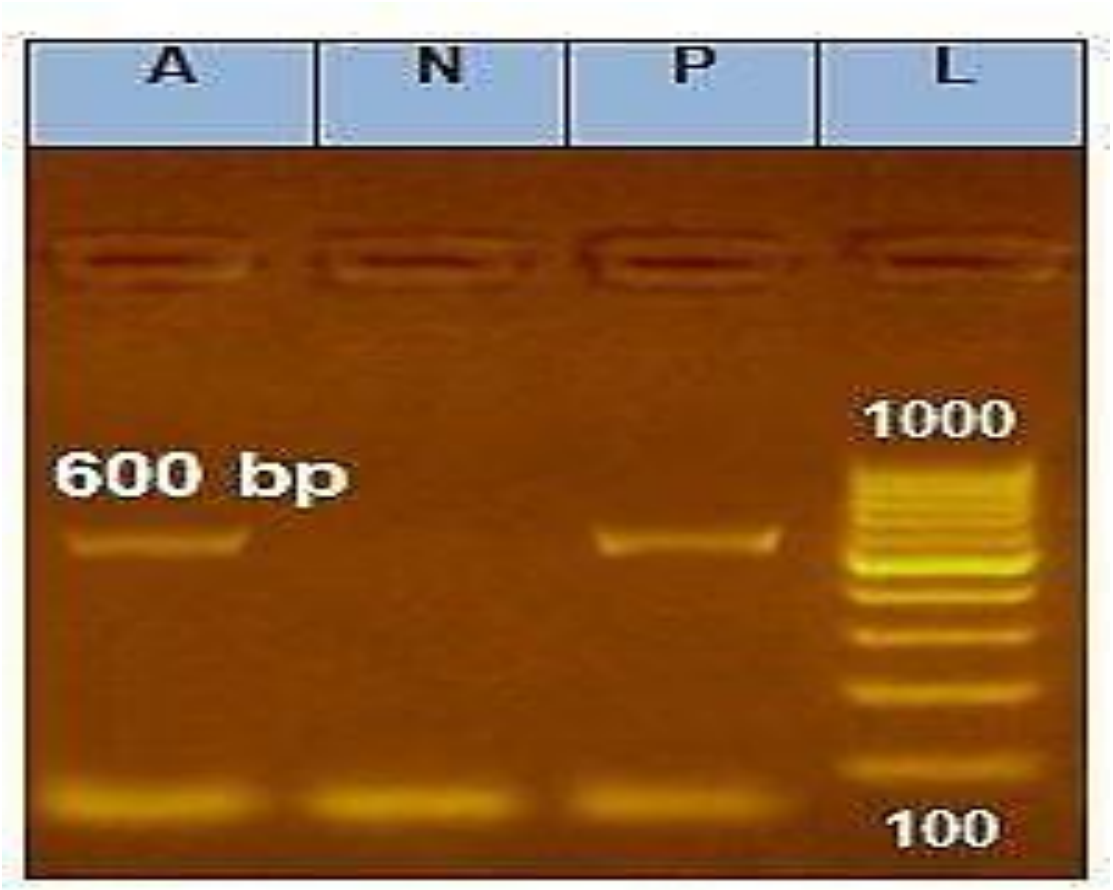


**Fig. 3.** Gel electrophoresis of the 18S rRNA gene of *S. fusiformis*. DNA size marker of 100bp.


**Fig. 4.** Phylogenetic tree of *Sarcocystis fusiformis* 18S rRNA gene from Menoufia, Egypt**.**

## DISCUSSION

The current study was showed the infection rate and molecular characterization of *Sarcocystis fusiformis* in Egyptian buffaloes in Menoufia province, Egypt. The total prevalence of *S. fusiforms* in slaughtered buffaloes was (12.87%) and this rate was decrease than that of Mohanty et al., (1995) who found that the infection rates with *S. fusiforms* was (87%) in India, Said, (1996) who found the infection rate of *S. fusiforms* was (76.8 %) in Beni-suef, Egypt, Fawaz, (1998) who found that the infection rate of *S. fusiforms* was in Qena (72.6 %). Latif et al., (1999) who found that the prevalence of *S. fusiforms* was (82.9%) in Iraq, and El-Dakhly et al., (2011) who mentioned that the infection rate of *S. fusiforms* was (78.6 %) in Beni-suef, Egypt,

The low infection rate of sarcocystosis in the current study may be due to

ways that used in diagnosis of infection that depend on the detection of sarcocysts by using naked eye during meat inspection.

Concerning to the allocation of sarcocysts in variant organs, the Sarcocystis species in the present study were infect different muscles as oesophagus, tongue, pharyngeal muscle and the esophagus was the most infected organs with Sarcocystis spp., this finding agreed with previous studies Huong, (1999); Latif et al., (1999); Oryan et al., (2010); Abdel-Baky's, (2011); Abu-Elwafa et al., (2015b); JyothiSree et al., (2017); El-Bahy et al., (2019); Ardalan (2020) and Gareh et al., (2020) who reported that the most infected organs were oesophagus, heart and tongue.

In this study, there was no parasite cysts were discovered in heart muscles, this finding disagree with El-Dakhly et al. (2011) and Ahmed et al., (2016) who recorded that occurrence of the parasitic *Sarocystis* in variant organs of buffaloes didn't hold a special pattern.

In current study, the prevalence was greater in females (20.23%) than males (10.42%) and this finding agreed with Ahmed et al., (2016); Ibrahim et al., (2018) and El Shanawany et al., (2019) who reported that the prevalence of Sarocystis cysts was affect females more than males, and also agreed with Ghorbanpoor et al., (2007); Oryan et al., (2010) and Ibrahim et al., (2018) who found that female animals was exhibited greater infection rate than males.

The difference between sex in the present study could be due to the stress factors that affect females as pregnancy, lactation and the immune system.

The infection rate of sarcocystosis in the present study was greater in winter (14.82 %) and spring (14.27%) than Autumn (10.67%) and summer (10.27%) seasons and the females were recorded greater prevalence than males, this detection disagreed with Abdel-Baky, (2011) who found that there was no contrast in the infection rate of Sarcocystis parasite species in buffalo carcasses per season.

In the current study, molecular characterization of Sarcocystis parasite by using primers of 18S rRNA gave a band at 600 base pair. The sequence of the primer was highly identical (about 94-99%) with sequences of *Sarcocystis fusiformis* from Egypt and other countries. The phylogenetic tree revealed that *Sarcocystis* in the current study was recorded in the same genetic clade with the *Sarcocystis fusiformis* in gene bank, this finding agrees with Holmdahl et al., (1999); Li et al., (2002) and Jehle et al., (2009) who reported that the18S had targeted gene for the characterization and detection of the related Sarcocystis species and for the phylogenic analysis also, also agreed with Olsen and Woese (1993) who found that the gene regions of 18S rRNA was used to increase the same gene region in similar parasite spp. Because of its structure which allowed the extension in different phylogenic analysis studies, and agreed with Holmdahl et al., (1999) who stated that the 18S primer gene was gave an identity value with other species, it was an important to detect Sarcocystis based on molecular analysis of its gene, and also agreed with Williams et al., (1990) who found that the molecular techniques had been useful in the determination of Sarcocystis spp., in intermediate hosts. These methods were provided specific and more sensitive ways for diagnosis than conventionally one in the epidemiological.

## REFERENCES


Abdel-Baky, A. A., 2011. Some Studies on *Sarcocystis fusiformis* in Egyptian Buffaloes, Ph. D. Thesis, Fac. of Vet. Med, Beni sueif Univ, Egypt.

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. International Journal of Advanced Research 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. Vet World, 9: 1461-1465.

Ardalan, N. M., 2020. The Light Microscopy and Ultrastructural Characteristics of *Sarcocystis fusiformis* Infecting Buffaloes in Iraq. Journal of the Egyptian Society of Parasitology, 50: 235-241.

Bancroft, J. D., and Gamble, M., 2002. "Theory and Practice of Histological Techniques". In: Swisher, B. (Ed.), Microorganisms. Churchill Livingstone, Philadelphia: 325–344.

Bhatia, B. B., 2000. Textbook of Veterinary Protozoology, 1st Edition (ICAR, New Delhi).

Dubey, J. P., Speer, C. A., and Fayer, R., 1989. Sarcocystosis in Animals and Man. 1st ed. CRC Press, Boca Raton, FL, pp 1–145.

Dubey, J. P., Hamir, A. N., Niezgoda, M., and Rupperch, C. E., 1996. A Sarcocyst Neurona-like Organism Associated with Encephalitis in A Stripped Stuck (Mephitis mephitis), Journal of Parasitology, 82, 172–174.

Dubey, J. P., Fayer, R., Rosenthal, B. M., Calero-Bernal, R., and Uggla, A., 2014. Identity of *Sarcocystis* Species of the Water Buffalo (*Bubalus bubalis*) and Cattle (Bos taurus) and the Suppression of *Sarcocystis sinensis* as a Nomen nudum. Veterinary parasitology, 205: 1-6.

Dubey, J. P., and Rosenthal, B. M., 2023. Bovine Sarcocystosis: *Sarcocystis* Species, Diagnosis, Prevalence, Economic and Public Health Considerations, and Association of *Sarcocystis* Species with Eosinophilic Myositis in Cattle. International Journal for Parasitology, 53(9), 463–475.

El Shanawany, E. 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, 69: 275-289.

El-Bahy, N., El-Bagory, A., AbouLaila, M., Elkhatam, A., and Mady, H., 2019. Prevalence of *Sarcocystis fusiformis* and Hydatid Cyst Among Different Ruminants at Menofia Governorate, Egypt. Journal of Current Veterinary Research, 1: 1-10.

El-Dakhly, K. M., El-Nesr, K. A., El-Nahass el-S, Hirata, A., Sakai, H., and Yanai, T., 2011. Prevalence and Distribution Patterns of *Sarcocystis* spp. in Buffaloes in Beni-Suef, Egypt. Trop Anim Health Prod 43:1549–1554.

El-Seify, M., El-Morsey, A., Hilali, M., Zayed, A., El-Dakhly, K.,

Haridy, M., Sakai, H., and Yanai, T., 2014. Molecular Characterization of *Sarcocytis fusiformis* and *Sarcocystis buffalonis* Infecting Water Buffaloes (*Bubalus bubalis*) from Egypt. American Journal of Animal and Veterinary Sciences, 9: 95-104.

Fawaz, A. A., 1998. Incidence of Toxoplasma and Sarcosporidia in Slaughtered Animals in Qena Governorate, (Unpublished PhD Thesis. Faculty of Veterinary Medicine, Assiut University).

Fayer, R., 2004. *Sarcocystis* spp. in Human Infections. Clinical Microbiology Reviews, 17(4): 894–902.

Fischer, S. K., and Odening, 1998. Characterization of Bovine *Sarcocystis* Species by Analysis of Their 18S ribosomal DNA Sequences. J. Parasitol. 84:50–54.

Gareh, A., Soliman, M., Saleh, A. A., El-Gohary, F. A., El-Sherbiny, H. M. M., Mohamed, R. H., and Elmahallawy, E. K., 2020. Epidemiological and Histopathological Investigation of *Sarcocystis* spp. in Slaughtered Dromedary Camels (*Camelus dromedarius*) in Egypt. Veterinary sciences, 7: 162.

Ghorbanpoor, M., Hamidinejat, H., Nabavi, L., Khadjeh, G. H., Razi, and Jalali, M., 2007. Evaluation of an ELISA for the Diagnosis of Sarcocystosis in Water Buffaloes. Bulletin of the Veterinary Institute in Pulawy, 51: 229-231.

Hilali, M., El-Seify, M., Zayed, A., El-Morsey, A., and Dubey, J. P., 2011. *Sarcocystis dubeyi* (Huong and Uggla, 1999) Infection in Water Buffaloes (*Bubalus bubalis*) from Egypt. The Journal of parasitology, 97: 527-528.

Holmdahl, O. J., Morrison, D. A., Ellis, J. T., and Huong, L. T., 1999. Evolution of Ruminant *Sarcocystis* (Sporozoa) Parasites Based on Small Subunit rDNA Sequences. Mol. Phylogenet. 11: 27-37.

Huong, L. T. T., 1999. Prevalence of *Sarcocystis* spp. in water buffaloes in Vietnam. Veterinary parasitology, 86: 33-39.

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 Veterinary Medical Journal, 35: 106-122.

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. in Cattle (*Bos taurus*) and Water Buffalo (*Bubalus bubalis*) in Northern Vietnam, Veterinary Parasitology, 166, 314–320.

Juyal, P. D., and Bhatia, B. B., 1989. Sarcocystosis: An Emerging Zoonosis, Indian Veterinary Medical Journal, 13, 66–69.

JyothiSree, C., 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. Journal of Parasitic Diseases, 41: 476-482.

Latif, B. M., Al-Delim, J. K., Mohamed, 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.

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. The Journal of parasitology, 88(6), 1259–1261.

Metwally, A. M., Abd Ellah, 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. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology, 38: 378-382.

Mohanty, B. N., Misra, S. C., Panda, D. N., and Panda, M. R., 1995. Prevalence of *Sarcocystis* Infection in Ruminants in Orissa. Indian Vet J 72:1026–1030.

Olsen, G. J., and Woese, C. R., 1993. Ribosomal RNA: A key to phylogeny FASEB, J., 1: 113-123.

Oryan, A., Ahmadi, N., and Mousavi, S. M. M., 2010. Prevalence, Biology and Distribution Pattern of *Sarcocystis* Infection in Water Buffalo (*Bubalus bubalis*) in Iran. Tropical Animal Health and Production, 42: 1513-1518.

Said, M. S., 1996. Muscular Parasites in Slaughtered Animals in Assiut Governorate. PhD thesis, Faculty of Veterinary Medicine Assiut University.

Soulsby, E. J. L., 1982. Helminthes, Arthropoda and Protozoa of Domesticated Animals, 6th Edition (Baillier, Tindall and Casell, London).

Urquhart, G. M., Armour, P., Duncan, J. L., Dunn, A. M., and Jennings, F. W., 1996. Veterinary Parastitology. ELBS. Longman. England 2nd ed.

Williams, J. G. K., Kubelik, A. R., Jivak, K. S., Rafalksi, J. A., and Tingey, S. V., 1990. DNA Polymorphisims Amplified by Arbitrary Primers are Useful as Genetic Markers, Nucleic acids Res, 18: 6531-6535.

Yang, Z. Q., Y. X., Zuo, B., Ding, X. W., Chen, J. Luo, Y. P., and Zhang, 2001. Identification of *Sarcocystis hominis*-like (Protozoa: Sarcocystidae) Cyst in Water Buffalo (*Bubalus bubalis*) Based on 18s rRNA Gene Sequences. J. Parasitol. 87:934–937.